RFA RENEWABLE FUELS ASSOCIATION

growth energy™
America's Ethanol Supporters

February 3, 2012

Byron Bunker
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Office of Transportation and Air Quality
U.S. Environmental Protection Agency
National Vehicle and Fuel Emissions Laboratory/OAR
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Re: Registration of E15

Dear Mr. Bunker:

On behalf of our member companies, the Renewable Fuels Association (RFA) and Growth Energy hereby submit the attached documentation in support of the registration of gasoline that contains up to 15 percent ethanol by volume (E15), as required by section 211(b) of the Clean Air Act. In an effort to support the introduction of E15 in the marketplace, RFA and Growth Energy have developed the attached health impact information to meet the Tier I and Tier 2 testing requirements under 40 CFR Part 79.

Enclosed are the combustion and evaporative emissions characterizations and the literature search in fulfillment of the Tier 1 requirements at 40 CFR 79.52. While we developed new data and information in fulfillment of the Tier 1 requirements, we relied on previously submitted data on E10 to meet the Tier 2 requirements for E15, as permitted under 40 CFR 79.53. As explained herein, the literature search conducted pursuant to 40 CFR 79.52 contains the results of adequately performed and adequately documented previous testing that provides information reasonably comparable to that which would be supplied by additional Tier II testing. Specifically, the results of previously conducted Tier 1 and Tier 2 testing on baseline gasoline and baseline gasoline blended with 10% ethanol (E10) are reasonably comparable to information that would be provided by testing E15 separately. The enclosed documents include:

- Combustion Emission Characterization of E0, E10, and E15 in Support of the Fuel and Fuel Additive Registration of E15 (Prepared by Southwest Research Institute, Project No. 03.15812, February 2011)
- Evaporative Emissions Characterization of E0, E10, and E15 in Support of the Fuel and Fuel Additive Registration of E15 (Prepared by Southwest Research Institute, Project No. 03.15812, February 2011)

- Evaporative and Exhaust Emissions Characterization of 2011 E0, E10, and E15: Comparison to Data Developed by the Section 211(b) Research Group (Prepared by Cambridge Environmental Inc., February 17, 2011)
- Addendum to Characterization of 2011 E0, E10, and E15: Comparison to Data Developed by the Section 211(b) Research Group (Prepared by ENVIRON International Corporation, February 3, 2012)
- Literature Review and Report: Ethanol-15 (E15) Emission Product Health and Welfare Effects Studies Summary (Prepared by ENVIRON International Corporation, October 19, 2011)

RFA and Growth Energy agree that the attached health impact test data and analysis should be made available free of charge to any fuel or fuel additive manufacturer required to register E15 before introducing E15 into commerce. Please consider this letter as a waiver of our right to proportional reimbursement of testing costs under 40 CFR 79.56. Please also waive the requirement under 40 CFR 79.59 that future registrants provide evidence that they have notified us of their use of the data and analysis and have complied or intend to comply with the above-noted proportional reimbursement requirements. We note that this waiver is only applicable to the Tier 1 test data and Tier 2 analysis we developed and that there may be other reimbursement requirements for potential E15 registrants regarding our reliance on previously submitted Tier 2 data for E10.

Respectfully submitted,

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COMBUSTION EMISSION CHARACTERIZATION OF E0, E10, AND E15 IN SUPPORT OF THE FUEL AND FUEL ADDITIVE REGISTRATION OF E15

FINAL REPORT

SwRI® Project No. 03.15812

Prepared for:

Renewable Fuels Association and Growth Energy

Prepared by:

E. Robert Fanick
Manager

February 2011

SAN ANTONIO, TEXAS
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SwRI® Project No. 03.15812
Emissions Research and Development Department

Prepared for:

Renewable Fuels Association and Growth Energy

February 2011

Prepared by:

E. Robert Fanick, Manager

Approved by:

Jeff J. White, Director

EMISSIONS RESEARCH AND DEVELOPMENT DEPARTMENT
VEHICLE, EMISSIONS, AND VEHICLE RESEARCH DIVISION

IDENTIFICATION OF TEST SUBSTANCE

Ethanol

MANUFACTURER

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FOREWORD

This project was performed for the Renewable Fuels Association and Growth Energy under SwRI Project 03-15812. Ms. Kristy Moore was the program director for the Renewable Fuels Association (RFA), and Ms. Erin Heupel was the program director on behalf of Growth Energy. The Principal Investigator and the Project Leader was Mr. E. Robert Fanick, Manager of the Emissions Chemistry Section in the Emissions Research and Development Department. SwRI technical personnel involved in testing included: Mr. Jeff A. Mathis, Ms. Svitlana Kroll, Ms. Yolanda Rodriguez, Mr. Chuan-yi (Joe) Tsai, Ms. Janelle N. Sancho, and Ms. Kelley L. Strate. Data reduction was performed by Ms. Linda De Salme, Ms. Amanda L. Korzekwa, and Ms. Rachel Clair. Southwest Research Institute is located at:

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TABLE OF CONTENTS

Page

Appendices **No. of Pages**

LIST OF FIGURES

LIST OF TABLES

TEST SUBSTANCE INFORMATION

The fuel additive of interest for this project was ethanol, chemical formula C_2H_5OH, that would be used in a fuel blend commonly referred to as E15, a blend of 15 percent ethanol and 85 percent unleaded gasoline. Ethanol is also commonly referred to as fuel ethanol or denatured fuel ethanol; all meaning ethanol suitable for use in spark-ignition engine systems. Additional fuels tested in this evaluation were an unleaded gasoline certification fuel with no ethanol added (baseline fuel or E0) and a gasoline certification fuel blended with 10 percent ethanol (E10).

EXECUTIVE SUMMARY

The combustion emission composition of three fuels (E0 - unleaded gasoline, E10 - ethanol blended at a concentrations of 10 percent in unleaded gasoline, and E15 - ethanol blended at a concentration of 15 percent in unleaded gasoline) was evaluated for Renewable Fuels Association (RFA) and Growth Energy to address a portion of the Environmental Protection Agency (EPA) requirements for registration of designated fuels and fuel additives (F/FA) as stipulated by sections 211(b) and 211(e) of the Clean Air Act (CAA). Under the Tier 1 requirements of this protocol, manufacturers of F/FAs are required to supply EPA with:

- The identity and concentration of emission products from the F/FA
- Any available information regarding the health and welfare effects of the whole and speciated emissions
- A characterization of the emission products which are generated by evaporation and by combustion of the F/FA.

This report covers the combustion emission portion of the testing. Three fuels were prepared by Haltermann Products:

- Base fuel (E0), EPA Tier II EEE with additives – Haltermann Product Code HF0872 (SwRI Code EM-7578-F)
- E10, 90 percent unleaded gasoline and 10 percent ethanol – Haltermann Product Code HF0869 (SwRI Code Fuel EM-7579-F)
- E15, 85 percent unleaded gasoline and 15 percent ethanol – Haltermann Product Code HF0870 (SwRI Code Fuel EM-7580-F)

The base fuel was a certification gasoline that met the specifications of Title 40 CFR Part 86, Subpart B, Section 86.113-04 with the addition of the required additives for a 211(b) base fuel. This same fuel was then used to blend the E10 and E15 test fuels. These same three fuels were also tested for evaporative emissions in a separate program for E15, and those results were reported separately.

Emission generation, collection, and analysis of the regulated emissions and of selected vapor- and particulate-phase unregulated emissions, and a speciation of volatile-phase hydrocarbon compounds were performed on a 2011 2.5 L Toyota Camry vehicle equipped with aftertreatment (sequential fuel injection, air/fuel sensor, close-coupled three way catalyst, heated oxygen sensor, underbody three way catalyst). The vehicle was tested after 4000 miles of vehicle operation with the base fuel. Testing involved a four bag cold-start Federal Test Procedure on each of three different days with the aftertreatment installed and then three additional days with the aftertreatment removed. Samples were collected and analyzed for regulated emissions including total hydrocarbons (THC), carbon monoxide (CO), oxides of nitrogen (NO_x), and total particulate; for a hydrocarbon speciation of volatile hydrocarbons; and for volatile- and particulate-phase polycyclic aromatic hydrocarbons (PAH) and nitrated polycyclic aromatic hydrocarbons (NPAH). The fuel was then changed to a 10 percent blend of ethanol with the baseline gasoline, and the vehicle was operated for 4000 miles with this fuel. At the completion of the mileage accumulation, the above emission characterization test sequence

was repeated. The fuel was then changed to a second concentration of 15 percent ethanol blended with the base gasoline. The vehicle was operated for 4000 miles with this fuel, and the above emission characterization test sequence was repeated.

In general, the vehicle was able to meet the 2011 emissions standard for the tests with aftertreatment. In this case, the composite emissions for the base fuel with the aftertreatment were 0.02 g/mi for NO_x, 0.03 g/mi for NMHC, and 0.09 g/mi for CO. The average composite emissions with 10 percent ethanol were 0.02 g/mi for NO_x, 0.01 g/mi for NMHC, and 0.06 g/mi for CO; and the average composite emissions with 15 percent ethanol were 0.03 g/mi for NO_x, 0.02 g/mi for NMHC, and 0.09 g/mi for CO. Without aftertreatment, all of the emissions increased significantly, and the emissions were not able to meet the standards for 2011. A higher level of emissions without aftertreatment was expected because the aftertreatment was necessary for the vehicle to meet these emissions standards.

In addition to the regulated emissions, a determination of the hydrocarbon speciation from C_1 to C_{12} hydrocarbons was conducted on the exhaust for each fuel. When the speciated emissions from the two ethanol blends with no aftertreatment were compared to the emissions with aftertreatment, no additional C_1 to C_{12} hydrocarbons, aldehydes, and ketones were present in the exhaust with aftertreatment at or above the detection limits of the analytical procedures. In addition, a number of compounds were detected in the tests without aftertreatment that were <u>not</u> detected in the tests with aftertreatment. Concentrations were typically lower with aftertreatment than without. Four compounds (1-pentene; ethanol; 1,3-diethylbenzene; and acetaldehyde) were detected at a higher concentration with the two ethanol blends and no aftertreatment when compared to the base fuel with no aftertreatment. Methanol and ethanol were the only alcohols detected above the detection limit except for 2-propanol which was only detected in one test with the base fuel and no aftertreatment. No other higher molecular weight alcohols or ethers were detected.

The measurement of volatile- and particulate-phase PAH and NPAH were required in this study. Analyses were conducted for individual compounds including: benzo[a]anthracene, benzo[b]fluoranthene, benzo[k]fluoranthene, benzo[a]pyrene, chrysene, dibenzo[a,h]anthracene, indeno[1,2,3-cd]pyrene, 7-nitrobenzo[a]anthracene, 6-nitrobenzo[a]pyrene, 6-nitrochrysene, 2-nitrofluorene, and 1-nitropyrene. In general, the concentrations of PAH and NPAH were lower with aftertreatment than without it, and the particulate-phase PAHs and NPAHs were generally higher in concentration than the volatile-phase PAHs and NPAHs. No additional PAHs or NPAHs were found with the two ethanol blends than were found in the base fuel.

1.0 INTRODUCTION

This work was performed for RFA and Growth Energy to address Environmental Protection Agency (EPA) requirements for registration of designated fuels and fuel additives (F/FA) as stipulated by sections 211(b) and 211(e) of the Clean Air Act (CAA). In general, standard mandatory requirements for F/FA registrations are contained in a three tiered structure. The first two tiers generally apply to most F/FA manufacturers, but there are special provisions for certain types of additives and for small businesses. Each manufacturer is required to submit basic registration data for each product being registered. Small businesses with less than $50 million of annual sales are excused from the first two tiers of requirements for F/FA which are considered baseline or non-baseline, and small businesses with less than $10 million annual sales are excused from Tier 2 requirements for "atypical" F/FA. Definitions of baseline, non-baseline, and "atypical" F/FA are discussed in detail below. Other special provisions include experimental F/FA, relabeled products, and products exclusively for off-road use.

Each F/FA is sorted into one of two broad "fuel families": conventional or alternative. The conventional fuel families are diesel and gasoline, and the alternative fuel families include methanol, ethanol, methane, and propane. Each fuel family is then subdivided into three "F/FA categories": baseline, non-baseline, and "atypical." The baseline category consists of fuels and associated fuel additives which resemble the respective baseline fuel for a particular fuel family in terms of elemental composition (no elements other than carbon, hydrogen, oxygen, nitrogen, and sulfur), and which conforms with certain quantitative limits for particular constituents. "Atypical" is defined as those fuels which contain metals; elements other than carbon, hydrogen, nitrogen, sulfur, and oxygen or do not meet the requirements under ASTM D4814 "Standard Specifications for Automotive Spark-Ignition Engine Fuel." The non-baseline category is an intermediate category between baseline and atypical. In the gasoline family, the distinction between baseline and non-baseline is based primarily on the presence of significant concentrations of oxygen-containing compounds (greater than 1.5 percent oxygen by weight). Ethanol in E15 is considered a non-baseline fuel additive.

Tier 1 testing for the generation, collection, and analysis of combustion emission samples was required. Regulated exhaust emissions for total hydrocarbons (THC), non-methane hydrocarbons (NMHC), carbon monoxide (CO), oxides of nitrogen (NO_x), and particulate (PM) and carbon dioxide (CO_2) were evaluated for all tests conducted. In addition, hydrocarbon speciation was performed to determine volatile-phase exhaust hydrocarbons, aldehydes, ketones, alcohols, and ethers; and samples were collected for volatile- and particulate-phase polycyclic aromatic hydrocarbons (PAH) and nitrated polycyclic aromatic hydrocarbons (NPAH). This report includes the emission measurements that were conducted as part of the requirements for the registration of an additive or fuel as stipulated by sections 211(b) and 211(e) of the CAA.

2.0 LIGHT-DUTY VEHICLE TESTING

2.1 Objective

The objective of this program was to provide RFA and Growth Energy with the generation, collection, and analysis of combustion emission samples from three fuels using a 2011 2.5 L Toyota Camry. One fuel (EM-7578-F) was the base fuel with no additive, the second fuel (EM-7579-F) contained 10 percent denatured fuel ethanol, and the third fuel (EM-7580-F) contained 15 percent denatured fuel ethanol. Emission testing was conducted according to Tier 1 requirements as identified in Title 40 CFR Part 79, Subpart F, Section 79.57. Protocols outlined in Title 40 CFR Part 86, Subpart D and in applicable Southwest Research Institute® (SwRI)® Test and Inspection Procedures (TIP) References 1 through 17 were followed.

2.2 Scope of Work

2.2.1 Light-Duty Test Protocol

The method for evaluating light-duty vehicle exhaust emissions is the FTP, which utilizes the Urban Dynamometer Driving Schedule (UDDS). The UDDS is the result of more than ten years of effort by various groups to translate the Los Angeles smog-producing driving conditions to dynamometer operations, and is a non-repetitive driving cycle covering 7.5 miles in 1372 seconds with an average speed of 19.7 mph. Its maximum speed is 56.7 mph. An FTP consists of a cold-start, 505-second transient phase (Bag 1), followed immediately by an 867-second stabilized phase (Bag 2). Following the 867 phase, the vehicle is allowed to soak for 10 minutes with the engine turned off before proceeding with a hot-start 505 phase (Bag 3) and a 867 phase (Bag 4) to allow sampling for toxic compounds. For a 4-bag FTP, the distance traveled is 15.0 miles. The emissions are mathematically weighted to represent the average of several 7.5 mile trips made from hot- and cold-starts. Table 1 summarizes the cycle duration, driving distance, and average speed for the FTP, and a speed versus time illustration of the 505 and 867 phases of the FTP driving cycle is given in Figure 1.

TABLE 1. SUMMARY OF FTP DRIVING SCHEDULE

SEGMENT	DURATION, SEC.	DISTANCE, MILES	AVERAGE SPEED, MPH
Transient Phase	505	3.6	25.7
Stabilized Phase	867	3.9	16.2
UDDS	1372	7.5	19.7

Exhaust emissions from the FTP cover three regimes of engine operation. The "cold-start" or "cold transient" emissions represent the effects of vehicle and emission control system warm-up as the vehicle is operated over the cycle. The "stabilized" phase produces emissions from a fully warmed up or stabilized vehicle and emission control system. "Hot-start" or "hot transient" emissions result when the vehicle is started after the vehicle and emission control systems have stabilized during operation and are then soaked (turned off) for 10 minutes.

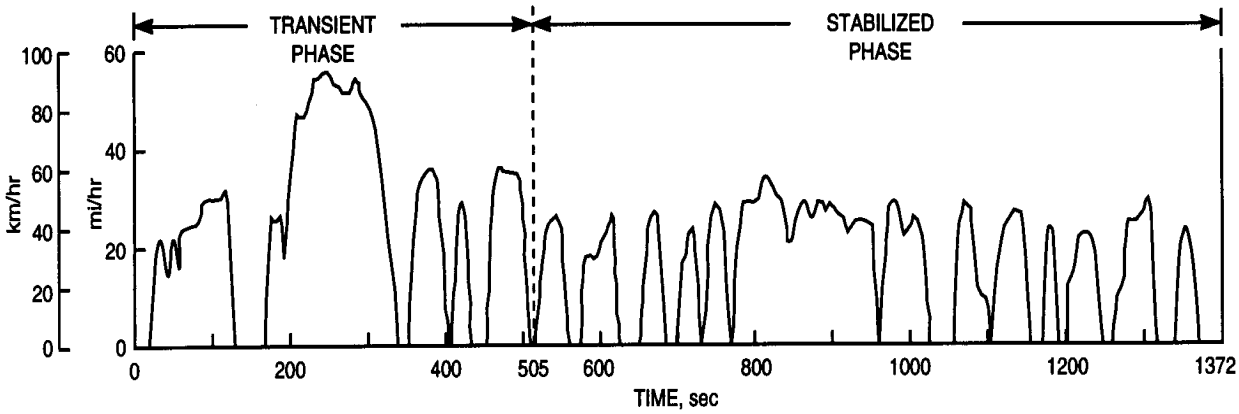

FIGURE 1. SPEED VERSUS TIME ILLUSTRATION OF 505 AND 867 PHASES OF FTP DRIVING CYCLE

The chassis dynamometer used in the vehicle testing was a Clayton Model ECE-50 passenger car dynamometer with a direct drive variable inertia flywheel system. The inertia weight simulates equivalent weights of vehicles from 454 kg (1000 lb) to 2213 kg (4875 lb) in 57 kg (125 lb) increments. A full-flow exhaust dilution tunnel was used in conjunction with a constant volume sampler (CVS). This unit had a nominal capacity of 9 m^3/min (320 scfm). The vehicle hood was maintained fully open during all cycles and closed during soak periods. A cooling fan of 143 m^3/min (5000 cfm) capacity was used in front of the test vehicle to provide air flow during all tests.

Emission testing for characterization of combustion emissions was conducted both with the aftertreatment device intact and with the aftertreatment device rendered non-functional. Table 2 lists the test plan for the light-duty vehicle testing. For both configurations, the emissions were generated on six different days. Two test sequences were conducted during testing (one with base fuel at 4000 miles and one with additive at 4000 miles), resulting in a total of 18 4-bag FTP emission tests (3 fuels × 2 configurations × 3 repeat tests). After vehicle selection was made, the vehicle's catalyst was removed to prepare a non-functioning aftertreatment device. After consulting with Mr. Jim W. Caldwell of EPA's Office of Mobile Sources, a decision was made to use a blank spool piece (hollow tube) in the place of the catalyst to serve as a non-functioning catalyst. This hollow tube simulated a "worst case" condition, where the catalyst has melted and has been blown out of the exhaust system.

The test vehicle was equipped with three oxygen sensors as part of the On-Board Diagnostic system (OBD II), two upstream and one downstream of the underbody catalyst. When the non-functioning catalyst was installed, the vehicle would have operated in a fuel-rich or "limp-home" mode because the signal from the downstream oxygen sensor to the OBD II system indicated a catalyst failure. To eliminate this problem, the voltage signal from the downstream oxygen sensor was recorded with the catalyst in place. During the tests without the catalyst, the downstream oxygen sensor was disconnected, and the recorded signal was replayed to the on-board computer. This step allowed the vehicle to operate as if the catalyst was present and prevented the "check engine" light from illuminating. Consistent emission results throughout the test program suggested that this step was successful.

TABLE 2. TEST PLAN FOR LIGHT-DUTY VEHICLE TESTING

STEP	DESCRIPTION
1	The test vehicle was obtained with less than 500 miles on the odometer. When received at SwRI, it was checked for an intact aftertreatment device, exhaust leaks, acceptable tires, proper oil level, proper transmission fluid level, and proper vehicle operation on the mileage accumulation dynamometer (MAD). The vehicle was drained and refueled with the base fuel. (**Note:** No other fuel was used until testing and mileage accumulations have been completed with this fuel. Mileage accumulation for 4000 miles was performed using a MAD. All scheduled maintenance will be performed according to the manufacturer's recommendations. Unscheduled maintenance or repairs were reported immediately. After unscheduled maintenance, repairs were made to Original Equipment Manufacturer (OEM) specifications using OEM or OEM approved parts. In addition, baseline emissions were measured after the unscheduled maintenance before resuming mileage accumulation, to ensure that the post-maintenance emission levels are within 20 percent of the pre-maintenance emission levels.
2	For emission testing after the first 4000 miles, the vehicle's exhaust system was prepared for connection to the Constant Volume Sampler (CVS), all necessary calibrations of the testing equipment were performed, and the vehicle was run over one UDDS sequence to prepare it for testing the following day.
3	Soak vehicle overnight (12 to 36 hours).
4	Perform a 4-bag FTP. Measure gaseous emissions, and sample for hydrocarbon speciation including aldehydes, ketones, alcohols, ethers, PAH, and NPAH.
5	Repeat Steps 3 and 4 two additional times on different days.
6	Remove the catalytic converter; replace with an uncoated, non-functioning catalyst monolith of similar size or a blank spool piece; prepare the vehicle with one UDDS sequence; and repeat Steps 3 through 5.
7	Perform fuel change procedure using the baseline fuel blended with 10 percent ethanol. Purge fuel supply, etc. (**Note:** No other fuel was used until testing with this fuel was completed.). Change oil. Perform mileage accumulation for 4000 miles using the same criteria listed in Step 1 above.
8	Prepare the vehicle with one UDDS sequence, and repeat Steps 3 through 6.
9	Perform fuel change procedure using the baseline fuel blended with 15 percent ethanol. Purge fuel supply, etc. (**Note:** No other fuel was used until testing with this fuel was completed.). Change oil. Perform mileage accumulation for 4000 miles using the same criteria listed in Step 1 above.
10	Repeat Step 8.
11	Analyze all samples collected, and prepare final report.

2.2.2 Light-Duty Vehicle Selection and Description

For the purpose of testing, a 2011 2.5 L Toyota Camry (VIN 4T4BF3EKXBR107329) was selected and purchased for RFA and Growth Energy. The vehicle was selected to meet the following criteria:

- Less than 500 miles of prior operation
- Same type, class, and subclass which consumed the most gallons of fuel in the fuel family over the past three years
- Represent the most common fuel metering system and the most common of the most important emission control system devices or characteristics with respect to the emission reduction performance for the model year in which testing began
- One of the five highest selling models from the current model year
- Unaltered from the specification of the original equipment manufacturer and to remain under the control of SwRI throughout the testing.

Table 3 lists the vehicle specifications and features.

TABLE 3. VEHICLE SPECIFICATIONS AND FEATURES

VEHICLE PARAMETER	COMMENT
Vehicle Type	Gasoline, 4-Cycle
Model	2011 Toyota Camry LE
VIN	4T4BF3EKXBR107329
Configuration	In-line, 4-cylinder, transverse, orientation, dual overhead cam, electronic ignition, 16-valve VVT-I, 5-speed automatic transmission
Displacement	2.5L (152 CID)
Bore and Stroke	3.5 X 3.9 in.
Compression Ratio	10.4:1
Fuel economy – city	22
Fuel economy – highway	33
Drag coefficient	0.28
Curb weight	3263 lbs
GVWR	4275 lbs
Rated Power at rpm	169 hp at 6000 rpm
Peak Torque at rpm	167 lb-ft at 4100 rpm
Vehicle Family	BTYXV02.5BEB
Aftertreatment	Sequential fuel injection, air/fuel sensor, close-coupled three way catalyst, heated oxygen sensor, underbody three way catalyst

2.2.3 Mileage Accumulation

After the vehicle was received, it was operated for 4000 miles with the baseline gasoline (EM-7578-F) using the Standard Road Cycle (SRC) on a mileage accumulation dynamometer (MAD). The SCR has a lap distance of 3.7 miles with an average speed of 46.3 mph, and the cycle was performed on a continuous basis to obtain 4000 miles of vehicle operation prior to conducting the baseline emission tests. At the completion of the baseline tests, the fuel was changed to the baseline fuel plus 10 percent ethanol (EM-7579-F), and the same mileage accumulation cycle was used. A second ethanol fuel blend (EM-7580-F) was also testing with the same test sequence. Table 4 details the SRC used for mileage accumulation.

TABLE 4. SRC FOR MILEAGE ACCUMULATION

Lap	Description	Cumulative Cycle, mi	No. of Laps	Ending, mph	Distance, miles	Typical Accel Rate, mph/s	Lapsed Time, sec.	Cumulative Cycle Time, sec.
1	(start engine) Idle 10 sec	0.00	0.00	0	0.00	0	10	10
1	Mod accel to 30 MPH	0.03	0.01	30	0.03	4	8	18
1	Cruise at 30 MPH for 1/4 lap	0.91	0.25	30	0.88	0	106	123
1	Mod. decel to 20 MPH	0.93	0.25	20	0.01	-5	2	125
1	Mod accel to 30 MPH	0.94	0.25	30	0.02	4	3	128
1	Cruise at 30 MPH for 1/4 lap	1.83	0.49	30	0.88	0	106	234
1	Mod. decel to stop	1.85	0.50	0	0.03	-5	6	240
1	Idle 5 sec	1.85	0.50	0	0.00	0	5	245
1	Mod accel to 35 MPH	1.89	0.51	35	0.04	4	9	253
1	Cruise at 35 MPH for 1/4 lap	2.76	0.75	35	0.87	0	89	342
1	Mod. decel to 25 MPH	2.78	0.75	25	0.02	-5	2	344
1	Mod accel to 35 MPH	2.80	0.76	35	0.02	4	3	347
1	Cruise at 35 MPH for 1/4 lap	3.67	0.99	35	0.87	0	90	436
1	Mod. decel to stop	3.70	1.00	0	0.03	-5	7	443
2	Idle 10 sec	3.70	1.00	0	0.00	0	10	453
2	Mod accel to 40 MPH	3.77	1.02	40	0.07	3	13	467
2	Cruise at 40 MPH for 1/4 lap	4.61	1.24	40	0.83	0	75	541
2	Mod. decel to 30 MPH	4.63	1.25	30	0.02	-5	2	543
2	Mod accel to 40 MPH	4.66	1.26	40	0.03	3	3	547
2	Cruise at 40 MPH for 1/4 lap	5.51	1.49	40	0.85	0	76	623
2	Mod. decel to stop	5.55	1.50	0	0.04	-5	8	631
2	Idle 5 sec	5.55	1.50	0	0.00	0	5	636
2	Mod accel to 45 MPH	5.64	1.53	45	0.09	3	15	651
2	Cruise at 45 MPH for 1/4 lap	6.45	1.74	45	0.81	0	65	716
2	Mod. decel to 35 MPH	6.48	1.75	35	0.02	-5	2	718
2	Mod accel to 45 MPH	6.51	1.76	45	0.04	3	3	721
2	Cruise at 45 MPH for 1/4 lap	7.34	1.98	45	0.83	0	67	788
2	Mod. decel to stop	7.40	2.00	0	0.06	-5	9	797
3	Idle 10 sec	7.40	2.00	0	0.00	0	10	807
3	Hard accel to 55 MPH	7.51	2.03	55	0.11	4	14	820
3	Cruise at 55 MPH for 1/4 lap	8.30	2.24	55	0.79	0	52	872
3	Mod. decel to 45 MPH	8.33	2.25	45	0.03	-5	2	874
3	Mod accel to 55 MPH	8.39	2.27	55	0.07	2	5	879
3	Cruise at 55 MPH for 1/4 lap	9.22	2.49	55	0.83	0	54	934
3	Mod. decel to 45 MPH	9.25	2.50	45	0.03	-5	2	936
3	Mod accel to 60 MPH	9.36	2.53	60	0.11	2	8	943
3	Cruise at 60 MPH for 1/4 lap	10.14	2.74	60	0.79	0	47	990
3	Mod. decel to 50 MPH	10.18	2.75	50	0.03	-5	2	992
3	Mod. accel to 60 MPH	10.25	2.77	60	0.08	2	5	997
3	Cruise at 60 MPH for 1/4 lap	10.98	2.97	60	0.72	0	43	1041
3	Mod. decel to stop	11.10	3.00	0	0.13	-4	15	1056

TABLE 4 (CONT'D). SRC FOR MILEAGE ACCUMULATION

Lap	Description	Cumulative Cycle, mi	No. of Laps	Ending, mph	Distance, miles	Typical Accel Rate, mph/s	Lapsed Time, sec.	Cumulative Cycle Time, sec.
4	Idle 10 sec	11.10	3.00	0	0.00	0	10	1066
4	Hard accel to 80 MPH	11.40	3.08	80	0.30	3	27	1092
4	Coastdown to 70 MPH	11.60	3.14	70	0.21	-1	10	1102
4	Cruise at 70 MPH for 1/2 Lap	12.84	3.47	70	1.23	0	69	1171
4	Mod. decel to 50 MPH	12.95	3.50	50	0.11	-3	7	1178
4	Mod accel to 65 MPH	13.07	3.53	65	0.12	2	7	1186
4	Cruise at 65 MPH for 1/2 lap	14.72	3.98	65	1.65	0	91	1277
4	Mod. decel to 50 MPH	14.80	4.00	50	0.08	-3	5	1282
5	Mod accel to 75 MPH	15.23	4.12	75	0.43	1	25	1307
5	Cruise at 75 MPH for 1/2 lap	16.51	4.46	75	1.27	0	61	1368
5	Mod. decel to 50 MPH	16.65	4.50	50	0.14	-3	8	1376
5	Lt. accel to 70 MPH	16.98	4.59	70	0.33	1	20	1396
5	Cruise at 70 MPH for 1/2 lap	18.39	4.97	70	1.41	0	72	1469
5	Mod. decel 50 MPH	18.50	5.00	50	0.11	-3	7	1475
6	Mod accel to 70 MPH	18.67	5.05	70	0.17	2	10	1485
6	Coastdown to 60 MPH	18.85	5.09	60	0.18	-1	10	1495
6	Cruise at 60 MPH for 1/2 lap	20.31	5.49	60	1.46	0	91	1586
6	Mod. decel to 50 MPH	20.35	5.50	50	0.04	-4	2	1589
6	Mod. accel to 65 MPH	20.59	5.56	65	0.24	1	15	1604
6	Cruise at 65 MPH for 1/2 lap	22.05	5.96	65	1.46	0	81	1685
6	Mod. decel to stop	22.20	6.00	0	0.15	-4	16	1701
7	Idle 45 sec	22.20	6.00	0	0.00	0	45	1746
7	Hard accel to 55 MPH	22.31	6.03	55	0.11	4	14	1760
7	Cruise at 55 MPH for 1/4 lap	23.09	6.24	55	0.78	0	51	1811
7	Mod. decel to 40 MPH	23.13	6.25	40	0.04	-5	3	1814
7	Mod accel to 55 MPH	23.22	6.28	55	0.10	2	7	1822
7	Cruise at 55 MPH for 1/4 lap	24.01	6.49	55	0.79	0	51	1873
7	Mod. decel to 40 MPH	24.05	6.50	40	0.04	-5	3	1876
7	Mod accel to 50 MPH	24.11	6.52	50	0.06	2	5	1881
7	Cruise at 50 MPH for 1/4 lap	24.95	6.74	50	0.84	0	60	1941
7	Mod. decel to 40 MPH	24.98	6.75	40	0.03	-5	2	1943
7	Mod. accel to 50 MPH	25.04	6.77	50	0.06	2	5	1948
7	Cruise at 50 MPH for 1/4 lap	25.83	6.98	50	0.79	0	57	2005
7	Mod. decel to stop	25.90	7.00	0	0.07	-5	10	2015

2.2.4 Fuel Blending and Analyses

The three fuels used for mileage accumulation and for emissions testing were obtained in a single batches from Haltermann Products. The baseline fuel was designated with SwRI Code EM-7578-F. Traditionally, test fuel for the fuel and fuel additive Tier 1 test program was required to meet the Title 40 CFR Part 79 specifications. However per EPA request for this test program, the test fuel was requested to meet Title 40 CFR Part 86, Subpart B, Section 86.113-04 specifications. The Title 40 CFR Part 86 specifications were required in this project by EPA because the gasoline fuel specifications changed in 2004. As a result, the fuel specifications that were established in Title 40 CFR Part 79 were no longer applicable for a base fuel in this project.

Additionally, this base fuel also included additives for deposit control, corrosion inhibitor, demulsifier, and anti-oxidant as required in Title 40 CFR Part 79, Subpart F, Section 79.55. Approval of the base fuel specifications were obtained from Mr. Jim Caldwell on July 20, 2010.[18] When compared to Title 40 CFR Part 86 specifications for a reference fuel, all requirements were met for the base fuel. The other two fuels with ethanol (EM-7579-F and EM-7580-F) met most of the requirements except for some distillation and RVP properties. Table 5 presents the test fuel properties and the Title 40 CFR Parts 86 and Part 79 fuel specifications for comparison.

2.2.5 Scheduled and Unscheduled Maintenance

For this project, the baseline tests were performed after operating the vehicle for 4000 miles. The original as-received engine oil was not changed prior to starting the durability and baseline emissions testing. After the baseline emission tests had been completed, the engine oil was changed prior to mileage accumulation with the 10 percent ethanol blend. The engine oil was changed again prior to mileage accumulation with the 15 percent ethanol blend. No other scheduled maintenance was performed on the vehicle.

Prior to starting the emission testing with the 15 percent ethanol blend, a code was set during the "prep." The code was DTC #P0441 for an evaporative purge flow fault code. Apparently, the gas cap had not been replaced after fueling the vehicle upon return from mileage accumulation. The gas cap was replaced, the fault code was clear, and the vehicle was operated over another "prep" cycle. The code did not return. While the fault code was considered unscheduled maintenance, the replacement of the gas cap was not considered a severe problem which would require the vehicle to be emission tested to determine if the unscheduled maintenance had returned the vehicle to within 20 percent of the baseline emission levels. Testing proceeded as usual, and no negative effects were observed.

TABLE 5. 211(b) GASOLINE FUEL SPECIFICATION AND ANALYSIS

Component	40 CFR 79.55 Specifications	40 CFR 86.113-04 Specifications	EM-7578-F	EM-7579-F	EM-7580-F
Fuel Properties					
Sulfur, ppm	339±25	15-80	31	25	25
Benzene, vol %	1.53±0.3	NS[a]	1.56	1.53	1.49
RVP, psi	8.7±0.3	8.7-9.2	9.2	9.6	9.5
API Gravity	57.4±0.3	NS	59.0	57.4	56.9
Octane, (R+M)/2	87.3±0.5	NS	92.8	95.5	95.7
Research Octane, min.	NS	93	97.0	100.8	100.6
Sensitivity, min.	NS	7.5	8.5	10.8	11.3
Lead, g/gal., max.	NS	0.050	<0.001	<0.001	<0.001
Phosphorus, g/gal., max.	NS	0.005	0.00011	0.00009	0.00007
Aromatics, vol %	32.0±2.7	35 max.	28	24.3	23.2
Olefins, vol %	9.2±2.5	10 max.	2	0.6	1.1
Saturates, vol %	58.8±2.0	Remainder	70	65.3	60.9
Distillation Parameters					
IBP, °F (ºC)	NS	75-95 (24-35)	85 (29.4)	95 (35.0)	96 (35.7)
10%, °F (ºC)	128±5	120-135 (49-57)	125 (51.7)	124 (51.0)	127 (52.9)
50%, °F (ºC)	218±5	200-230 (93-110)	223 (106.1)	209 (98.3)	165 (73.8)
90%, °F (ºC)	330±5	300-325 (149-163)	319 (159.4)	316 (157.9)	317 (158.1)
EP, °F (ºC), max.	NS	415 (213)	393 (200.6)	392 (199.7)	386 (196.5)
Required and Permissible Additive Types					
Deposit Control, ppb	Required	NR[b]	44.04	UR[c]	UR
Corrosion Inhibitor, ppb	Required	NR	4.5	UR	UR
Demulsifier, ppb	Required	NR	1.0	UR	UR
Antioxidant, ppb	Required	NR	2.0	UR	UR
Metal Deactivator, ppb	Required	NR	2.0	UR	UR
Anti-Static	Permissible	NR	NI[d]	UR	UR

[a]NS – No standard for this property with EPA reference fuel

[b]NR – No requirement for additives with EPA reference fuel

[c]UR – Unreported

[d]NI– Not included in the fuel

3.0 DESCRIPTION OF ANALYTICAL METHODS

Regulated and unregulated emission measurements conducted for this program, as required by the EPA, included the following:

- Measurement of regulated emissions including THC, NMHC, CO, NO_x, and total particulate
- Speciation of volatile-phase hydrocarbon compounds, aldehydes, ketones, alcohols, and ethers
- Semi-volatile emissions for both volatile- and particulate-phase PAH and NPAH including: benzo[a]anthracene, benzo[b]fluoranthene, benzo[k]fluoranthene, benzo[a]pyrene, chrysene, dibenzo[a,h]anthracene, indeno[1,2,3-c,d]pyrene, 7-nitrobenzo[a]anthracene, 6-nitrobenzo[a]pyrene, 6-nitrochrysene, 2-nitrofluorene, and 1-nitropyrene.

Regulated emissions (THC, NMHC, CO, NO_x, and particulate) and CO_2 were analyzed according to Title 40 CFR, Subpart D specifications, and all applicable accuracy and calibration requirements were met. All filters were conditioned and weighed in accordance with the appropriate sections of the CFR for light-duty vehicles. Two sizes of filters were used to collect particulate samples. These filters included the following for each test:

- One set of 47-mm Pallflex (fluorocarbon-coated glass fiber) filters for determination of the regulated total particulate mass rate
- One 20×20-inch Pallflex filter for dilute exhaust filtration of particulate and subsequent extraction for PAH and NPAH.

Analyses of unregulated emissions were conducted according to Coordinating Research Council (CRC), EPA, and SwRI analytical procedures. Applicable SwRI TIPs are listed in References 1 through 17. Table 6 presents the sampling requirements for each of the measured emissions. Test numbers in Table 6 are coded based on the project, fuel, and aftertreatment according to the following formula RFAXXXXYYY where the fuel XXXX is indicated by BASE, E10, or E15 and the presence of aftertreatment YYY is indicated by CAT. In the absence of aftertreatment, YYY is omitted. Dinitrophenylhydrozene (DNPH) cartridges were employed to collect aldehydes and ketones, and Tedlar® bags and wet absorption techniques were used for alcohols and ethers. These sampling techniques are discussed in more detail below.

3.1 Regulated Emissions

Regulated emissions were quantified in a manner consistent with EPA protocols for light-duty emissions testing as given in Title 40 CFR Part 86, Subpart D. Analysis of the regulated emissions was performed continuously throughout the entire test. The exhaust gas samples for CO and CO_2 were analyzed using non-dispersive infrared (NDIR) instruments, and the THC and NO_x were monitored using a flame ionization detector and a chemiluminescent instrument, respectively.

TABLE 6. SAMPLE COLLECTION METHODS

TEST NUMBER	REGULATED THC, CO, NO_X, and PARTICULATE	SPECIATION $C_1 - C_{12}$	PAH/NPAH		ALCOHOLS and ETHERS	ALDEHYDES and KETONES
			Particulate	Volatile		
Base Fuel With Aftertreatment[a]						
RFABASECAT-2	Cont., Bag, 47 mm Filter	Bag	20X20 Filter	PUF	Bag and Bubbler	Cartridge
RFABASECAT-3	Cont., Bag, 47 mm Filter	Bag	20X20 Filter	PUF	Bag and Bubbler	Cartridge
RFABASECAT-6	Cont., Bag, 47 mm Filter	Bag	20X20 Filter	PUF	Bag and Bubbler	Cartridge
Base Fuel Without Aftertreatment[b]						
RFABASE-1	Cont., Bag, 47 mm Filter	Bag	20X20 Filter	PUF	Bag and Bubbler	Cartridge
RFABASE-2	Cont., Bag, 47 mm Filter	Bag	20X20 Filter	PUF	Bag and Bubbler	Cartridge
RFABASE-4	Cont., Bag, 47 mm Filter	Bag	20X20 Filter	PUF	Bag and Bubbler	Cartridge
10 Percent Blend With Aftertreatment[a]						
RFAE10CAT-1	Cont., Bag, 47 mm Filter	Bag	20X20 Filter	PUF	Bag and Bubbler	Cartridge
RFAE10CAT-2	Cont., Bag, 47 mm Filter	Bag	20X20 Filter	PUF	Bag and Bubbler	Cartridge
RFAE10CAT-3	Cont., Bag, 47 mm Filter	Bag	20X20 Filter	PUF	Bag and Bubbler	Cartridge
10 Percent Blend Without Aftertreatment[b]						
RFAE10-1	Cont., Bag, 47 mm Filter	Bag	20X20 Filter	PUF	Bag and Bubbler	Cartridge
RFAE10-2	Cont., Bag, 47 mm Filter	Bag	20X20 Filter	PUF	Bag and Bubbler	Cartridge
RFAE10-3	Cont., Bag, 47 mm Filter	Bag	20X20 Filter	PUF	Bag and Bubbler	Cartridge
15 Percent Blend With Aftertreatment[a]						
RFAE15CAT-1	Cont., Bag, 47 mm Filter	Bag	20X20 Filter	PUF	Bag and Bubbler	Cartridge
RFAE15CAT-3	Cont., Bag, 47 mm Filter	Bag	20X20 Filter	PUF	Bag and Bubbler	Cartridge
RFAE15CAT-4	Cont., Bag, 47 mm Filter	Bag	20X20 Filter	PUF	Bag and Bubbler	Cartridge
15 Percent Blend Without Aftertreatment[b]						
RFAE15-1	Cont., Bag, 47 mm Filter	Bag	20X20 Filter	PUF	Bag and Bubbler	Cartridge
RFAE15-2	Cont., Bag, 47 mm Filter	Bag	20X20 Filter	PUF	Bag and Bubbler	Cartridge
RFAE15-3	Cont., Bag, 47 mm Filter	Bag	20X20 Filter	PUF	Bag and Bubbler	Cartridge

[a]Tests conducted with the aftertreatment in place
[b]Tests conducted with the aftertreatment removed
Please note: Test numbers are coded based on the project - RFA, fuel, and aftertreatment according to the following formula RFAXXXXYYY where the fuel XXXX is indicated by BASE, E10, or E15; and the presence of aftertreatment YYY is indicated by CAT. In the absence of aftertreatment, YYY is omitted.

3.2 Speciation of Volatile Hydrocarbon Compounds

Volatile hydrocarbon compounds were determined by hydrocarbon speciation. Analytical procedures for conducting the hydrocarbon speciation (C_1 to C_{12} hydrocarbons, aldehydes, ketones, alcohols, and ethers) were similar to the CRC Auto/Oil Phase II methods. The SwRI TIPs for this determination are listed in the Reference Section. With these methods, exhaust emissions samples are analyzed for the presence of more than 200 different exhaust species. Five gas chromatography (GC) procedures and one High Performance Liquid Chromatography (HPLC) procedure were used to identify and quantify specific compounds. One GC is used for the measurement of methane, a second for C_2-C_4 species, and a third for C_5-C_{12} species including some of the higher molecular weight alcohols and ethers. A fourth GC was used to measure 1-methylcyclopentene, benzene, toluene, 2,3-dimethylhexane, cyclohexane, and 2,3,3-trimethylpentane, which co-elute and cannot be accurately quantified by other methods. A fifth GC was used to determine the C_1 to C_6 alcohols and ethers while the higher molecular weight alcohols were determined with the CRC Auto/Oil Phase II methods. Analysis of all emission "sample" bags were begun within 30 minutes of sampling and before the "background" bags, so that reactive exhaust compounds could be analyzed as quickly as possible. Data were reported as background corrected. A brief description of these procedures is given in the following sections.

3.2.1 Methane Speciation

Methane levels were determined for proportional exhaust gas samples collected in Tedlar® bags and as described in SwRI TIP 07C-002. A GC equipped with a flame ionization detector (FID) was utilized for the analyses, and was used in accordance with SAE J1151 procedures. The GC system was equipped with a packed column to resolve methane from other hydrocarbons in the sample. Samples were introduced into a 5-mL sample loop via a diaphragm pump. For analysis, the valve was switched to the inject position, and the helium carrier gas swept the sample from the loop toward the detector through a 61 cm × 0.3 cm Porapak N column in series with a 122 cm × 0.3 cm molecular sieve 13X column. As soon as the methane peak passed into the molecular sieve column, the helium flow was reversed through the Porapak N column to vent. For quantification, sample peak areas were compared to those of external calibration standards. Detection limits for the procedure were on the order of 0.05 mg/mi in dilute exhaust.

3.2.2 C_2-C_4 Species

SwRI TIP 07C-013 describes the analytical procedure for determining the C_2-C_4 hydrocarbons. With the aid of a DB-WAX pre-column and a 10-port switching valve, this procedure allowed the separation and determination of exhaust concentrations of C_2-C_4 individual hydrocarbon species, including: ethane; ethylene; acetylene; propane; propylene; trans-2-butene; butane; 1-butene; 2-methylpropene (isobutylene); 2,2-dimethypropane (neopentane); propyne; 1,3-butadiene; 2-methylpropane; 1-butyne; and cis-2-butene. Bag samples were analyzed with a GC system which utilized a Hewlett-Packard Model 5890 Series II GC with an FID, two pneumatically operated and electrically controlled valves, and two analytical columns. The first column separated the C_2-C_4 hydrocarbons from the higher molecular weight hydrocarbons and the polar compounds. These higher molecular weight hydrocarbons (and water and alcohols) were retained on the pre-column while the C_2-C_4 hydrocarbons were passed through to the analytical column (50 m Alumina PLOT/KCl with 10 μm film thickness and 0.53 mm i.d.). At the same time, the C_2-C_4 hydrocarbons were separated on the analytical column, the pre-column was back-flushed with helium to prepare for the next analysis. The carrier gas for this analysis was helium. The GC was calibrated daily using a CRC Auto/Oil 23-component calibration mixture. Analysis for the C_2-C_4 hydrocarbons was typically begun within 30 minutes after sample collection was completed. Detection limits for the procedure were on the order of 0.05 mg/mi in dilute exhaust for all compounds, and the limit of quantification was 0.1 mg/mi.

3.2.3 C_5-C_{12} Species

SwRI TIP 07C-013 describes the analytical procedures for the C_5-C_{12} hydrocarbons. This procedure provides separation and exhaust concentrations for more than 200 C_5-C_{12} individual hydrocarbon compounds. Bag samples were analyzed using a gas chromatograph equipped with an FID. The GC system utilized a Hewlett-Packard Model 5890 Series II GC with an FID, a pneumatically operated and electrically controlled valve, and a 60 m DB-1 fused silica open tubular (FSOT) column with a 1.0 μm film thickness and a 0.32 mm i.d. The carrier gas was helium. Gaseous samples were pumped from the bag through a sample loop and then introduced into a liquid nitrogen cooled column. The column oven was then programmed to a maximum temperature of 200°C. The analog signal from the FID was sent to a networked

computer system via a buffered analog to digital converter. The GC was calibrated daily using a CRC Auto/Oil 23-component calibration mixture. Detection limits for the procedure were on the order of 0.05 mg/mi in dilute exhaust for all compounds, and the limit of quantification was 0.1 mg/mi.

3.2.4 Benzene and Toluene

The analytical procedure for benzene and toluene is also described in SwRI TIP 07C-013. This procedure used a separate system configured similarly to the C_5-C_{12} GC method (with a 30 m DB-5 analytical column in place of the DB-1 FSOT column) to resolve individual concentrations of benzene and toluene according to the CRC Auto/Oil Phase II Protocols. Separation of benzene and toluene from co-eluting peaks was carried out by fine-tuning the column head pressure to give benzene a retention time of 22 to 23 minutes. The GC was calibrated daily using a CRC 7-component calibration mixture. Detection limits for the procedure were 0.05 mg/mi in dilute exhaust for all compounds, and the limit of quantification was 0.1 mg/mi.

3.2.5 Aldehydes and Ketones

An HPLC procedure was used for the analysis of aldehydes and ketones. SwRI TIP 07C-006 describes the analytical procedure. Samples were collected in DNPH cartridges at a nominal flowrate of 2 L/min and eluted with acetonitrile. SwRI TIP 07C-018 covers the sampling of aldehydes and ketones with DNPH cartridges. Samples were extracted from the cartridges using pure acetonitrile, transferred into volumetric flasks with ground glass joints, and analyzed immediately or stored in ground glass stopped vials at 0°C for no more than one week prior to analysis. For analysis, a portion of the acetonitrile solution was injected into a liquid chromatograph equipped with an ultra-violet (UV) detector. External standards of the aldehyde and ketone DNPH derivatives were used to quantify the results. The aldehydes and ketones include: formaldehyde, acetaldehyde, acrolein, acetone, propionaldehyde, crotonaldehyde, methacrolein, n- and isobutyraldehyde (not resolved during normal operating conditions, and so reported together), methyl ethyl ketone, benzaldehyde, isovaleraldehyde, valeraldehyde, o-tolualdehyde, m-tolualdehyde/p-tolualdehyde (not resolved during normal operating conditions, and so reported together), methyl isobutyl ketone, hexanaldehyde, and 2,5-dimethylbenzaldehyde. Detection limits for this procedure were on the order of 0.05 mg/mi aldehyde or ketone in dilute exhaust, and the limit of quantification was 0.1 mg/mi.

3.2.6 Alcohols and Ethers

The measurement of alcohols in exhaust was accomplished by bubbling the exhaust through glass impingers containing deionized water and in Tedlar® bags. Water soluble alcohols with a carbon number up to about C_6 were detected with wet absorption techniques, and the higher molecular weight alcohols were determined as part of the volatile hydrocarbon speciation as explained above. For the water soluble alcohols, two glass impingers in series were used to collect exhaust samples for the analysis. The two glass impingers contained 25 ml of deionized water in each and were able to collect 99+ percent of the lower molecular weight alcohols which are soluble in water. Table 7 lists a number of alcohols that range in solubility from miscible to slightly soluble in water. The temperature of the collection impingers is maintained at 0 to 5°C

TABLE 7. SELECTED C_1 TO C_6 ALCOHOLS THAT HAVE SOME SOLUBILITY IN WATER

COMPOUND	EMPIRICAL FORMULA	COMPOUND	EMPIRICAL FORMULA
Water Soluble Alcohols			
Methanol	CH_4O	3-Methyl-2-butanol	$C_5H_{12}O$
Ethanol	C_2H_6O	Neopentanol (2,2-dimethyl-1-propanol)	$C_5H_{12}O$
2-Propyn-1-ol	C_3H_4O	1-Pentanol	$C_5H_{12}O$
Allyl alcohol (2-propen-1-ol)	C_3H_6O	2-Pentanol	$C_5H_{12}O$
Isopropanol	C_3H_8O	3-Pentanol	$C_5H_{12}O$
n-Propanol	C_3H_8O	tert-Pentanol (2-methyl-2-butanol)	$C_5H_{12}O$
Crotyl alcohol (2-buten-1-ol)	C_4H_8O	Phenol	C_6H_6O
n-Butanol	$C_4H_{10}O$	3-Methyl-1-pentyn-3-ol	$C_6H_{10}O$
Isobutanol (2-methyl-1-propanol)	$C_4H_{10}O$	Cyclohexanol	$C_6H_{12}O$
sec-Butanol (2-butanol)	$C_4H_{10}O$	4-Hydroxy-4-methyl-2-pentanone	$C_6H_{12}O_2$
tert-Butanol (2-methyl-2-propanol)	$C_4H_{10}O$	2,2-Dimethyl-1,3-dioxolane-4-methanol	$C_6H_{12}O_3$
Furfuryl alcohol	$C_5H_6O_2$	2,5-Tetrahydrofurandimethanol	$C_6H_{12}O_3$
2-Methyl-3-butyn-2-ol	C_5H_8O	1-Hexanol	$C_6H_{14}O$
Cyclopentanol	$C_5H_{10}O$	2-Methyl-1-pentanol	$C_6H_{14}O$
Tetrahydrofurfuryl alcohol	$C_5H_{10}O_2$	3-Methyl-3-pentanol	$C_6H_{14}O$
Isopentanol (3-methyl-1-butanol)	$C_5H_{12}O$	4-Methyl-2-pentanol	$C_6H_{14}O$
2-Methyl-1-butanol	$C_5H_{12}O$	3,3-Dimethyl-2-butanol	$C_6H_{14}O$
3-Methyl-1-butanol	$C_5H_{12}O$	2-Ethyl-1-butanol	$C_6H_{14}O$
Water Soluble Ethers			
Methyl ether	C_2H_6O	Ethyl ether	$C_4H_{10}O$
Methyl ethyl ether	C_3H_8O	Methyl propyl ether	$C_4H_{10}O$
Vinyl ether	C_4H_6O	Isopropyl ether	$C_6H_{14}O$
Cyclopropyl methyl ether	C_4H_8O	Propyl ether	$C_6H_{14}O$

with an ice water bath, and the flow rate through the impingers is maintained at 4 L/min by the sample pump. A dry gas meter is used to determine the total flow through the impingers. The temperature of the gas stream is monitored by a thermocouple immediately prior to the dry gas meter. A drier is included in the system to prevent condensation in the pump, flow meter, dry gas meter, etc. The flow meter in the system allows continuous monitoring of the sample to ensure proper flow rates during the sampling. The Teflon® line connecting the CVS and the solenoid valve is heated to approximately 235°F to prevent water from condensing in the sample line.

The exhaust sample was collected continuously during each cold- and hot-start test cycle. Upon completion of each transient cycle, the impingers were removed, and the contents were transferred to a 30 ml polypropylene bottle and capped. Analysis of samples was begun within four hours after sampling. For analysis, a 1.0 µl portion of the aqueous solution was injected into a GC equipped with a FID and an autosampler. The analytical column was a 30 m × 0.53 mm i.d. capillary column of 1 µm DB-Wax film thickness. The carrier gas was helium and was set to give optimum separation (18 ml/min.). To quantify the results, the sample peak areas were compared to the peak areas of standard solutions. External standards containing methanol, ethanol, isopropanol, n-propanol, isobutanol, and n-butanol in deionized water were used to quantify the results. Sample chromatograms were also searched for the presence of a number of other alcohols using predetermined retention times. The search list included: tert-butanol (CAS# 75-65-0), 2-methyl-2-butanol (CAS# 75-85-4), 2-butanol (CAS# 78-92-2), 3-pentanol

(CAS# 584-02-1), 3-methyl-3-pentanol (CAS# 77-74-7), 3,3-dimethyl-2-butanol (CAS# 464-07-3), 2-pentanol (CAS# 6032-29-7), 4-methyl-2-pentanol (CAS# 108-11-2), 2-methyl-1-butanol (CAS# 137-32-6), 3-methyl-1-butanol (CAS# 123-51-3), 1-pentanol (CAS# 71-41-0), 2-methyl-1-pentanol (CAS# 105-30-6), and 2-ethyl-1-butanol (CAS# 97-95-0). Detection limits with this procedure were on the order of 0.05 mg/mi in dilute exhaust, and the limit of quantification was 0.1 mg/mi.

3.3 PAH and NPAH

In addition to the regulated and C_1 to C_{12} hydrocarbon exhaust emissions, semi-volatile (volatile- and particulate-phase) PAH and NPAH compounds were also determined for each fuel. Seven PAH compounds were quantified: benzo[a]anthracene, benzo[b]fluoranthene, benzo[k]fluoranthene, benzo[a]pyrene, chrysene, dibenzo[a,h]anthracene, and indeno[1,2,3-cd]pyrene; and five NPAH compounds: 7-nitrobenzo[a]anthracene, 6-nitrobenzo[a]pyrene, 6-nitrochrysene, 2-nitrofluorene, and 1-nitropyrene. A 400 in^2 fluorocarbon-coated glass fiber filter (20×20-inch Pallflex filter) was used to collect the particulate-phase PAH and NPAH, and a PUF/XAD/PUF sandwich adsorbent trap was used to collect the volatile-phase PAH and NPAH. The PUF/XAD/PUF traps contained a layered sampling media consisting of a 1.25 inch deep layer of polyurethane foam (PUF), a 0.5 inch deep layer of XAD-2 resin, and a second 1.25 inch deep layer of PUF. The XAD-2 resin was incorporated to improve the trapping efficiency for the lighter PAH and NPAH compounds.

Volatile-phase PAH and NPAH samples presented a particular problem for light-duty sampling because conventional sampling techniques would not allow for sufficient sample to be gathered to meet EPA detection requirements. Commercially available sampling media and hardware were of insufficient size to allow for the collection of sample volumes needed to meet these detection limits. Sampling media size was also limited by the ability to extract and concentrate samples obtained. Therefore, an approach was devised involving both custom built sampling hardware and a modified sampling plan. The PUF/XAD/PUF traps were sized to allow a media diameter of 4 inches, rather than the conventional 2.5 inches. This larger diameter allowed a much higher flowrate to be used, while maintaining the face velocity within recommended levels for the smaller, conventional sampling media. This volume of dilute exhaust sample was sufficient for the analysis to meet a detection threshold of 0.01 ng/mi for PAH and 0.1 ng/mi for NPAH.

Prior to sampling, both XAD-2 and PUF sample media were cleaned. For cleaning the XAD-2, the material was cleaned by siphoning four times with water using a Soxhlet. The residual water was then removed under vacuum. The XAD-2 was then Soxhlet extracted three times: once with methanol for 24 hours, once with acetone for 48 hours, and finally with methylene chloride again for 48 hours. The residual methylene chloride was removed by purging with heated nitrogen. For cleaning the PUF material, each foam disk was washed with soapy water, rinsed with deionized water, air dried, Soxhlet extracted with acetone for 48 hours, and Soxhlet extracted with methylene chloride for 48 hours. The cleaned PUF was then air dried for 12 hours.

Volatile- and particulate-phase PAH and NPAH samples were obtained using a separate secondary dilution tunnel, which was operated in parallel with the smaller secondary dilution tunnel used to obtain the 90-mm filter samples for particulate mass determinations. The PAH

and NPAH tunnel was considerably larger than the 90-mm system in order to allow for the use of 20×20-inch Pallflex sampling media to collect particulate-phase PAH and NPAH compounds and to allow the use of a specially designed PUF/XAD/PUF trap to collect the volatile-phase PAH and NPAH compounds. Filter and PUF/XAD/PUF trap samples were generated during each cold-start and a hot-start test. Background PAH and NPAH sample sets were obtained by operating the sampling systems for about two hours with sampling media loaded, but without the vehicle operating.

Following testing, sample sets were delivered to the analytical laboratory for extraction and analysis. In cases where immediate extraction was not possible, samples were stored at 4°C. One half of each filter and the entire PUF/XAD/PUF sample material were extracted separately. Prior to extraction of the filters, each filter was spiked with an internal standard solution containing 100 ng each of seven deuterated PAH:

- Benzo[a]anthracene-d12
- Chrysene-d12
- Benzo[b]fluoranthene-d12
- Benzo[k]fluoranthene-d12
- Benzo[a]pyrene-d12
- Indeno[1,2,3-cd]pyrene-d12
- Dibenzo[a,h]anthracene-d14

and four deuterated NPAH:

- 2-nitrofluorene-d9
- 1-nitropyrene-d9
- 6-nitrochrysene-d11
- 6-nitrobenzo[a]pyrene-d11.

This spiked internal standard was used to quantify the target PAH and NPAH in the sample. The filters were then Soxhlet extracted with methylene chloride for 10 hours and again with toluene for another 10 hours. For the PUF/XAD/PUF traps, each was spiked with the same amount of deuterated PAH and NPAH as used for the filters. The trap samples were then Soxhlet extracted for at 16 hours with methylene chloride. After extraction, the methylene chloride extract was reduced to 20 mL with a rotary evaporator with a water bath held at 35^{o}C. The concentrated extract was then split into two portions: 16 ml (80 percent) and 4 ml (20 percent). The 80 percent portion was blown down to about 0.5 ml, diluted to 6.0 ml with hexane, acid- and base-washed, and then column fractionated with a one inch silica gel column. The final sample extract was blown down to 100 µL, and this concentrated sample was analyzed for both PAH and NPAH. The 4 mL extract was saved as a reserve. The filter extract was treated similarly, and the unextracted filter half was saved as a reserve.

Samples for both the volatile- and the particulate-phase PAH and NPAH were analyzed by GC/MS (gas chromatograph/mass spectroscopy) using an Agilent 5973N MSD with a 30 m by 0.25 mm i.d. DB-5 column and a 0.25 µm film thickness. For each analysis, a 2 µL aliquot of the sample extract was injected into the instrument. A calibration curve consisting of at least five points was obtained prior to sample analysis to ensure linearity, and a mid-point continuing

calibration was performed each day after the initial five point calibration. Analysis of NPAH compounds was performed using the negative ion/chemical ionization (NI/CI) mode and analysis for PAH compounds was performed using the positive ion/electron ionization (PI/EI) mode. Two or three characteristic ions for each PAH and NPAH were monitored. Separate GC/MS analyses were necessary to acquire both the PAH and NPAH data. Each target compound met the criterion of a 30 percent relative response factor (RRF) and 30 percent deviation in relation to the mean RRF obtained in the initial and continuing calibration.

4.0 QUALITY CONTROL AND QUALITY ASSURANCE

In order to demonstrate SwRI's constant goal to provide quality emissions data in our project efforts, the Vehicle, Emissions, and Vehicle Research Division (EEVRD) maintains certification to ISO 9001:2000 and accreditation to ISO/IEC 17025:2005 standards. Standard operating procedures and routine instrument calibration and calibration records are included in these standards. Based on the successful completion of third party audits, the EEVRD is able to maintain registration under ISO 9001:2000, "Quality Management System," and accreditation by ISO/IEC 17025:2005, General Requirements for the Competence of Testing and Calibration Laboratories." The SwRI Office of Automotive Engineering (OAE) Quality Policy Statement:

SwRI OAE Quality Policy Statement

> *"The Office of Automotive Engineering provides unequaled capabilities for the research, development, evaluation, and qualification of transportation systems, vehicles, vehicles, fuels, lubricants, and emissions-related products. Quality excellence is the foundation for the management of our business and the keystone to customer satisfaction. It is our objective to ensure that our final products are internationally recognized with unquestioned quality and are delivered to our clients in a professional, cost effective, and timely manner.*
>
> *We are committed to comply with ISO 17025, ISO 9001:2000, and all customer-required standards of excellence. Continual improvement of this policy occurs through regular review of the quality system's suitability to meet customer, employee, and supplier needs."*

Throughout this project, SwRI implemented our QA/QC plan in a manner consistent with the program objectives, including spot-checking of records, accuracy/precision charts, notebooks, calibration tags, and other quality control elements including chain of custody of samples. Listed below are a few of the key process that ensure the quality standards are implemented.

Senior Scientist/Technician Review - A system for formal data review is in place in the SwRI Department of Emissions R & D (DER&D). All technicians review their work prior to submitting it to the data computations laboratory for calculation of final concentrations. The Project Leader performs the final review before test results are accepted.

Interlaboratory Comparisons/Round Robins - SwRI has participated in numerous Round Robin exercises to correlate the results of our laboratory with other accepted facilities. Those Round Robin studies which are directly related to this project include: CRC Round Robin Analysis of Alcohol and Carbonyl Synthetic Exhaust Samples (results published in SAE Paper No. 941944); and CRC Round Robin Hydrocarbon Speciation Analysis of Synthetic Exhaust Gas (results not published).

Project Records - Documents directly associated with a technical project, such as: correspondence, proposals, contracts, work orders, interim and final reports, and follow-up contacts are maintained. These records are handled in accordance with Standard Operating Procedure (SOP), document SOP-4.16 "Quality Records" (Reference 17).

Calibration Records - Data sheets, chart recordings, computer printouts, logbooks, calibration and maintenance logs, and spreadsheets associated with the calibration of measurement equipment are maintained. Calibration results from external suppliers are also included.

Vehicle and Vehicle Testing Records - Data sheets, chart recordings, logbooks, start/stop logs, and computer printouts associated with evaluation and testing of vehicles and vehicles are retained.

Chemistry Calibration and Analysis Records - Data sheets, logbooks, and spreadsheets associated with calibration and analyses performed in the chemistry areas are retained.

Data Reduction and Test Result Records - Computed results, tables, and spreadsheets generated using information obtained from emissions testing and chemical analysis are maintained. Records developed in the areas specified in SOP 4.16 are retained for a period of ten years.

Training and Competency Evaluation: Personnel are trained to applicable SOP, TIP, and Design and Analysis Procedures and Safety Requirements. Staff members that perform an individual test are certified before performing these tests without supervision. Management encourages personnel to avail themselves to attend appropriate seminars, conferences, and continuing/higher education opportunities to continually enhance their skill set.

5.0 TEST RESULTS

Emissions testing was performed using a 2011 2.5 L Toyota Camry light-duty gasoline vehicle. Three fuels were evaluated in this project. One fuel (EM-7578-F) was the base fuel with no additive, the second fuel (EM-7579-F) contained 10 percent denatured fuel ethanol, and the third fuel (EM-7580-F) contained 15 percent denatured fuel ethanol. The vehicle was operated for 4000 miles with the base gasoline. Three 4-bag cold-start test sequences were conducted with the vehicle exhaust aftertreatment in place, and three 4-bag cold-start test sequences were performed with the aftertreatment removed. The fuel was then changed to the 10 percent blend of ethanol with the baseline gasoline, and the test sequence was repeated. Finally, the fuel was changed to the 15 percent blend, and the mileage accumulation and test sequence were repeated. Results of these tests are presented below.

5.1 Regulated Emissions

The 2011 emission standards for NMOG (non-methane organic gases), CO, and NO_x were 0.075, 3.4, and 0.05 g/mi, respectively. (Please note: Emission standards are in terms of NMOG, but data reported here are in terms of NMHC.) After 4000 miles of vehicle operation with the base gasoline, the average composite emissions with aftertreatment were significantly lower than the corresponding 2011 emission standard and similar to the 50,000 mile certification data. The average of the three composite tests was 0.03 g/mi NMHC, 0.09 g/mi CO, and 0.02 g/mi NO_x. Without aftertreatment, all of the emissions increased significantly as expected. The average of the three composites was 1.4 g/mi NMHC, 11.4 g/mi CO, and 3.8 g/mi NO_x. In general, operating the vehicle without aftertreatment would not be expected to meet these standards since the vehicle was designed to employ aftertreatment. The average fuel economy for the baseline fuel with aftertreatment was 26.72 mpg, and the average fuel economy without aftertreatment was 26.31 mpg. Table 8 summarizes the regulated emissions at each test condition. Appendix A contains the individual and composite emission test results.

After 4000 miles of vehicle operation with 10 percent ethanol, the test sequence was repeated. The average of the three composites with aftertreatment was 0.01 g/mi NMHC, 0.06 g/mi CO, and 0.02 g/mi NO_x. Both NMHC and CO were lower than the base fuel, and all of the emission results were well below the 2011 emission standards and similar to the 50,000 mile certification data. The average fuel economy with aftertreatment was 25.78 mpg. When the average composite emissions were compared without aftertreatment, the averages were again significantly higher than with aftertreatment. The average of the three composites without aftertreatment was 1.4 g/mi NMHC, 13.0 g/mi CO, and 3.8 g/mi NO_x. The average fuel economy was 24.99 mpg. Table 8 summarizes the regulated emissions at each test condition, and Appendix A contains the individual and composite emission test results.

The fuel was then changed to the 15 percent ethanol blend, the vehicle was operated for 4000 miles, and the test sequence was repeated. When the average of the three composite emissions was compared to the baseline fuel with aftertreatment, the CO was similar to the initial tests using the baseline fuel, the NMHC was 0.01 g/mi lower, and the NO_x was 0.01 g/mi higher. The average of the three composites with aftertreatment was 0.02 g/mi NMHC, 0.09 g/mi CO, and 0.03 g/mi NO_x. All of the emission results were well below the 2011 emission standards and

TABLE 8. SUMMARY OF 211(b) REGULATED EMISSIONS FOR TOYOTA CAMRY

Bag or Composite	Aftertreatment Present	Emission Results, g/mi						Fuel Economy, mpg
		THC	NMHC	CO	NO_x	CO_2	Particulate	
2011 Bin 5 Standard		**NS[a]**	**0.075[b]**	**3.4**	**0.05**	**NS**	**NS**	**NS**
50,000 mile Certification Data		**0.01**	**--[c]**	**0.09**	**0.02**	**--**	**--**	**28.1**
RFABASECAT 2								
1	Y[d]	0.116	0.105	0.379	0.032	377.75	0.0009	23.55
2	Y	0.003	0.003	0.000	0.013	345.64	0.0008	25.80
3	Y	0.004	0.002	0.005	0.029	294.28	0.0005	30.31
4	Y	0.003	0.002	0.000	0.017	338.23	0.0007	26.37
Composite	Y	0.027	0.024	0.080	0.022	336.00	0.0007	26.64
RFABASECAT 3								
1	Y	0.119	0.106	0.384	0.051	372.04	0.0026	23.91
2	Y	0.003	0.002	0.005	0.015	338.76	0.0021	26.33
3	Y	0.004	0.002	0.001	0.034	305.75	0.0016	29.17
4	Y	0.003	0.002	0.001	0.013	339.55	0.0020	26.27
Composite	Y	0.027	0.024	0.081	0.027	336.87	0.0021	26.52
RFABASECAT 6								
1	Y	0.173	0.163	0.462	0.038	367.67	0.0014	24.17
2	Y	0.003	0.000	0.000	0.013	341.54	0.0019	26.11
3	Y	0.004	0.000	0.000	0.048	295.03	0.0015	30.23
4	Y	0.003	0.000	0.001	0.000	331.31	0.0002	26.92
Composite	Y	0.038	0.034	0.096	0.024	331.15	0.0012	27.01
Average of Composites		0.031	0.027	0.086	0.024	334.67	0.0010	26.72
RFABASE 1								
1	N[e]	1.527	1.477	10.581	4.218	355.76	0.0041	23.65
2	N	1.601	1.547	11.954	3.350	329.49	0.0021	25.64
3	N	1.178	1.140	9.178	4.112	282.35	0.0023	29.68
4	N	1.543	1.490	11.471	3.491	307.53	0.0020	26.99
Composite	N	1.452	1.404	10.764	3.781	315.49	0.0026	26.60
RFABASE 2								
1	N	1.657	1.603	10.367	4.207	316.52	0.0056	26.38
2	N	1.555	1.504	11.714	3.330	335.66	0.0040	24.85
3	N	1.183	1.145	9.070	4.186	296.47	0.0020	28.36
4	N	1.517	1.466	11.301	3.415	318.82	0.0022	26.13
Composite	N	1.463	1.415	10.585	3.772	315.94	0.0033	26.47
RFABASE 3								
1	N	1.667	1.613	12.097	4.297	356.21	0.0055	23.44
2	N	1.617	1.563	13.084	3.245	324.58	0.0029	25.46
3	N	1.230	1.191	11.684	4.125	294.05	0.0032	28.30
4	N	1.658	1.604	14.109	3.468	317.68	0.0026	25.85
Composite	N	1.533	1.483	12.798	3.770	320.70	0.0034	25.86
Average of Composites		1.483	1.434	11.382	3.774	317.38	0.0031	26.31

[a]NS – No standard for THC or CO_2
[b]Standard reported in terms of NMOG; data in table reported as NMHC
[c]No value or cell intentionally left blank
[d]Y – Yes aftertreatment present
[e]N – No aftertreatment removed

TABLE 8 (CONT'D). SUMMARY OF 211(b) REGULATED EMISSIONS FOR TOYOTA CAMRY

Bag or Composite	Aftertreatment Present	Emission Results, g/mi						Fuel Economy, mpg
		THC	NMHC	CO	NO_x	CO_2	Particulate	
RFAE10CAT 1								
1	Y[d]	0.088	0.075	0.341	0.041	368.96	0.0017	23.29
2	Y	0.003	0.003	0.004	0.014	347.92	0.0008	24.75
3	Y	0.004	0.001	0.003	0.012	300.19	0.0009	28.68
4	Y	0.001	0.000	0.004	0.014	331.99	0.0007	25.94
Composite	Y	0.020	0.017	0.074	0.019	334.46	0.0010	25.83
RFAE10CAT 2								
1	Y	0.054	0.043	0.262	0.048	370.14	0.0020	23.23
2	Y	0.003	0.002	0.012	0.014	348.74	0.0006	24.69
3	Y	0.004	0.000	0.000	0.032	298.86	0.0015	28.81
4	Y	0.003	0.002	0.000	0.016	340.27	0.0010	25.31
Composite	Y	0.014	0.010	0.057	0.027	337.00	0.0013	25.63
RFAE10CAT 3								
1	Y	0.061	0.050	0.289	0.053	365.39	0.0019	23.53
2	Y	0.003	0.002	0.001	0.009	341.90	0.0002	25.19
3	Y	0.003	0.000	0.001	0.014	295.29	0.0009	29.16
4	Y	0.003	0.002	0.004	0.015	333.73	0.0000	25.80
Composite	Y	0.015	0.012	0.061	0.021	331.56	0.0007	26.04
Average of Composites		0.016	0.013	0.064	0.022	334.34	0.0010	25.78
RFAE10 1								
1	N[e]	1.469	1.415	11.762	4.193	350.69	0.0029	23.05
2	N	1.501	1.444	13.086	3.280	329.50	0.0017	24.28
3	N	1.112	1.072	10.497	3.929	286.25	0.0020	28.13
4	N	1.474	1.417	13.003	3.405	319.87	0.0019	24.98
Composite	N	1.380	1.328	12.076	3.685	319.17	0.0021	25.23
RFAE10 2								
1	N	1.506	1.395	13.671	4.217	351.52	0.0029	22.81
2	N	1.540	1.525	14.691	3.380	331.53	0.0017	23.97
3	N	1.154	1.142	11.730	4.101	291.00	0.0018	27.52
4	N	1.492	1.477	14.031	3.492	320.13	0.0020	24.84
Composite	N	1.413	1.368	13.473	3.784	321.18	0.0021	24.91
RFAE10 3								
1	N	1.535	1.475	13.837	4.187	347.52	0.0029	23.03
2	N	1.535	1.483	14.707	3.418	335.18	0.0019	23.72
3	N	1.154	1.110	11.993	4.138	295.31	0.0015	27.11
4	N	1.507	1.448	14.076	3.466	318.86	0.0020	24.92
Composite	N	1.422	1.369	13.596	3.790	321.97	0.0020	24.83
Average of Composites		1.405	1.355	13.048	3.753	320.77	0.0021	24.99

[d]Y – Yes aftertreatment present
[e]N – No aftertreatment removed

TABLE 8 (CONT'D). SUMMARY OF 211(b) REGULATED EMISSIONS FOR TOYOTA CAMRY

Bag or Composite	Aftertreatment Present	Emission Results, g/mi						Fuel Economy, mpg
		THC	NMHC	CO	NO_x	CO_2	Particulate	
RFAE15CAT 1								
1	Y[d]	0.066	0.053	0.395	0.048	379.01	0.0026	22.43
2	Y	0.000	0.000	0.004	0.009	338.75	0.0017	25.16
3	Y	0.008	0.005	0.003	0.016	302.10	0.0035	28.20
4	Y	0.003	0.002	0.000	0.015	336.15	0.0036	25.35
Composite	Y	0.017	0.013	0.084	0.021	336.24	0.0030	25.41
RFAE15CAT 3								
1	Y	0.079	0.063	0.371	0.052	367.38	0.0048	23.14
2	Y	0.006	0.004	0.016	0.010	341.98	0.0029	24.91
3	Y	0.005	0.000	0.000	0.048	298.36	0.0045	28.56
4	Y	0.004	0.002	0.001	0.016	330.00	0.0029	25.82
Composite	Y	0.020	0.015	0.081	0.031	331.72	0.0037	25.76
RFAE15CAT 4								
1	Y	0.096	0.080	0.484	0.065	369.68	0.0084	22.98
2	Y	0.003	0.000	0.004	0.011	344.04	0.0025	24.77
3	Y	0.008	0.003	0.000	0.035	300.99	0.0059	28.31
4	Y	0.000	0.000	0.004	0.013	330.64	0.0035	25.77
Composite	Y	0.024	0.017	0.102	0.029	333.59	0.0049	25.62
Average of Composites		0.020	0.015	0.089	0.027	333.85	0.0034	25.60
RFAE15 1								
1	N[e]	1.489	1.440	8.521	4.365	351.90	0.0036	23.05
2	N	1.419	1.371	9.384	3.563	320.94	0.0017	25.07
3	N	1.036	1.002	7.187	4.373	280.51	0.0024	28.90
4	N	1.385	1.338	9.227	3.865	314.34	0.0005	25.59
Composite	N	1.319	1.275	8.556	4.041	314.32	0.0019	25.78
RFAE15 2								
1	N	1.445	1.394	8.564	4.332	354.11	0.0032	22.91
2	N	1.478	1.431	9.031	3.492	314.63	0.0030	25.57
3	N	1.074	1.038	7.083	4.180	280.66	0.0017	28.88
4	N	1.338	1.292	8.909	3.610	308.28	0.0013	26.12
Composite	N	1.319	1.274	8.364	3.889	311.61	0.0022	26.02
RFAE15 3								
1	N	1.465	1.421	8.525	4.423	350.76	0.0033	23.12
2	N	1.452	1.406	9.138	3.568	322.06	0.0017	25.01
3	N	1.097	1.058	7.779	4.369	281.05	0.0019	28.73
4	N	1.405	1.360	9.479	3.668	310.06	0.0025	25.89
Composite	N	1.343	1.300	8.740	3.995	313.23	0.0023	25.84
Average of Composites		1.327	1.283	8.553	3.975	313.05	0.0021	25.88

[d]Y – Yes, aftertreatment present
[e]N – No, aftertreatment removed

similar to the 50,000 mile certification data. The average fuel economy with aftertreatment was 25.60 mpg. When the average composite emissions were compared without aftertreatment, the averages were again significantly higher than with aftertreatment. The average of the three composites without aftertreatment was 1.3 g/mi NMHC, 8.6 g/mi CO, and 4.0 g/mi NO_x. The average fuel economy was 25.88 mpg. Table 8 summarizes the regulated emissions at each test condition, and Appendix A contains the individual and composite emission test results.

5.2 Speciation of Volatile Hydrocarbon Compounds

Speciation results for volatile hydrocarbon compounds with carbon numbers from C_1 to C_{12} plus aldehydes, ketones, alcohols, and ethers are included in this section. Speciation was performed on samples collected during each cold- and hot-start test sequence both with and without aftertreatment. More than 200 compounds were checked for their presence in the dilute exhaust. Data for the individual compounds, corrected for background dilution air contributions, are included in Appendix B.

Some compounds were found in either one or more of the cold, stabilize, or hot-start tests during the triplicate test sequence with each fuel. Values were reported when these compounds were detected at the limit of quantification which was more than two times the detection limit (0.05 mg/mi). In other cases, compounds that were detected at less than two times the limit of quantification and above the detection limit were labeled as "trace" in the appendix tables. Where compounds were not detected (below the detection limit), ND was used to indicate that no quantitative value could be assigned to the compound for that test. Average values were also reported for the composite results with each fuel. For these averages, values reported as ND and "trace" were set to zero for calculation purposes.

With the large number of compounds detected in the exhaust, there are a number of ways to compare the fuels both with and without aftertreatment. The first comparison was for the base fuel with aftertreatement and the two ethanol blends with aftertreatment. In this case, several compounds were detected in the exhaust with base fuel that were <u>not</u> in the additive. These compounds included:

- 3-methyl-1-butene
- trans-2-pentene
- cyclopentadiene
- 1-cis,2-trans,3-trimethylcyclopentane
- Ethylbenzene
- 1,2,4-trimethylbenzene
- Unidentified C9-C12
- n-/isobutyraldehyde
- o-tolualdehyde

When comparing the base fuel without aftertreatment and the two ethanol concentrations without aftertreatment, several compounds were found in the exhaust with the base fuel that were <u>not</u> found with the additive. These compounds included:

- 3-methyl-1-butene
- 1-trans,2-cis,3-trimethylcyclopentane
- cis-2-octene
- 1,2-diethylbenzene
- 1-methyl-2-n-propylbenzene
- 1,4-dimethyl-2-ethylbenzene
- 1,3-dimethyl-2-ethylbenzene
- undecane

In addition, 1-pentene; ethanol; 1,3-diethylbenzene; and acetaldehyde were detected at higher concentrations with the additive and no aftertreatment. When the aftertreatment was present, the aftertreatment was able to maintain the concentrations near the base fuel levels except for the compounds listed above.

Another comparison was for the same fuels both with and without aftertreatment. In general, the concentrations for the tests with aftertreatment were generally lower than the tests without aftertreatment regardless of the fuel. With all three fuels, a large number of compounds were present without aftertreatment that were not detected with aftertreatment. No additional compounds were detected with two ethanol blends which were not detected in the base fuel.

5.3 Alcohols and Ethers

Methanol and ethanol were detected in the exhaust of the base fuel when testing without the aftertreatment in place; and 2-propanol was detected in the exhaust of the base fuel without aftertreatment in the first test only. A trace amount of methanol was found in the base fuel during only one test with aftertreatment. No 2-propanol was detected in the exhaust with the three ethanol concentrations, and no other lower molecular weight alcohols or ethers were detected with either of the fuels. The concentration of methanol and ethanol was greater without aftertreatment, and the concentrations of methanol and ethanol increased with the ethanol concentration of the fuel.

For this analysis, the minimum detection limit for the alcohols and ethers was 0.05 mg/mi. While no additional alcohols or ethers were detected, one cannot conclude that these compounds were present or not present; but if present, they were below the limits of detection. Table 9 lists the results for the alcohol analyses.

5.4 PAH and NPAH

Volatile- and particulate-phase PAH and NPAH compounds were determined for each test condition. Individual compounds included: benzo[a]anthracene, benzo[b]fluoranthene, benzo[k]fluoranthene, benzo[a]pyrene, chrysene, dibenzo[a,h]anthracene, indeno[1,2,3-cd]pyrene, 7-nitrobenzo[a]anthracene, 6-nitrobenzo[a]pyrene, 6-nitrochrysene, 2-nitrofluorene, and 1-nitropyrene. The analytical procedure used to measure these compounds was able to detect less than 1 ng/mi. Values have been reported in terms of ng/mi for each compound. Table 10 presents the individual volatile-phase results, and Table 11 presents the individual particulate-phase results. Table 12 summarizes the combined volatile- and particulate-phase results.

In general, the concentrations of PAH were significantly lower with aftertreatment than without it. Benzo(a)anthracene, chrysene, and benzo(b)fluoreanthene were generally detected at the highest concentrations for the PAHs, and the concentrations tended to decrease with the higher molecular weight compounds. In general, the concentrations for the PAHs and NPAHs were higher in the particulate-phase than the volatile-phase. Without aftertreatment, the total concentration of the individual PAHs were similar between the base fuel and E15, while the E10 fuel produced higher concentrations. 2-nitrofluorene and 1-nitropyrene were detected in all three fuels both with and without aftertreatment, but only 7-nitrobenz(a)anthracene was found in the base fuel with aftertreatment. This compound was not detected without aftertreatment, and no other NPAHs were detected in any of the samples. For all of the NPAHs, 2-nitrofluorene was

present at the highest concentration in the volatile-phase, and 1-nitropyrene was higher in the particulate-phase. For this analysis, the minimum detection limit for PAH was 0.01 ng/mi and 0.6 ng/mi for NPAH. While some of the PAH and NPAH were not detected, one cannot conclude that these compounds were present or not present; but if present, they were below the limits of detection.

TABLE 10. VOLATILE-PHASE PAH AND NPAH RESULTS

COMPOUND[a]	EMISSIONS, ng/mi			AVERAGE COMPOSITE, ng/mi	EMISSIONS, ng/mi			AVERAGE COMPOSITE, ng/mi
	BASE 2	BASE 3	BASE 6		E10-1	E10-2	E10-3	
WITH AFTERTREATMENT								
2-Nitrofluorene	Trace[b]	Trace	Trace	Trace	Trace	Trace	Trace	Trace
1-Nitropyrene	Trace	Trace	Trace	Trace	Trace	Trace	Trace	Trace
7-Nitrobenz(a)anthracene	ND[c]	ND	ND	ND	ND	ND	ND	ND
6-Nitrochrysene	ND	ND	ND	ND	ND	ND	ND	ND
6-Nitrobenz(a)pyrene	ND	ND	ND	ND	ND	ND	ND	ND
Benzo(a)anthracene	26	22	37	28	47	33	27	36
Chrysene	22	19	28	23	30	22	18	23
Benzo(b)fluoranthene	3.7	3.1	6.2	4.3	2.8	2.9	2.6	2.8
Benzo(k)fluoranthene	1.1	0.9	1.8	1.3	0.9	1.0	0.8	0.9
Benzo(a)pyrene	0.8	0.7	1.8	1.1	0.5	0.4	0.5	0.5
Indeno[1,2,3-cd]pyrene	0.9	0.8	1.5	1.1	0.5	0.3	0.5	0.4
Dibenz(a,h)anthracene	0.2	0.2	0.3	0.3	0.2	0.2	0.3	0.2
WITHOUT AFTERTREATMENT								
2-Nitrofluorene	7.9	7.6	6.7	7.4	5.5	5.0	4.9	5.2
1-Nitropyrene	Trace	Trace	Trace	Trace	ND	ND	ND	ND
7-Nitrobenz(a)anthracene	ND	ND	ND	ND	ND	ND	ND	ND
6-Nitrochrysene[a]	ND	ND	ND	ND	ND	ND	ND	ND
6-Nitrobenz(a)pyrene	ND	ND	ND	ND	ND	ND	ND	ND
Benzo(a)anthracene	415	512	428	452	339	340	293	324
Chrysene	223	278	226	242	214	221	201	212
Benzo(b)fluoranthene	39	55	56	50	32	44	36	37
Benzo(k)fluoranthene	14	20	19	18	14	20	16	17
Benzo(a)pyrene	14	20	20	18	13	19	16	16
Indeno[1,2,3-cd]pyrene	8.5	13	13	11	7.8	12	10	9.9
Dibenz(a,h)anthracene	2.3	3.1	3.1	2.8	1.7	3.3	2.5	2.5

[a]Detection limit in ng/mi calculated using the minimum value that could be quantified by the analytical procedure; if present, the PAH was at a concentration less than 0.01 ng/mi, and the NPAH was at a concentration of 0.6 ng/mi.

[b]Trace - Value not quantifiable at detection limit; concentration less than twice the detection limit

[c]ND - None detected at the detection limit.

TABLE 10 (CONT'D). VOLATILE-PHASE PAH AND NPAH RESULTS

COMPOUND[a]	EMISSIONS, ng/mi			AVERAGE COMPOSITE, ng/mi
	E15-1	E15-3	E15-4	
WITH AFTERTREATMENT				
2-Nitrofluorene	Trace[b]	Trace	Trace	Trace
1-Nitropyrene	Trace	Trace	Trace	Trace
7-Nitrobenz(a)anthracene	ND[c]	ND	ND	ND
6-Nitrochrysene	ND	ND	ND	ND
6-Nitrobenz(a)pyrene	ND	ND	ND	ND
Benzo(a)anthracene	107	102	75	95
Chrysene	69	62	49	60
Benzo(b)fluoranthene	6.1	5.7	5.6	5.8
Benzo(k)fluoranthene	2.1	2.0	1.9	2.0
Benzo(a)pyrene	1.0	0.8	1.0	1.0
Indeno[1,2,3-cd]pyrene	0.5	0.4	0.6	0.5
Dibenz(a,h)anthracene	0.4	0.2	0.3	0.3
WITHOUT AFTERTREATMENT				
2-Nitrofluorene	6.7	7.5	6.8	7.0
1-Nitropyrene	Trace	Trace	Trace	Trace
7-Nitrobenz(a)anthracene	ND	ND	ND	ND
6-Nitrochrysene[a]	ND	ND	ND	ND
6-Nitrobenz(a)pyrene	ND	ND	ND	ND
Benzo(a)anthracene	361	329	345	345
Chrysene	198	196	202	199
Benzo(b)fluoranthene	31	35	45	37
Benzo(k)fluoranthene	12	14	18	15
Benzo(a)pyrene	11	15	16	14
Indeno[1,2,3-cd]pyrene	6.2	8.0	9.1	7.7
Dibenz(a,h)anthracene	1.8	1.7	2.1	1.9

[a]Detection limit in ng/mi calculated using the minimum value that could be quantified by the analytical procedure; if present, the PAH was at a concentration less than 0.01 ng/mi, and the NPAH was at a concentration of 0.6 ng/mi.

[b]Trace - Value not quantifiable at detection limit; concentration less than twice the detection limit

[c]ND - None detected at the detection limit.

TABLE 11. PARTICULATE-PHASE PAH AND NPAH RESULTS

COMPOUND[a]	EMISSIONS, ng/mi			AVERAGE COMPOSITE, ng/mi	EMISSIONS, ng/mi			AVERAGE COMPOSITE, ng/mi
	BASE 2	BASE 3	BASE 6		E10-1	E10-2	E10-3	
WITH AFTERTREATMENT								
2-Nitrofluorene	ND[b]	ND	Trace[c]	Trace	ND	ND	ND	ND
1-Nitropyrene	Trace	Trace	Trace	Trace	Trace	Trace	Trace	Trace
7-Nitrobenz(a)anthracene	Trace	Trace	Trace	Trace	ND	ND	ND	ND
6-Nitrochrysene	ND	ND	ND	ND	ND	ND	ND	ND
6-Nitrobenz(a)pyrene	ND	ND	ND	ND	ND	ND	ND	ND
Benzo(a)anthracene	27	29	20	25	23	20	18	20
Chrysene	23	23	15	20	19	15	16	17
Benzo(b)fluoranthene	20	23	41	28	22	18	18	19
Benzo(k)fluoranthene	7.1	7.5	15	9.8	8.7	6.5	6.8	7.3
Benzo(a)pyrene	8.6	7.9	22	13	8.0	5.5	5.8	6.4
Indeno[1,2,3-cd]pyrene	8.5	8.4	24	14	5.8	3.9	4.2	4.6
Dibenz(a,h)anthracene	1.5	2.2	4.0	2.6	1.4	1.0	1.0	1.1
WITHOUT AFTERTREATMENT								
2-Nitrofluorene	1.1	1.4	1.3	1.3	ND	ND	ND	ND
1-Nitropyrene	3.2	3.1	2.9	3.1	3.7	4.6	5.0	4.4
7-Nitrobenz(a)anthracene	ND	ND	ND	ND	ND	ND	ND	ND
6-Nitrochrysene[a]	ND	ND	ND	ND	ND	ND	ND	ND
6-Nitrobenz(a)pyrene	ND	ND	ND	ND	ND	ND	ND	ND
Benzo(a)anthracene	479	722	681	627	1593	1653	1446	1564
Chrysene	323	433	402	386	1116	1083	1050	1083
Benzo(b)fluoranthene	464	722	616	601	1105	1174	1129	1136
Benzo(k)fluoranthene	152	203	173	176	405	395	369	390
Benzo(a)pyrene	173	275	254	234	478	468	466	471
Indeno[1,2,3-cd]pyrene	130	230	191	184	357	400	421	393
Dibenz(a,h)anthracene	24	36	32	31	77	95	96	89

[a]Detection limit in ng/mi calculated using the minimum value that could be quantified by the analytical procedure; if present, the PAH was at a concentration less than 0.01 ng/mi, and the NPAH was at a concentration of 0.6 ng/mi.

[b]ND - None detected at the detection limit.

[c]Trace - Value not quantifiable at detection limit; concentration less than twice the detection limit

TABLE 11 (CONT'D). PARTICULATE-PHASE PAH AND NPAH RESULTS

COMPOUND[a]	EMISSIONS, ng/mi			AVERAGE COMPOSITE, ng/mi
	E15-1	E15-3	E15-4	
WITH AFTERTREATMENT				
2-Nitrofluorene	ND[b]	ND	ND	ND
1-Nitropyrene	Trace[c]	Trace	Trace	Trace
7-Nitrobenz(a)anthracene	ND	ND	ND	ND
6-Nitrochrysene	ND	ND	ND	ND
6-Nitrobenz(a)pyrene	ND	ND	ND	ND
Benzo(a)anthracene	60	43	36	46
Chrysene	55	35	38	43
Benzo(b)fluoranthene	28	24	22	25
Benzo(k)fluoranthene	11	9.5	8.3	9.5
Benzo(a)pyrene	8.9	7.8	7.1	7.9
Indeno[1,2,3-cd]pyrene	4.3	5.0	5.1	4.8
Dibenz(a,h)anthracene	1.0	1.0	1.3	1.1
WITHOUT AFTERTREATMENT				
2-Nitrofluorene	ND	ND	ND	ND
1-Nitropyrene	5.0	4.6	4.3	4.7
7-Nitrobenz(a)anthracene	ND	ND	ND	ND
6-Nitrochrysene[a]	ND	ND	ND	ND
6-Nitrobenz(a)pyrene	ND	ND	ND	ND
Benzo(a)anthracene	636	715	639	663
Chrysene	419	461	436	439
Benzo(b)fluoranthene	419	502	446	456
Benzo(k)fluoranthene	162	182	187	177
Benzo(a)pyrene	160	208	193	187
Indeno[1,2,3-cd]pyrene	132	166	157	152
Dibenz(a,h)anthracene	38	50	48	46

[a]Detection limit in ng/mi calculated using the minimum value that could be quantified by the analytical procedure; if present, the PAH was at a concentration less than 0.01 ng/mi, and the NPAH was at a concentration of 0.6 ng/mi.

[b]Trace - Value not quantifiable at detection limit; concentration less than twice the detection limit

[c]ND - None detected at the detection limit.

TABLE 12. COMBINED VOLATILE- AND PARTICULATE-PHASE PAH AND NPAH RESULTS

COMPOUND[a]	BASELINE, ng/mi			E10, ng/mi		
	VOLATILE	PARTICULATE	TOTAL	VOLATILE	PARTICULATE	TOTAL
With Aftertreatment						
2-Nitrofluorene	Trace[b]	Trace	Trace	Trace	ND[c]	Trace
1-Nitropyrene	Trace	Trace	Trace	Trace	Trace	Trace
7-Nitrobenz(a)anthracene	ND	Trace	Trace	ND	ND	ND
6-Nitrochrysene	ND	ND	ND	ND	ND	ND
6-Nitrobenz(a)pyrene	ND	ND	ND	ND	ND	ND
Benzo(a)anthracene	28	25	53	36	20	56
Chrysene	23	20	43	23	17	40
Benzo(b)fluoranthene	4.3	28	32	2.8	19	23
Benzo(k)fluoranthene	1.3	9.8	11	0.9	7.3	8.2
Benzo(a)pyrene	1.1	13	14	0.5	6.4	6.9
Indeno[1,2,3-cd]pyrene	1.1	14	15	0.4	4.6	5.0
Dibenz(a,h)anthracene	0.3	2.6	2.9	0.2	1.1	1.3
Without Aftertreatment						
2-Nitrofluorene	7.4	1.3	8.6	5.2	ND	5.2
1-Nitropyrene	Trace	3.1	3.1	ND	4.4	4.4
7-Nitrobenz(a)anthracene	ND	ND	ND	ND	ND	ND
6-Nitrochrysene	ND	ND	ND	ND	ND	ND
6-Nitrobenz(a)pyrene	ND	ND	ND	ND	ND	ND
Benzo(a)anthracene	452	627	1079	324	1564	1888
Chrysene	242	386	628	212	1083	1295
Benzo(b)fluoranthene	50	601	651	37	1136	1173
Benzo(k)fluoranthene	18	176	194	17	390	407
Benzo(a)pyrene	18	234	252	16	471	487
Indeno[1,2,3-cd]pyrene	11	184	195	9.9	393	403
Dibenz(a,h)anthracene	2.8	31	34	2.5	89	93

[a]Detection limit in ng/mi calculated using the minimum value that could be quantified by the analytical procedure; if present, the PAH was at a concentration less than 0.01 ng/mi, and the NPAH was at a concentration of 0.6 ng/mi.

[b]Trace - Value not quantifiable at detection limit; concentration less than twice the detection limit

[c]ND - None detected at the detection limit.

TABLE 12 (CONT'D). COMBINED VOLATILE- AND PARTICULATE-PHASE PAH AND NPAH RESULTS

COMPOUND[a]	E15, ng/mi		
	VOLATILE	PARTICULATE	TOTAL
With Aftertreatment			
2-Nitrofluorene	Trace[b]	ND[c]	Trace
1-Nitropyrene	Trace	Trace	Trace
7-Nitrobenz(a)anthracene	ND	ND	ND
6-Nitrochrysene	ND	ND	ND
6-Nitrobenz(a)pyrene	ND	ND	ND
Benzo(a)anthracene	95	46	141
Chrysene	60	43	103
Benzo(b)fluoranthene	5.8	25	31
Benzo(k)fluoranthene	2.0	9.5	12
Benzo(a)pyrene	1.0	7.9	8.9
Indeno[1,2,3-cd]pyrene	0.5	4.8	5.3
Dibenz(a,h)anthracene	0.3	1.1	1.4
Without Aftertreatment			
2-Nitrofluorene	7.0	ND	7.0
1-Nitropyrene	Trace	4.7	4.7
7-Nitrobenz(a)anthracene	ND	ND	ND
6-Nitrochrysene	ND	ND	ND
6-Nitrobenz(a)pyrene	ND	ND	ND
Benzo(a)anthracene	345	663	1008
Chrysene	199	439	638
Benzo(b)fluoranthene	37	456	493
Benzo(k)fluoranthene	15	177	192
Benzo(a)pyrene	14	187	201
Indeno[1,2,3-cd]pyrene	7.7	152	160
Dibenz(a,h)anthracene	1.9	46	48

[a]Detection limit in ng/mi calculated using the minimum value that could be quantified by the analytical procedure; if present, the PAH was at a concentration less than 0.01 ng/mi, and the NPAH was at a concentration of 0.6 ng/mi.

[b]Trace - Value not quantifiable at detection limit; concentration less than twice the detection limit

[c]ND - None detected at the detection limit.

6.0 SUMMARY

Testing was performed on a light-duty vehicle to provide RFA and Growth Energy with data to address EPA requirements for registration of a designated F/FA as stipulated by section 211 (b) and 211 (e) of the CAA. A 2011 2.5 L Toyota Camry vehicle was tested according to procedures established in Title 40 CFR Part 79, Subpart F, Section 79.57 and Title 40 CFR Part 86, Subpart D. Emissions characterization was performed on the vehicle after 4000 miles of operation with the base gasoline, with a 10 percent ethanol blend, and with a 15 percent ethanol blend.

With aftertreatment, both fuels were able to meet the 2011 emissions standards. When the composite emissions for the three fuels (E0, E10, and E15) with aftertreatment were compared, the corresponding composite emissions for NO_x were 0.02 g/mi, 0.02 g/mi, and 0.03 g/mi respectively. The CO emissions were 0.09 g/mi for the baseline fuel, 0.06 g/mi for E10, and 0.09 g/mi with E15. Composite emissions for NMHC were 0.03 g/mi, 0.01 g/mi, and 0.02 g/mi, respectively. Based on the three replicate tests with aftertreatment, the fuel economy decreased by about 0.9 mpg with E10 and 1.1 mpg with E15; and the fuel economy with no aftertreatment decreased by about 1.3 mpg with E10 and 0.4 mpg with E15.

Speciation of the C_1 to C_{12} hydrocarbons, aldehydes, ketones, alcohols, and ethers was performed during each cold- and hot-segment of the EPA transient cycle. In general,

- Some compounds were found in either one or more of the cold, stabilize, or hot-start tests
- Several compounds were detected with the base fuel plus aftertreatment that were not detected with the additive plus aftertreatment
- Concentrations were lower with aftertreatment than without it
- With the additive, 1-pentene; ethanol; 1,3-diethylbenzene; and acetaldehyde were detected at a higher concentration with no aftertreatment; with aftertreatment, the concentrations were near the base fuel levels except for several compounds
- Methanol and ethanol were the only alcohols detected above the detection limit in both fuels except for 2-propanol was only detected in one test with the base fuel without aftertreatment; no additional alcohols or ethers were detected with any of the fuels
- More compounds were detected with no aftertreatment than with aftertreatment.

While some C_1 to C_{12} hydrocarbons were below the detection limit, one cannot necessarily conclude that these compounds were present or not present; however if present, the compounds were below the limits of detection.

Volatile- and particulate-phase PAH and NPAH compounds were determined for each test condition. Individual compounds included: benzo[a]anthracene, benzo[b]fluoranthene, benzo[k]fluoranthene, benzo[a]pyrene, chrysene, dibenzo[a,h]anthracene, indeno[1,2,3-cd]pyrene, 7-nitrobenzo[a]anthracene, 6-nitrobenzo[a]pyrene, 6-nitrochrysene, 2-nitrofluorene, and 1-nitropyrene. In general, exhaust PAH and NPAH concentrations without aftertreatment were higher than with aftertreatment, and the concentration of the particulate-phase PAH and NPAH compounds were higher than the volatile-phase compounds. As a result, the

aftertreatment was capable of removing the majority of the PAHs and NPAHs. Three PAHs (benzo(a)anthracene, chrysene, and benzo(b)fluoranthene) were present at the highest concentration for the six PAHs detected. In general, no additional PAH and NPAH were present with the two ethanol blends.

TABLE 9. ALCOHOL RESULTS

COMPOUND[a]	COLD	STABILIZE	HOT	COMPOSITE	COLD	STABILIZE	HOT	COMPOSITE	COLD	STABILIZE	HOT	COMPOSITE	AVERAGE COMPOSITE, mg/mi
	mg/mi	mg/mi	mg/mi	mg/mi	mg/mi	mg/mi	mg/mi	mg/mi	mg/mi	mg/mi	mg/mi	mg/mi	
Baseline with Aftertreatment													
Methanol	ND	ND	ND	ND	ND	ND	ND	ND	0.1	ND	ND	Trace	Trace
Ethanol	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
2-propanol	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
Isobutanol	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
Baseline without Aftertreatment													
Methanol	7.9	5.1	5.3	5.7	9.7	8.2	5.5	7.8	7.6	6.6	5.5	6.5	6.7
Ethanol	0.6	0.9	0.4	0.7	1.1	0.6	0.6	0.8	0.6	0.6	0.5	0.5	0.7
2-propanol	0.2	0.3	ND	0.2	ND	ND	ND	ND	ND	ND	ND	ND	Trace
E10 with Aftertreatment													
Methanol	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
Ethanol	1.2	ND	ND	0.2	0.8	ND	ND	0.2	1.3	ND	ND	0.3	0.2
2-propanol	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
E10 without Aftertreatment													
Methanol	8.6	8.9	5.8	8.0	8.9	9.3	6.0	8.3	8.6	8.7	6.1	8.0	8.1
Ethanol	71.9	70.5	51.4	65.5	72.0	65.8	50.7	62.9	72.6	66.2	51.9	63.6	64.0
2-propanol	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
E15 with Aftertreatment													
Methanol	ND	ND	ND	ND	ND	ND	ND	ND	0.3	0.2	ND	0.2	Trace
Ethanol	1.8	0.9	ND	0.4	2.9	ND	ND	0.6	4.4	0.1	ND	0.9	0.6
2-propanol	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
E15 without Aftertreatment													
Methanol	11.3	11.8	8.2	10.7	12.2	11.7	8.7	11.0	12.7	9.9	9.1	10.2	10.6
Ethanol	85.2	78.6	56.1	73.8	84.2	78.2	56.0	73.3	85.0	74.4	56.9	71.8	73.0
2-propanol	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND

Note: No other alcohols or ethers were detected within the limits of detection

[a]Minimum detection limit is 0.05 mg/mi

[b]ND - None detected

[c]Trace - Value not quantifiable at detection limit; concentration less than twice the detection limit

[d]NA - not available because water absorbing solution was contaminated with alcohol prior to sampling

7.0 REFERENCES

1. DEER SwRI TIP 06-001, “Traceability to Standards.”
2. DEER SwRI TIP 06-002, “NO_x Converter Efficiency Determination.”
3. DEER SwRI TIP 06-003, “Linearity Verification of Gas Dividers.”
4. DEER SwRI TIP 06-010, “Barometric Pressure Verification.”
5. DEER SwRI TIP 06-011, “Propane Recovery Check.”
6. DEER SwRI TIP 06-013, “Temperature Calibration and Verification.”
7. DEER SwRI TIP 06-016, “Wet CO2 Interference Check for CO Analyzers.”
8. DEER SwRI TIP 06-020, “Pressure Calibration and Verification.”
9. DEER SwRI TIP 06-022, “CVS Blower Calibration.”
10. DEER SwRI TIP 06-023, “Calibration of Analyzers Using Digital Readout.”
11. DEER SwRI TIP 07-023, “Operation of Bag Cart.”
12. DEER SwRI TIP 07C-002, “Methane Quantitative Analysis.”
13. DEER SwRI TIP 07C-006, “Analysis of Aldehydes and Ketones in Exhaust by Liquid Chromatography.”
14. DEER SwRI TIP 07C-018, “Cartridge Sampling of Exhaust Emissions.”
15. DEER SwRI TIP 07C-011, “Preparation of Impingers Used in Collection of Unregulated Emissions.”
16. DEER SwRI TIP 07C-013, “Hydrocarbon Speciation.”
17. DEER SwRI SOP-4.16, "Quality Records.”
18. Caldwell, J.W., e-mail to Mr. E. Robert Fanick, July 20, 2010, 12:47 p.m.

APPENDIX A

LIGHT-DUTY EMISSION TEST RESULTS

SOUTHWEST RESEARCH INSTITUTE - DEPARTMENT OF EMISSIONS RESEARCH

COMPUTER PROGRAM LDT 3.2-R 4-BAG EPA FTP VEHICLE EMISSION RESULTS PROJECT NO. 03-15812-00

VEHICLE NUMBER 7329	TEST RF211BASECAT2	GASOLINE +EPATIER EM-7578-F
VEHICLE MODEL 2011 TOYOTA CAMRY	DATE 9/ 9/2010 RUN	FUEL DENSITY 6.200 LB/GAL
ENGINE 2.5 L (153 CID)-4	DYNO 3 BAG CART 1	H .134 C .866 O .000 X .000
TRANSMISSION A	ACTUAL ROAD LOAD 11.30 HP (8.43 KW)	FTP
ODOMETER 4125 MILES (6637 KM)	TEST WEIGHT 3625 LBS (1643 KG)	

BAROMETER 29.02 IN HG (737.1 MM HG) DRY BULB TEMPERATURE 73.0°F (22.8°C) NOX HUMIDITY C.F. 1.056

RELATIVE HUMIDITY 68.6 PCT.

BAG NUMBER	1	2	3	4
BAG DESCRIPTION	COLD TRANSIENT (0-505 SEC.)	STABILIZED (505-1372 SEC.)	HOT TRANSIENT (0- 505 SEC.)	HOT STABILIZED (505-1372 SEC.)
RUN TIME SECONDS	505.4	868.3	505.1	868.3
DRY/WET CORRECTION FACTOR, SAMP/BACK	.972/.980	.975/.980	.973/.980	.975/.980
MEASURED DISTANCE MILES (KM)	3.60 (5.79)	3.87 (6.23)	3.59 (5.78)	3.86 (6.21)
BLOWER FLOW RATE SCFM (SCMM)	292.9 (8.30)	291.3 (8.25)	291.7 (8.26)	292.0 (8.27)
20X20 FLOW RATE SCFM (SCMM)	45.7 (1.30)	45.8 (1.30)	46.7 (1.32)	46.3 (1.31)
GAS METER FLOW RATE SCFM (SCMM)	.86 (.02)	.83 (.02)	.86 (.02)	.84 (.02)
TOTAL FLOW SCF (SCM)	2860. (81.0)	4890. (138.5)	2856. (80.9)	4907. (139.0)
HC SAMPLE METER/RANGE/PPM (BAG)	12.2/ 2/ 12.21	3.3/ 2/ 3.30	3.4/ 2/ 3.40	3.1/ 2/ 3.10
HC BCKGRD METER/RANGE/PPM	3.5/ 2/ 3.50	3.3/ 2/ 3.30	3.3/ 2/ 3.30	3.1/ 2/ 3.10
CO SAMPLE METER/RANGE/PPM	15.9/ 12/ 15.34	.1/ 12/ .10	.3/ 12/ .29	.2/ 12/ .19
CO BCKGRD METER/RANGE/PPM	.3/ 12/ .29	.2/ 12/ .19	.1/ 12/ .10	.2/ 12/ .19
CO2 SAMPLE METER/RANGE/PCT	51.2/ 2/ .9617	58.9/ 1/ .5743	76.8/ 1/ .7595	57.4/ 1/ .5590
CO2 BCKGRD METER/RANGE/PCT	2.7/ 2/ .0481	5.1/ 1/ .0488	5.1/ 1/ .0488	5.0/ 1/ .0479
NOX SAMPLE METER/RANGE/PPM (BAG) (D)	3.1/ 1/ .78	1.0/ 1/ .25	2.7/ 1/ .68	1.2/ 1/ .30
NOX BCKGRD METER/RANGE/PPM	.3/ 1/ .08	.3/ 1/ .08	.2/ 1/ .05	.3/ 1/ .08
CH4 SAMPLE PPM (1.180)	2.77	2.11	2.16	2.11
CH4 BCKGRD PPM	2.22	2.19	2.15	2.19
DILUTION FACTOR	13.98	23.46	17.74	24.10
HC CONCENTRATION PPM	8.96	.14	.29	.13
CO CONCENTRATION PPM	14.45	-.09	.19	.01
CO2 CONCENTRATION PCT	.9170	.5276	.7135	.5132
NOX CONCENTRATION PPM	.71	.18	.63	.23
CH4 CONCENTRATION PPM	.71	.02	.14	.01
NMHC CONCENTRATION PPM	8.12	.12	.13	.11
HC MASS GRAMS	.418	.011	.013	.010
CO MASS GRAMS	1.363	.000	.018	.001
CO2 MASS GRAMS	1359.91	1337.64	1056.47	1305.56
NOX MASS GRAMS	.115	.050	.103	.064
PM MASS MILLIGRAMS	3.4	3.0	1.7	2.6
CH4 MASS GRAMS	.038	.002	.007	.001
NMHC MASS GRAMS (FID)	.379	.010	.006	.009
FUEL MASS KG	.430	.422	.333	.412
FUEL ECONOMY MPG (L/100KM)	23.55 (9.99)	25.80 (9.12)	30.31 (7.76)	26.37 (8.92)

4-BAG COMPOSITE RESULTS

HC	G/MI	.027	CH4	G/MI	.003
CO	G/MI	.080	NMHC	G/MI	.024
NOX	G/MI	.022			
PM	MG/MI	.7			
FUEL ECONOMY MPG (L/100KM)		26.64 (8.83)			

SOUTHWEST RESEARCH INSTITUTE - DEPARTMENT OF EMISSIONS RESEARCH

COMPUTER PROGRAM LDT 3.2-R 4-BAG EPA FTP VEHICLE EMISSION RESULTS PROJECT NO. 03-15812-00

VEHICLE NUMBER	7329	TEST RF211BASECAT3	GASOLINE +EPATIER EM-7578-F
VEHICLE MODEL	2011 TOYOTA CAMRY	DATE 9/10/2010 RUN	FUEL DENSITY 6.200 LB/GAL
ENGINE	2.5 L (153 CID)-4	DYNO 3 BAG CART 2	H .134 C .866 O .000 X .000
TRANSMISSION	A	ACTUAL ROAD LOAD 11.30 HP (8.43 KW)	FTP
ODOMETER	4140 MILES (6661 KM)	TEST WEIGHT 3625 LBS (1643 KG)	

BAROMETER 29.01 IN HG (736.9 MM HG) DRY BULB TEMPERATURE 72.5°F (22.5°C) NOX HUMIDITY C.F. 1.039

RELATIVE HUMIDITY 67.2 PCT.

BAG NUMBER	1	2	3	4
BAG DESCRIPTION	COLD TRANSIENT (0-505 SEC.)	STABILIZED (505-1372 SEC.)	HOT TRANSIENT (0- 505 SEC.)	HOT STABILIZED (505-1372 SEC.)
RUN TIME SECONDS	505.0	868.9	504.8	868.0
DRY/WET CORRECTION FACTOR, SAMP/BACK	.972/.981	.976/.981	.974/.981	.976/.981
MEASURED DISTANCE MILES (KM)	3.60 (5.79)	3.83 (6.16)	3.57 (5.74)	3.85 (6.19)
BLOWER FLOW RATE SCFM (SCMM)	290.6 (8.23)	290.9 (8.24)	291.7 (8.26)	290.0 (8.21)
20X20 FLOW RATE SCFM (SCMM)	45.7 (1.30)	45.3 (1.28)	46.5 (1.32)	46.4 (1.31)
GAS METER FLOW RATE SCFM (SCMM)	.91 (.03)	.83 (.02)	.85 (.02)	.83 (.02)
TOTAL FLOW SCF (SCM)	2838. (80.4)	4880. (138.2)	2852. (80.8)	4879. (138.2)
HC SAMPLE METER/RANGE/PPM (BAG) (D)	12.1/ 2/ 12.10	3.1/ 2/ 3.10	3.2/ 2/ 3.20	3.1/ 2/ 3.10
HC BCKGRD METER/RANGE/PPM	3.1/ 2/ 3.10	3.1/ 2/ 3.10	3.1/ 2/ 3.10	3.1/ 2/ 3.10
CO SAMPLE METER/RANGE/PPM	16.4/ 12/ 15.86	.6/ 12/ .59	.4/ 12/ .39	.4/ 12/ .39
CO BCKGRD METER/RANGE/PPM	.5/ 12/ .49	.5/ 12/ .49	.4/ 12/ .39	.4/ 12/ .39
CO2 SAMPLE METER/RANGE/PCT	52.6/ 1/ .9557	32.1/ 1/ .5599	44.0/ 1/ .7859	32.4/ 1/ .5655
CO2 BCKGRD METER/RANGE/PCT	3.0/ 1/ .0492	3.0/ 1/ .0492	3.1/ 1/ .0508	3.1/ 1/ .0508
NOX SAMPLE METER/RANGE/PPM (BAG) (D)	5.1/ 1/ 1.28	1.3/ 1/ .33	3.5/ 1/ .88	1.3/ 1/ .33
NOX BCKGRD METER/RANGE/PPM	.6/ 1/ .15	.5/ 1/ .13	.5/ 1/ .13	.6/ 1/ .15
CH4 SAMPLE PPM (1.180)	2.73	1.96	1.98	1.96
CH4 BCKGRD PPM	2.03	2.02	1.97	2.02
DILUTION FACTOR	14.06	24.06	17.14	23.82
HC CONCENTRATION PPM	9.22	.13	.28	.13
CO CONCENTRATION PPM	14.78	.11	.02	.01
CO2 CONCENTRATION PCT	.9101	.5128	.7381	.5168
NOX CONCENTRATION PPM	1.14	.21	.76	.18
CH4 CONCENTRATION PPM	.84	.02	.13	.02
NMHC CONCENTRATION PPM	8.23	.11	.13	.11
HC MASS GRAMS	.427	.010	.013	.010
CO MASS GRAMS	1.383	.018	.002	.002
CO2 MASS GRAMS	1339.34	1297.44	1091.54	1307.26
NOX MASS GRAMS	.182	.056	.122	.050
PM MASS MILLIGRAMS	9.5	7.9	5.8	7.7
CH4 MASS GRAMS	.045	.002	.007	.002
NMHC MASS GRAMS (FID)	.381	.009	.006	.009
FUEL MASS KG	.423	.409	.344	.412
FUEL ECONOMY MPG (L/100KM)	23.91 (9.84)	26.33 (8.93)	29.17 (8.06)	26.27 (8.96)

4-BAG COMPOSITE RESULTS

HC	G/MI	.027	CH4	G/MI	.003
CO	G/MI	.081	NMHC	G/MI	.024
NOX	G/MI	.027			
PM	MG/MI	2.1			
FUEL ECONOMY MPG (L/100KM)		26.52 (8.87)			

SOUTHWEST RESEARCH INSTITUTE - DEPARTMENT OF EMISSIONS RESEARCH

COMPUTER PROGRAM LDT 3.2-R 4-BAG EPA FTP VEHICLE EMISSION RESULTS PROJECT NO. 03-15812-00

VEHICLE NUMBER 7329	TEST RFABASECAT-T6	GASOLINE +EPATIER EM-7578-F
VEHICLE MODEL 2011 TOYOTA CAMRY	DATE 9/15/2010 RUN	FUEL DENSITY 6.200 LB/GAL
ENGINE 2.5 L (153 CID)-4	DYNO 3 BAG CART 2	H .134 C .866 O .000 X .000
TRANSMISSION A	ACTUAL ROAD LOAD 11.30 HP (8.43 KW)	FTP
ODOMETER 4197 MILES (6752 KM)	TEST WEIGHT 3625 LBS (1643 KG)	

BAROMETER 29.09 IN HG (738.9 MM HG) DRY BULB TEMPERATURE 71.1°F (21.7°C) NOX HUMIDITY C.F. 1.050

RELATIVE HUMIDITY 72.3 PCT.

BAG NUMBER	1	2	3	4
BAG DESCRIPTION	COLD TRANSIENT (0-505 SEC.)	STABILIZED (505-1372 SEC.)	HOT TRANSIENT (0- 505 SEC.)	HOT STABILIZED (505-1372 SEC.)
RUN TIME SECONDS	504.4	868.9	505.3	868.0
DRY/WET CORRECTION FACTOR, SAMP/BACK	.972/.981	.976/.981	.974/.981	.976/.981
MEASURED DISTANCE MILES (KM)	3.56 (5.73)	3.82 (6.15)	3.57 (5.74)	3.83 (6.16)
BLOWER FLOW RATE SCFM (SCMM)	292.2 (8.27)	292.1 (8.27)	293.2 (8.30)	292.5 (8.28)
20X20 FLOW RATE SCFM (SCMM)	45.7 (1.29)	45.5 (1.29)	46.7 (1.32)	46.6 (1.32)
GAS METER FLOW RATE SCFM (SCMM)	.86 (.02)	.82 (.02)	.87 (.02)	.82 (.02)
TOTAL FLOW SCF (SCM)	2847. (80.6)	4900. (138.8)	2870. (81.3)	4918. (139.3)
HC SAMPLE METER/RANGE/PPM (BAG)	16.3/ 2/ 16.30	3.3/ 2/ 3.30	3.6/ 2/ 3.60	3.5/ 2/ 3.50
HC BCKGRD METER/RANGE/PPM	3.3/ 2/ 3.30	3.3/ 2/ 3.30	3.5/ 2/ 3.50	3.5/ 2/ 3.50
CO SAMPLE METER/RANGE/PPM	19.2/ 12/ 18.54	.2/ 12/ .20	.4/ 12/ .39	.4/ 12/ .39
CO BCKGRD METER/RANGE/PPM	.3/ 12/ .30	.3/ 12/ .30	.4/ 12/ .39	.4/ 12/ .39
CO2 SAMPLE METER/RANGE/PCT	51.2/ 1/ .9277	31.8/ 1/ .5543	42.2/ 1/ .7511	31.2/ 1/ .5432
CO2 BCKGRD METER/RANGE/PCT	2.7/ 1/ .0442	2.6/ 1/ .0426	2.8/ 1/ .0459	2.9/ 1/ .0475
NOX SAMPLE METER/RANGE/PPM (BAG) (D)	3.4/ 1/ .85	.8/ 1/ .20	4.4/ 1/ 1.10	.2/ 1/ .05
NOX BCKGRD METER/RANGE/PPM	.1/ 1/ .03	.1/ 1/ .03	.2/ 1/ .05	.2/ 1/ .05
CH4 SAMPLE PPM (1.180)	2.47	2.03	2.53	2.03
CH4 BCKGRD PPM	2.01	1.99	2.01	1.99
DILUTION FACTOR	14.48	24.30	17.94	24.80
HC CONCENTRATION PPM	13.23	.14	.30	.14
CO CONCENTRATION PPM	17.51	-.09	.02	.01
CO2 CONCENTRATION PCT	.8865	.5135	.7078	.4976
NOX CONCENTRATION PPM	.83	.18	1.05	.00
CH4 CONCENTRATION PPM	.60	.12	.64	.12
NMHC CONCENTRATION PPM	12.53	-.01	-.46	-.00
HC MASS GRAMS	.615	.011	.014	.011
CO MASS GRAMS	1.643	.000	.001	.002
CO2 MASS GRAMS	1308.90	1304.69	1053.24	1268.92
NOX MASS GRAMS	.134	.049	.172	.001
PM MASS MILLIGRAMS	4.9	7.2	5.5	.9
CH4 MASS GRAMS	.032	.012	.034	.011
NMHC MASS GRAMS (FID)	.582	.000	.000	.000
FUEL MASS KG	.414	.411	.332	.400
FUEL ECONOMY MPG (L/100KM)	24.17 (9.73)	26.11 (9.01)	30.23 (7.78)	26.92 (8.74)

4-BAG COMPOSITE RESULTS

HC	G/MI	.038	CH4	G/MI	.006
CO	G/MI	.096	NMHC	G/MI	.034
NOX	G/MI	.024			
PM	MG/MI	1.2			
FUEL ECONOMY MPG (L/100KM)		27.01 (8.71)			

SOUTHWEST RESEARCH INSTITUTE - DEPARTMENT OF EMISSIONS RESEARCH

COMPUTER PROGRAM LDT 3.2-R 4-BAG EPA FTP VEHICLE EMISSION RESULTS PROJECT NO. 03-15812-00

VEHICLE NUMBER	7329	TEST RFABASE-T1	GASOLINE +EPATIER EM-7578-F
VEHICLE MODEL	2011 TOYOTA CAMRY	DATE 9/16/2010 RUN	FUEL DENSITY 6.200 LB/GAL
ENGINE	2.5 L (153 CID)-4	DYNO 3 BAG CART 2	H .134 C .866 O .000 X .000
TRANSMISSION	A	ACTUAL ROAD LOAD 11.30 HP (8.43 KW)	FTP
ODOMETER	4245 MILES (6830 KM)	TEST WEIGHT 3625 LBS (1643 KG)	

BAROMETER 29.08 IN HG (738.6 MM HG) DRY BULB TEMPERATURE 72.4°F (22.4°C) NOX HUMIDITY C.F. 1.039

RELATIVE HUMIDITY 67.6 PCT.

BAG NUMBER	1	2	3	4
BAG DESCRIPTION	COLD TRANSIENT (0-505 SEC.)	STABILIZED (505-1372 SEC.)	HOT TRANSIENT (0- 505 SEC.)	HOT STABILIZED (505-1372 SEC.)
RUN TIME SECONDS	505.2	867.6	504.9	868.4
DRY/WET CORRECTION FACTOR, SAMP/BACK	.973/.981	.976/.981	.974/.981	.976/.981
MEASURED DISTANCE MILES (KM)	3.57 (5.74)	3.84 (6.18)	3.56 (5.73)	3.82 (6.15)
BLOWER FLOW RATE SCFM (SCMM)	292.8 (8.29)	293.7 (8.32)	292.5 (8.28)	292.2 (8.28)
20X20 FLOW RATE SCFM (SCMM)	46.2 (1.31)	46.0 (1.30)	46.7 (1.32)	46.6 (1.32)
GAS METER FLOW RATE SCFM (SCMM)	.87 (.02)	.85 (.02)	.86 (.02)	.82 (.02)
TOTAL FLOW SCF (SCM)	2861. (81.0)	4924. (139.4)	2862. (81.0)	4915. (139.2)
HC SAMPLE METER/RANGE/PPM (BAG)	12.0/ 3/ 120.46	79.8/ 2/ 79.82	93.2/ 2/ 93.22	77.2/ 2/ 77.22
HC BCKGRD METER/RANGE/PPM	.4/ 3/ 4.02	3.5/ 2/ 3.50	3.7/ 2/ 3.70	3.9/ 2/ 3.90
CO SAMPLE METER/RANGE/PPM	43.5/ 1/ 417.64	62.3/ 14/ 292.57	74.8/ 14/ 359.52	59.9/ 14/ 280.04
CO BCKGRD METER/RANGE/PPM	.1/ 1/ .93	.1/ 14/ .42	.1/ 14/ .42	.2/ 14/ .83
CO2 SAMPLE METER/RANGE/PCT	49.9/ 1/ .9018	31.0/ 1/ .5395	40.7/ 1/ .7222	29.3/ 1/ .5082
CO2 BCKGRD METER/RANGE/PCT	3.0/ 1/ .0492	2.8/ 1/ .0459	2.9/ 1/ .0475	3.0/ 1/ .0492
NOX SAMPLE METER/RANGE/PPM (BAG) (D)	37.5/ 3/ 93.75	46.4/ 2/ 46.62	90.9/ 2/ 91.00	48.2/ 2/ 48.40
NOX BCKGRD METER/RANGE/PPM	.1/ 3/ .25	.2/ 2/ .21	.1/ 2/ .10	.2/ 2/ .21
CH4 SAMPLE PPM (1.180)	5.23	4.19	4.41	4.19
CH4 BCKGRD PPM	2.10	2.09	2.07	2.09
DILUTION FACTOR	14.13	23.41	17.59	24.82
HC CONCENTRATION PPM	116.73	76.47	89.73	73.47
CO CONCENTRATION PPM	400.44	282.76	346.29	270.40
CO2 CONCENTRATION PCT	.8561	.4956	.6774	.4610
NOX CONCENTRATION PPM	93.51	46.42	90.90	48.21
CH4 CONCENTRATION PPM	3.28	2.19	2.45	2.18
NMHC CONCENTRATION PPM	112.85	73.89	86.84	70.90
HC MASS GRAMS	5.453	6.148	4.193	5.896
CO MASS GRAMS	37.774	45.902	32.673	43.818
CO2 MASS GRAMS	1270.05	1265.26	1005.18	1174.76
NOX MASS GRAMS	15.057	12.863	14.640	13.334
PM MASS MILLIGRAMS	14.5	8.2	8.3	7.7
CH4 MASS GRAMS	.177	.203	.133	.202
NMHC MASS GRAMS (FID)	5.272	5.940	4.058	5.690
FUEL MASS KG	.425	.428	.337	.398
FUEL ECONOMY MPG (L/100KM)	23.65 (9.95)	25.24 (9.32)	29.68 (7.93)	26.99 (8.72)

4-BAG COMPOSITE RESULTS

HC	G/MI	1.452	CH4	G/MI	.048
CO	G/MI	10.764	NMHC	G/MI	1.404
NOX	G/MI	3.781			
PM	MG/MI	2.6			
FUEL ECONOMY MPG (L/100KM)		26.60 (8.84)			

SOUTHWEST RESEARCH INSTITUTE - DEPARTMENT OF EMISSIONS RESEARCH

COMPUTER PROGRAM LDT 3.2-R 4-BAG EPA FTP VEHICLE EMISSION RESULTS PROJECT NO. 03-15812-00

VEHICLE NUMBER	7329	TEST RFABASE-T2	GASOLINE +EPATIER EM-7578-F
VEHICLE MODEL	2011 TOYOTA CAMRY	DATE 9/17/2010 RUN	FUEL DENSITY 6.200 LB/GAL
ENGINE	2.5 L (153 CID)-4	DYNO 3 BAG CART 2	H .134 C .866 O .000 X .000
TRANSMISSION	A	ACTUAL ROAD LOAD 11.30 HP (8.43 KW)	FTP
ODOMETER	4259 MILES (6852 KM)	TEST WEIGHT 3625 LBS (1643 KG)	

BAROMETER 29.06 IN HG (738.1 MM HG) DRY BULB TEMPERATURE 71.0°F (21.7°C) NOX HUMIDITY C.F. 1.026

RELATIVE HUMIDITY 68.6 PCT.

BAG NUMBER	1	2	3	4
BAG DESCRIPTION	COLD TRANSIENT (0-505 SEC.)	STABILIZED (505-1372 SEC.)	HOT TRANSIENT (0- 505 SEC.)	HOT STABILIZED (505-1372 SEC.)
RUN TIME SECONDS	505.5	867.8	505.1	868.4
DRY/WET CORRECTION FACTOR, SAMP/BACK	.973/.982	.977/.982	.975/.982	.977/.982
MEASURED DISTANCE MILES (KM)	3.57 (5.74)	3.83 (6.16)	3.57 (5.74)	3.83 (6.16)
BLOWER FLOW RATE SCFM (SCMM)	292.4 (8.28)	292.7 (8.29)	291.9 (8.27)	293.5 (8.31)
20X20 FLOW RATE SCFM (SCMM)	46.2 (1.31)	46.1 (1.30)	46.6 (1.32)	46.4 (1.31)
GAS METER FLOW RATE SCFM (SCMM)	.87 (.02)	.83 (.02)	.87 (.02)	.82 (.02)
TOTAL FLOW SCF (SCM)	2860. (81.0)	4912. (139.1)	2857. (80.9)	4932. (139.7)
HC SAMPLE METER/RANGE/PPM (BAG)	12.9/ 3/ 129.49	77.4/ 2/ 77.42	93.7/ 2/ 93.72	75.0/ 2/ 75.02
HC BCKGRD METER/RANGE/PPM	.3/ 3/ 3.01	3.3/ 2/ 3.30	3.4/ 2/ 3.40	3.0/ 2/ 3.00
CO SAMPLE METER/RANGE/PPM	84.9/ 14/ 415.64	61.2/ 14/ 286.81	74.4/ 14/ 357.33	59.1/ 14/ 275.88
CO BCKGRD METER/RANGE/PPM	.1/ 4/ 7.40	.1/ 14/ .42	.1/ 14/ .42	.2/ 14/ .83
CO2 SAMPLE METER/RANGE/PCT	91.8/ 11/ .8994	67.8/ 11/ .5533	83.3/ 11/ .7651	65.8/ 11/ .5290
CO2 BCKGRD METER/RANGE/PCT	8.9/ 1/ .1478	8.8/ 11/ .0508	9.3/ 11/ .0537	9.3/ 11/ .0537
NOX SAMPLE METER/RANGE/PPM (BAG) (D)	37.8/ 3/ 94.50	46.5/ 2/ 46.72	94.1/ 2/ 94.23	47.6/ 2/ 47.81
NOX BCKGRD METER/RANGE/PPM	.0/ 3/ .00	.0/ 2/ .00	.1/ 2/ .10	.1/ 2/ .10
CH4 SAMPLE PPM (1.180)	5.30	3.95	4.26	3.95
CH4 BCKGRD PPM	1.95	1.96	1.95	1.96
DILUTION FACTOR	14.15	22.90	16.66	23.93
HC CONCENTRATION PPM	126.69	74.26	90.53	72.14
CO CONCENTRATION PPM	392.51	277.01	343.77	266.18
CO2 CONCENTRATION PCT	.7620	.5047	.7145	.4775
NOX CONCENTRATION PPM	94.50	46.72	94.13	47.71
CH4 CONCENTRATION PPM	3.48	2.08	2.43	2.07
NMHC CONCENTRATION PPM	122.58	71.81	87.66	69.70
HC MASS GRAMS	5.916	5.957	4.223	5.810
CO MASS GRAMS	37.009	44.866	32.379	43.282
CO2 MASS GRAMS	1129.97	1285.56	1058.40	1221.07
NOX MASS GRAMS	15.019	12.755	14.945	13.078
PM MASS MILLIGRAMS	20.1	15.4	7.3	8.6
CH4 MASS GRAMS	.188	.193	.131	.193
NMHC MASS GRAMS (FID)	5.724	5.760	4.089	5.613
FUEL MASS KG	.381	.434	.354	.412
FUEL ECONOMY MPG (L/100KM)	26.38 (8.92)	24.85 (9.47)	28.36 (8.29)	26.13 (9.00)

4-BAG COMPOSITE RESULTS

HC	G/MI	1.463	CH4	G/MI	.047
CO	G/MI	10.585	NMHC	G/MI	1.415
NOX	G/MI	3.772			
PM	MG/MI	3.3			
FUEL ECONOMY MPG (L/100KM)		26.47 (8.89)			

SOUTHWEST RESEARCH INSTITUTE - DEPARTMENT OF EMISSIONS RESEARCH

COMPUTER PROGRAM LDT 3.2-R 4-BAG EPA FTP VEHICLE EMISSION RESULTS PROJECT NO. 03-15812-00

VEHICLE NUMBER	7329	TEST RFABASE-T4	GASOLINE +EPATIER EM-7578-F
VEHICLE MODEL	2011 TOYOTA CAMRY	DATE 9/21/2010 RUN	FUEL DENSITY 6.200 LB/GAL
ENGINE	2.5 L (153 CID)-4	DYNO 3 BAG CART 2	H .134 C .866 O .000 X .000
TRANSMISSION	A	ACTUAL ROAD LOAD 11.30 HP (8.43 KW)	FTP
ODOMETER	4312 MILES (6938 KM)	TEST WEIGHT 3625 LBS (1643 KG)	

BAROMETER 29.11 IN HG (739.4 MM HG) DRY BULB TEMPERATURE 71.3°F (21.8°C) NOX HUMIDITY C.F. 1.075

RELATIVE HUMIDITY 75.9 PCT.

BAG NUMBER	1	2	3	4
BAG DESCRIPTION	COLD TRANSIENT (0-505 SEC.)	STABILIZED (505-1372 SEC.)	HOT TRANSIENT (0- 505 SEC.)	HOT STABILIZED (505-1372 SEC.)
RUN TIME SECONDS	505.4	868.0	505.1	868.0
DRY/WET CORRECTION FACTOR, SAMP/BACK	.971/.980	.975/.980	.973/.980	.975/.980
MEASURED DISTANCE MILES (KM)	3.57 (5.74)	3.85 (6.19)	3.57 (5.74)	3.84 (6.18)
BLOWER FLOW RATE SCFM (SCMM)	291.4 (8.25)	293.4 (8.31)	293.6 (8.31)	294.9 (8.35)
20X20 FLOW RATE SCFM (SCMM)	46.5 (1.32)	46.4 (1.31)	47.0 (1.33)	46.9 (1.33)
GAS METER FLOW RATE SCFM (SCMM)	.85 (.02)	.83 (.02)	.87 (.02)	.83 (.02)
TOTAL FLOW SCF (SCM)	2854. (80.8)	4928. (139.6)	2874. (81.4)	4958. (140.4)
HC SAMPLE METER/RANGE/PPM (BAG)	13.0/ 3/ 130.49	80.7/ 2/ 80.72	9.7/ 3/ 97.37	82.0/ 2/ 82.02
HC BCKGRD METER/RANGE/PPM	.3/ 3/ 3.01	3.5/ 2/ 3.50	.4/ 3/ 4.02	3.5/ 2/ 3.50
CO SAMPLE METER/RANGE/PPM	49.6/ 1/ 479.15	67.8/ 14/ 321.68	92.4/ 14/ 458.45	71.9/ 14/ 343.74
CO BCKGRD METER/RANGE/PPM	.0/ 1/ .00	.1/ 14/ .42	.1/ 14/ .42	.1/ 14/ .42
CO2 SAMPLE METER/RANGE/PCT	92.5/ 11/ .9110	66.9/ 11/ .5423	82.7/ 11/ .7560	65.6/ 11/ .5266
CO2 BCKGRD METER/RANGE/PCT	9.6/ 11/ .0555	9.6/ 11/ .0555	9.5/ 11/ .0549	9.4/ 11/ .0543
NOX SAMPLE METER/RANGE/PPM (BAG) (D)	92.4/ 2/ 92.51	43.6/ 2/ 43.84	88.1/ 2/ 88.18	46.2/ 2/ 46.42
NOX BCKGRD METER/RANGE/PPM	.2/ 2/ .21	.3/ 2/ .31	.2/ 2/ .21	.3/ 2/ .31
CH4 SAMPLE PPM (1.180)	5.34	4.10	4.48	4.10
CH4 BCKGRD PPM	2.00	2.01	2.06	2.01
DILUTION FACTOR	13.90	23.19	16.64	23.73
HC CONCENTRATION PPM	127.70	77.37	93.60	78.67
CO CONCENTRATION PPM	459.01	310.06	440.17	331.44
CO2 CONCENTRATION PCT	.8595	.4891	.7044	.4746
NOX CONCENTRATION PPM	92.32	43.54	87.99	46.12
CH4 CONCENTRATION PPM	3.48	2.18	2.55	2.18
NMHC CONCENTRATION PPM	123.59	74.80	90.59	76.10
HC MASS GRAMS	5.950	6.225	4.392	6.368
CO MASS GRAMS	43.185	50.372	41.711	54.177
CO2 MASS GRAMS	1271.66	1249.64	1049.75	1219.89
NOX MASS GRAMS	15.342	12.495	14.727	13.317
PM MASS MILLIGRAMS	19.8	11.1	11.4	9.9
CH4 MASS GRAMS	.188	.203	.138	.204
NMHC MASS GRAMS (FID)	5.759	6.018	4.251	6.160
FUEL MASS KG	.428	.425	.356	.418
FUEL ECONOMY MPG (L/100KM)	23.44 (10.04)	25.46 (9.24)	28.20 (8.34)	25.85 (9.10)

4-BAG COMPOSITE RESULTS

HC	G/MI	1.533	CH4	G/MI	.049
CO	G/MI	12.798	NMHC	G/MI	1.483
NOX	G/MI	3.770			
PM	MG/MI	3.4			
FUEL ECONOMY MPG (L/100KM)		25.86 (9.10)			

SOUTHWEST RESEARCH INSTITUTE - DEPARTMENT OF EMISSIONS RESEARCH

COMPUTER PROGRAM LDT 3.2-R 4-BAG EPA FTP VEHICLE EMISSION RESULTS PROJECT NO. 03-15812-00

VEHICLE NUMBER	7329	TEST RFAE10CAT-T1	GASOLINE EPA TIER EM-7579-F
VEHICLE MODEL	2011 TOYOTA CAMRY	DATE 10/ 5/2010 RUN	FUEL DENSITY 6.250 LB/GAL
ENGINE	2.5 L (153 CID)-4	DYNO 3 BAG CART 2	H .134 C .829 O .037 X .000
TRANSMISSION	A	ACTUAL ROAD LOAD 11.30 HP (8.43 KW)	FTP
ODOMETER	8348 MILES (13431 KM)	TEST WEIGHT 3625 LBS (1643 KG)	

BAROMETER 29.37 IN HG (746.0 MM HG) DRY BULB TEMPERATURE 69.5°F (20.8°C) NOX HUMIDITY C.F. 1.010

RELATIVE HUMIDITY 70.2 PCT.

BAG NUMBER	1	2	3	4
BAG DESCRIPTION	COLD TRANSIENT (0-505 SEC.)	STABILIZED (505-1372 SEC.)	HOT TRANSIENT (0- 505 SEC.)	HOT STABILIZED (505-1372 SEC.)
RUN TIME SECONDS	505.2	868.4	505.1	868.0
DRY/WET CORRECTION FACTOR, SAMP/BACK	.974/.982	.977/.982	.975/.982	.977/.982
MEASURED DISTANCE MILES (KM)	3.57 (5.74)	3.84 (6.18)	3.58 (5.76)	3.85 (6.19)
BLOWER FLOW RATE SCFM (SCMM)	291.3 (8.25)	292.4 (8.28)	294.3 (8.34)	291.3 (8.25)
20X20 FLOW RATE SCFM (SCMM)	47.2 (1.34)	47.0 (1.33)	48.0 (1.36)	47.5 (1.35)
GAS METER FLOW RATE SCFM (SCMM)	.87 (.02)	.82 (.02)	.85 (.02)	.83 (.02)
TOTAL FLOW SCF (SCM)	2857. (80.9)	4926. (139.5)	2889. (81.8)	4914. (139.2)
HC SAMPLE METER/RANGE/PPM (BAG)	9.8/ 2/ 9.80	3.6/ 2/ 3.60	3.7/ 2/ 3.70	3.2/ 2/ 3.20
HC BCKGRD METER/RANGE/PPM	3.6/ 2/ 3.60	3.6/ 2/ 3.60	3.6/ 2/ 3.60	3.3/ 2/ 3.30
CO SAMPLE METER/RANGE/PPM	14.5/ 12/ 14.04	.5/ 12/ .49	.6/ 12/ .59	.3/ 12/ .30
CO BCKGRD METER/RANGE/PPM	.6/ 12/ .59	.4/ 12/ .39	.5/ 12/ .49	.2/ 12/ .20
CO2 SAMPLE METER/RANGE/PCT	51.4/ 1/ .9317	32.4/ 1/ .5655	42.7/ 1/ .7607	31.5/ 1/ .5488
CO2 BCKGRD METER/RANGE/PCT	2.8/ 1/ .0459	2.7/ 1/ .0442	2.8/ 1/ .0459	3.0/ 1/ .0492
NOX SAMPLE METER/RANGE/PPM (BAG) (D)	3.7/ 1/ .93	.8/ 1/ .20	1.3/ 1/ .33	1.0/ 1/ .25
NOX BCKGRD METER/RANGE/PPM	.0/ 1/ .00	.0/ 1/ .00	.2/ 1/ .05	.2/ 1/ .05
CH4 SAMPLE PPM (1.170)	3.06	2.33	2.38	2.33
CH4 BCKGRD PPM	2.39	2.41	2.33	2.41
DILUTION FACTOR	14.34	23.67	17.59	24.39
HC CONCENTRATION PPM	6.45	.15	.30	.04
CO CONCENTRATION PPM	12.93	.11	.11	.10
CO2 CONCENTRATION PCT	.8890	.5231	.7175	.5016
NOX CONCENTRATION PPM	.93	.20	.28	.20
CH4 CONCENTRATION PPM	.84	.02	.18	.02
NMHC CONCENTRATION PPM	5.47	.13	.10	.01
HC MASS GRAMS	.314	.013	.015	.003
CO MASS GRAMS	1.218	.017	.011	.016
CO2 MASS GRAMS	1317.19	1336.01	1074.69	1278.15
NOX MASS GRAMS	.145	.054	.044	.054
PM MASS MILLIGRAMS	6.0	3.0	3.1	2.7
CH4 MASS GRAMS	.045	.002	.010	.002
NMHC MASS GRAMS (FID)	.267	.011	.005	.001
FUEL MASS KG	.435	.440	.354	.421
FUEL ECONOMY MPG (L/100KM)	23.29 (10.10)	24.75 (9.50)	28.68 (8.20)	25.94 (9.07)

4-BAG COMPOSITE RESULTS

HC	G/MI	.020	CH4	G/MI	.004
CO	G/MI	.074	NMHC	G/MI	.017
NOX	G/MI	.019			
PM	MG/MI	1.0			
FUEL ECONOMY MPG (L/100KM)		25.83 (9.11)			

SOUTHWEST RESEARCH INSTITUTE - DEPARTMENT OF EMISSIONS RESEARCH

COMPUTER PROGRAM LDT 3.2-R 4-BAG EPA FTP VEHICLE EMISSION RESULTS PROJECT NO. 03-15812-00

VEHICLE NUMBER	7329	TEST RFAE10CAT-T2	GASOLINE EPA TIER EM-7579-F
VEHICLE MODEL	2011 TOYOTA CAMRY	DATE 10/ 6/2010 RUN	FUEL DENSITY 6.250 LB/GAL
ENGINE	2.5 L (153 CID)-4	DYNO 3 BAG CART 2	H .134 C .829 O .037 X .000
TRANSMISSION	A	ACTUAL ROAD LOAD 11.30 HP (8.43 KW)	FTP
ODOMETER	8362 MILES (13454 KM)	TEST WEIGHT 3625 LBS (1643 KG)	

BAROMETER 29.43 IN HG (747.5 MM HG) DRY BULB TEMPERATURE 70.9°F (21.6°C) NOX HUMIDITY C.F. .998

RELATIVE HUMIDITY 64.6 PCT.

BAG NUMBER	1	2	3	4
BAG DESCRIPTION	COLD TRANSIENT (0-505 SEC.)	STABILIZED (505-1372 SEC.)	HOT TRANSIENT (0- 505 SEC.)	HOT STABILIZED (505-1372 SEC.)
RUN TIME SECONDS	505.1	868.1	504.5	868.2
DRY/WET CORRECTION FACTOR, SAMP/BACK	.974/.983	.978/.983	.976/.983	.978/.983
MEASURED DISTANCE MILES (KM)	3.58 (5.76)	3.85 (6.19)	3.57 (5.74)	3.86 (6.21)
BLOWER FLOW RATE SCFM (SCMM)	293.6 (8.31)	295.4 (8.37)	291.5 (8.26)	293.1 (8.30)
20X20 FLOW RATE SCFM (SCMM)	46.8 (1.33)	46.8 (1.32)	47.6 (1.35)	47.6 (1.35)
GAS METER FLOW RATE SCFM (SCMM)	.88 (.02)	.84 (.02)	.87 (.02)	.83 (.02)
TOTAL FLOW SCF (SCM)	2873. (81.4)	4962. (140.5)	2859. (81.0)	4943. (140.0)
HC SAMPLE METER/RANGE/PPM (BAG)	7.0/ 2/ 7.00	3.3/ 2/ 3.30	3.4/ 2/ 3.40	3.2/ 2/ 3.20
HC BCKGRD METER/RANGE/PPM	3.3/ 2/ 3.30	3.3/ 2/ 3.30	3.3/ 2/ 3.30	3.2/ 2/ 3.20
CO SAMPLE METER/RANGE/PPM	11.1/ 12/ 10.78	.6/ 12/ .59	.3/ 12/ .30	.3/ 12/ .30
CO BCKGRD METER/RANGE/PPM	.5/ 12/ .49	.3/ 12/ .30	.3/ 12/ .30	.3/ 12/ .30
CO2 SAMPLE METER/RANGE/PCT	51.5/ 1/ .9337	32.5/ 1/ .5673	42.9/ 1/ .7646	32.0/ 1/ .5580
CO2 BCKGRD METER/RANGE/PCT	2.9/ 1/ .0475	2.9/ 1/ .0475	2.9/ 1/ .0475	2.9/ 1/ .0475
NOX SAMPLE METER/RANGE/PPM (BAG) (D)	4.5/ 1/ 1.13	.8/ 1/ .20	3.1/ 1/ .78	.9/ 1/ .23
NOX BCKGRD METER/RANGE/PPM	.1/ 1/ .03	.0/ 1/ .00	.1/ 1/ .03	.0/ 1/ .00
CH4 SAMPLE PPM (1.170)	2.62	1.99	2.12	1.99
CH4 BCKGRD PPM	2.07	2.04	2.02	2.04
DILUTION FACTOR	14.32	23.59	17.51	23.98
HC CONCENTRATION PPM	3.93	.14	.29	.13
CO CONCENTRATION PPM	9.91	.29	.01	.01
CO2 CONCENTRATION PCT	.8895	.5218	.7198	.5125
NOX CONCENTRATION PPM	1.10	.20	.75	.23
CH4 CONCENTRATION PPM	.69	.04	.22	.04
NMHC CONCENTRATION PPM	3.12	.09	.03	.09
HC MASS GRAMS	.193	.012	.014	.011
CO MASS GRAMS	.939	.048	.001	.001
CO2 MASS GRAMS	1325.09	1342.65	1066.93	1313.44
NOX MASS GRAMS	.171	.054	.116	.060
PM MASS MILLIGRAMS	7.0	2.5	5.5	3.8
CH4 MASS GRAMS	.037	.004	.012	.004
NMHC MASS GRAMS (FID)	.153	.008	.001	.007
FUEL MASS KG	.437	.442	.351	.432
FUEL ECONOMY MPG (L/100KM)	23.23 (10.13)	24.69 (9.53)	28.81 (8.16)	25.31 (9.30)

4-BAG COMPOSITE RESULTS

HC	G/MI	.014	CH4	G/MI	.004
CO	G/MI	.057	NMHC	G/MI	.010
NOX	G/MI	.027			
PM	MG/MI	1.3			
FUEL ECONOMY MPG (L/100KM)		25.63 (9.18)			

SOUTHWEST RESEARCH INSTITUTE - DEPARTMENT OF EMISSIONS RESEARCH

COMPUTER PROGRAM LDT 3.2-R 4-BAG EPA FTP VEHICLE EMISSION RESULTS PROJECT NO. 03-15812-00

VEHICLE NUMBER	7329	TEST RFAE10CAT-T3	GASOLINE EPA TIER EM-7579-F
VEHICLE MODEL	2011 TOYOTA CAMRY	DATE 10/ 7/2010 RUN	FUEL DENSITY 6.250 LB/GAL
ENGINE	2.5 L (153 CID)-4	DYNO 3 BAG CART 2	H .134 C .829 O .037 X .000
TRANSMISSION	A	ACTUAL ROAD LOAD 11.30 HP (8.43 KW)	FTP
ODOMETER	8376 MILES (13476 KM)	TEST WEIGHT 3625 LBS (1643 KG)	

BAROMETER 29.37 IN HG (746.0 MM HG) DRY BULB TEMPERATURE 72.0°F (22.2°C) NOX HUMIDITY C.F. 1.038
RELATIVE HUMIDITY 68.9 PCT.

BAG NUMBER	1	2	3	4
BAG DESCRIPTION	COLD TRANSIENT (0-505 SEC.)	STABILIZED (505-1372 SEC.)	HOT TRANSIENT (0- 505 SEC.)	HOT STABILIZED (505-1372 SEC.)
RUN TIME SECONDS	505.1	867.7	504.9	867.7
DRY/WET CORRECTION FACTOR, SAMP/BACK	.972/.981	.976/.981	.974/.981	.976/.981
MEASURED DISTANCE MILES (KM)	3.58 (5.76)	3.86 (6.21)	3.58 (5.76)	3.86 (6.21)
BLOWER FLOW RATE SCFM (SCMM)	291.2 (8.25)	292.0 (8.27)	291.0 (8.24)	292.0 (8.27)
20X20 FLOW RATE SCFM (SCMM)	46.8 (1.32)	46.7 (1.32)	47.5 (1.34)	47.4 (1.34)
GAS METER FLOW RATE SCFM (SCMM)	.87 (.02)	.82 (.02)	.87 (.02)	.82 (.02)
TOTAL FLOW SCF (SCM)	2853. (80.8)	4910. (139.1)	2856. (80.9)	4919. (139.3)
HC SAMPLE METER/RANGE/PPM (BAG)	7.6/ 2/ 7.60	3.4/ 2/ 3.40	3.4/ 2/ 3.40	3.5/ 2/ 3.50
HC BCKGRD METER/RANGE/PPM	3.3/ 2/ 3.30	3.4/ 2/ 3.40	3.4/ 2/ 3.40	3.5/ 2/ 3.50
CO SAMPLE METER/RANGE/PPM	12.3/ 12/ 11.93	.6/ 12/ .59	.5/ 12/ .49	.4/ 12/ .39
CO BCKGRD METER/RANGE/PPM	.5/ 12/ .49	.6/ 12/ .59	.5/ 12/ .49	.3/ 12/ .30
CO2 SAMPLE METER/RANGE/PCT	51.4/ 1/ .9317	32.4/ 1/ .5655	42.6/ 1/ .7588	31.6/ 1/ .5506
CO2 BCKGRD METER/RANGE/PCT	3.1/ 1/ .0508	3.0/ 1/ .0492	2.9/ 1/ .0475	2.9/ 1/ .0475
NOX SAMPLE METER/RANGE/PPM (BAG) (D)	4.8/ 1/ 1.20	.7/ 1/ .18	1.4/ 1/ .35	.9/ 1/ .23
NOX BCKGRD METER/RANGE/PPM	.1/ 1/ .03	.2/ 1/ .05	.2/ 1/ .05	.1/ 1/ .03
CH4 SAMPLE PPM (1.170)	2.63	1.99	2.09	1.99
CH4 BCKGRD PPM	2.03	2.04	2.03	2.04
DILUTION FACTOR	14.34	23.67	17.64	24.30
HC CONCENTRATION PPM	4.53	.14	.19	.14
CO CONCENTRATION PPM	11.00	.02	.02	.10
CO2 CONCENTRATION PCT	.8844	.5184	.7140	.5051
NOX CONCENTRATION PPM	1.18	.13	.30	.20
CH4 CONCENTRATION PPM	.74	.03	.17	.03
NMHC CONCENTRATION PPM	3.67	.11	-.00	.11
HC MASS GRAMS	.220	.012	.009	.012
CO MASS GRAMS	1.035	.003	.002	.017
CO2 MASS GRAMS	1308.11	1319.75	1057.15	1288.18
NOX MASS GRAMS	.189	.035	.049	.056
PM MASS MILLIGRAMS	6.9	.6	3.2	.0
CH4 MASS GRAMS	.040	.003	.009	.003
NMHC MASS GRAMS (FID)	.178	.009	.000	.009
FUEL MASS KG	.431	.435	.348	.424
FUEL ECONOMY MPG (L/100KM)	23.53 (10.00)	25.19 (9.34)	29.16 (8.07)	25.80 (9.12)

4-BAG COMPOSITE RESULTS

HC	G/MI	.015	CH4	G/MI	.003
CO	G/MI	.061	NMHC	G/MI	.012
NOX	G/MI	.021			
PM	MG/MI	.7			
FUEL ECONOMY MPG (L/100KM)		26.04 (9.03)			

SOUTHWEST RESEARCH INSTITUTE - DEPARTMENT OF EMISSIONS RESEARCH

COMPUTER PROGRAM LDT 3.2-R 4-BAG EPA FTP VEHICLE EMISSION RESULTS PROJECT NO. 03-15812-00

VEHICLE NUMBER 7329	TEST RFAE10-T1	GASOLINE EPA TIER EM-7579-F
VEHICLE MODEL 2011 TOYOTA CAMRY	DATE 10/ 8/2010 RUN	FUEL DENSITY 6.250 LB/GAL
ENGINE 2.5 L (153 CID)-4	DYNO 3 BAG CART 2	H .134 C .829 O .037 X .000
TRANSMISSION A	ACTUAL ROAD LOAD 11.30 HP (8.43 KW)	FTP
ODOMETER 8423 MILES (13552 KM)	TEST WEIGHT 3625 LBS (1643 KG)	

BAROMETER 29.29 IN HG (744.0 MM HG) DRY BULB TEMPERATURE 71.0°F (21.7°C) NOX HUMIDITY C.F. .999

RELATIVE HUMIDITY 64.4 PCT.

BAG NUMBER	1	2	3	4
BAG DESCRIPTION	COLD TRANSIENT (0-505 SEC.)	STABILIZED (505-1372 SEC.)	HOT TRANSIENT (0- 505 SEC.)	HOT STABILIZED (505-1372 SEC.)
RUN TIME SECONDS	505.0	867.8	505.1	868.2
DRY/WET CORRECTION FACTOR, SAMP/BACK	.974/.983	.978/.983	.976/.983	.978/.983
MEASURED DISTANCE MILES (KM)	3.59 (5.78)	3.86 (6.21)	3.59 (5.78)	3.86 (6.21)
BLOWER FLOW RATE SCFM (SCMM)	290.3 (8.22)	290.1 (8.22)	289.4 (8.20)	291.1 (8.24)
20X20 FLOW RATE SCFM (SCMM)	47.0 (1.33)	46.8 (1.32)	47.4 (1.34)	47.2 (1.34)
GAS METER FLOW RATE SCFM (SCMM)	.85 (.02)	.82 (.02)	.86 (.02)	.81 (.02)
TOTAL FLOW SCF (SCM)	2846. (80.6)	4884. (138.3)	2842. (80.5)	4908. (139.0)
HC SAMPLE METER/RANGE/PPM (BAG)	11.2/ 3/ 112.43	73.5/ 2/ 73.52	86.3/ 2/ 86.32	71.8/ 2/ 71.82
HC BCKGRD METER/RANGE/PPM	.4/ 3/ 4.02	4.1/ 2/ 4.10	4.2/ 2/ 4.20	4.0/ 2/ 4.00
CO SAMPLE METER/RANGE/PPM	48.5/ 1/ 468.01	68.3/ 14/ 324.36	85.2/ 14/ 417.33	67.6/ 14/ 320.62
CO BCKGRD METER/RANGE/PPM	.0/ 1/ .00	.1/ 14/ .42	.1/ 14/ .42	.1/ 14/ .42
CO2 SAMPLE METER/RANGE/PCT	49.6/ 1/ .8959	67.6/ 11/ .5508	82.0/ 11/ .7456	66.2/ 11/ .5338
CO2 BCKGRD METER/RANGE/PCT	2.8/ 1/ .0459	8.8/ 11/ .0508	8.9/ 11/ .0513	8.8/ 11/ .0508
NOX SAMPLE METER/RANGE/PPM (BAG) (D)	39.2/ 3/ 97.99	47.9/ 2/ 48.11	91.7/ 2/ 91.80	49.4/ 2/ 49.59
NOX BCKGRD METER/RANGE/PPM	.1/ 3/ .25	.2/ 2/ .21	.1/ 2/ .10	.1/ 2/ .10
CH4 SAMPLE PPM (1.170)	5.24	4.18	4.48	4.18
CH4 BCKGRD PPM	1.99	2.02	2.05	2.02
DILUTION FACTOR	14.07	22.72	16.86	23.41
HC CONCENTRATION PPM	108.70	69.60	82.37	67.99
CO CONCENTRATION PPM	450.04	313.71	402.16	310.19
CO2 CONCENTRATION PCT	.8533	.5023	.6973	.4852
NOX CONCENTRATION PPM	97.76	47.91	91.71	49.50
CH4 CONCENTRATION PPM	3.39	2.25	2.55	2.25
NMHC CONCENTRATION PPM	104.73	66.96	79.39	65.35
HC MASS GRAMS	5.274	5.795	3.991	5.689
CO MASS GRAMS	42.224	50.513	37.684	50.193
CO2 MASS GRAMS	1258.98	1271.88	1027.63	1234.70
NOX MASS GRAMS	15.054	12.661	14.104	13.145
PM MASS MILLIGRAMS	10.5	6.7	7.2	7.3
CH4 MASS GRAMS	.182	.208	.137	.208
NMHC MASS GRAMS (FID)	5.081	5.575	3.847	5.468
FUEL MASS KG	.442	.451	.362	.438
FUEL ECONOMY MPG (L/100KM)	23.05 (10.21)	24.28 (9.69)	28.13 (8.36)	24.98 (9.42)

4-BAG COMPOSITE RESULTS

HC	G/MI	1.380	CH4	G/MI	.049
CO	G/MI	12.076	NMHC	G/MI	1.328
NOX	G/MI	3.685			
PM	MG/MI	2.1			
FUEL ECONOMY MPG (L/100KM)		25.23 (9.32)			

SOUTHWEST RESEARCH INSTITUTE - DEPARTMENT OF EMISSIONS RESEARCH

COMPUTER PROGRAM LDT 3.2-R 4-BAG EPA FTP VEHICLE EMISSION RESULTS PROJECT NO. 03-15812-00

VEHICLE NUMBER 7329	TEST RFAE10-T2	GASOLINE EPA TIER EM-7579-F
VEHICLE MODEL 2011 TOYOTA CAMRY	DATE 10/11/2010 RUN	FUEL DENSITY 6.250 LB/GAL
ENGINE 2.5 L (153 CID)-4	DYNO 3 BAG CART 2	H .134 C .829 O .037 X .000
TRANSMISSION A	ACTUAL ROAD LOAD 11.30 HP (8.43 KW)	FTP
ODOMETER 8452 MILES (13599 KM)	TEST WEIGHT 3625 LBS (1643 KG)	

BAROMETER 29.04 IN HG (737.6 MM HG) DRY BULB TEMPERATURE 71.2°F (21.8°C) NOX HUMIDITY C.F. 1.050

RELATIVE HUMIDITY 72.0 PCT.

BAG NUMBER	1	2	3	4
BAG DESCRIPTION	COLD TRANSIENT (0-505 SEC.)	STABILIZED (505-1372 SEC.)	HOT TRANSIENT (0- 505 SEC.)	HOT STABILIZED (505-1372 SEC.)
RUN TIME SECONDS	505.2	868.3	505.5	867.7
DRY/WET CORRECTION FACTOR, SAMP/BACK	.972/.981	.975/.981	.973/.981	.975/.981
MEASURED DISTANCE MILES (KM)	3.58 (5.76)	3.86 (6.21)	3.58 (5.76)	3.86 (6.21)
BLOWER FLOW RATE SCFM (SCMM)	289.6 (8.20)	289.3 (8.19)	288.5 (8.17)	289.2 (8.19)
20X20 FLOW RATE SCFM (SCMM)	45.8 (1.30)	46.3 (1.31)	46.6 (1.32)	46.5 (1.32)
GAS METER FLOW RATE SCFM (SCMM)	.84 (.02)	.81 (.02)	.85 (.02)	.81 (.02)
TOTAL FLOW SCF (SCM)	2832. (80.2)	4868. (137.9)	2830. (80.2)	4866. (137.8)
HC SAMPLE METER/RANGE/PPM (BAG)	11.5/ 3/ 115.44	75.7/ 2/ 75.72	90.1/ 2/ 90.12	74.1/ 2/ 74.12
HC BCKGRD METER/RANGE/PPM	.4/ 3/ 4.02	4.3/ 2/ 4.30	4.8/ 2/ 4.80	4.9/ 2/ 4.90
CO SAMPLE METER/RANGE/PPM	56.3/ 1/ 547.55	76.1/ 14/ 366.64	94.1/ 14/ 468.29	73.1/ 14/ 350.25
CO BCKGRD METER/RANGE/PPM	.1/ 1/ .93	.2/ 14/ .83	.1/ 14/ .42	.2/ 14/ .83
CO2 SAMPLE METER/RANGE/PCT	50.1/ 1/ .9058	68.3/ 11/ .5594	83.1/ 11/ .7620	67.0/ 11/ .5435
CO2 BCKGRD METER/RANGE/PCT	3.2/ 1/ .0525	9.5/ 11/ .0549	9.6/ 11/ .0555	9.7/ 11/ .0561
NOX SAMPLE METER/RANGE/PPM (BAG) (D)	37.5/ 3/ 93.75	47.0/ 2/ 47.21	91.2/ 2/ 91.30	48.6/ 2/ 48.80
NOX BCKGRD METER/RANGE/PPM	.0/ 3/ .00	.1/ 2/ .10	.1/ 2/ .10	.1/ 2/ .10
CH4 SAMPLE PPM (1.170)	8.98	2.57	2.69	4.23
CH4 BCKGRD PPM	2.05	2.07	2.07	2.33
DILUTION FACTOR	13.81	22.23	16.41	22.90
HC CONCENTRATION PPM	111.71	71.61	85.61	69.43
CO CONCENTRATION PPM	524.24	353.31	450.01	337.59
CO2 CONCENTRATION PCT	.8571	.5070	.7099	.4898
NOX CONCENTRATION PPM	93.75	47.12	91.20	48.70
CH4 CONCENTRATION PPM	7.08	.59	.75	2.00
NMHC CONCENTRATION PPM	103.43	70.93	84.73	67.09
HC MASS GRAMS	5.393	5.944	4.131	5.760
CO MASS GRAMS	48.943	56.709	41.993	54.159
CO2 MASS GRAMS	1258.45	1279.69	1041.78	1235.72
NOX MASS GRAMS	15.098	13.046	14.682	13.479
PM MASS MILLIGRAMS	10.3	6.5	6.5	7.9
CH4 MASS GRAMS	.378	.054	.040	.184
NMHC MASS GRAMS (FID)	4.993	5.887	4.089	5.566
FUEL MASS KG	.445	.457	.369	.441
FUEL ECONOMY MPG (L/100KM)	22.81 (10.31)	23.97 (9.81)	27.52 (8.55)	24.84 (9.47)

4-BAG COMPOSITE RESULTS

HC	G/MI	1.413	CH4	G/MI	.042
CO	G/MI	13.473	NMHC	G/MI	1.368
NOX	G/MI	3.784			
PM	MG/MI	2.1			
FUEL ECONOMY MPG (L/100KM)		24.91 (9.44)			

SOUTHWEST RESEARCH INSTITUTE - DEPARTMENT OF EMISSIONS RESEARCH

COMPUTER PROGRAM LDT 3.2-R 4-BAG EPA FTP VEHICLE EMISSION RESULTS PROJECT NO. 03-15812-00

VEHICLE NUMBER	7329	TEST RFAE10-T3	GASOLINE EPA TIER EM-7579-F
VEHICLE MODEL	2011 TOYOTA CAMRY	DATE 10/12/2010 RUN	FUEL DENSITY 6.250 LB/GAL
ENGINE	2.5 L (153 CID)-4	DYNO 3 BAG CART 2	H .134 C .829 O .037 X .000
TRANSMISSION	A	ACTUAL ROAD LOAD 11.30 HP (8.43 KW)	FTP
ODOMETER	8466 MILES (13621 KM)	TEST WEIGHT 3625 LBS (1643 KG)	

BAROMETER 29.12 IN HG (739.6 MM HG) DRY BULB TEMPERATURE 71.0°F (21.7°C) NOX HUMIDITY C.F. 1.051

RELATIVE HUMIDITY 72.8 PCT.

BAG NUMBER	1	2	3	4
BAG DESCRIPTION	COLD TRANSIENT (0-505 SEC.)	STABILIZED (505-1372 SEC.)	HOT TRANSIENT (0- 505 SEC.)	HOT STABILIZED (505-1372 SEC.)
RUN TIME SECONDS	505.3	867.9	504.8	868.0
DRY/WET CORRECTION FACTOR, SAMP/BACK	.972/.981	.975/.981	.973/.981	.975/.981
MEASURED DISTANCE MILES (KM)	3.59 (5.78)	3.85 (6.19)	3.58 (5.76)	3.85 (6.19)
BLOWER FLOW RATE SCFM (SCMM)	287.8 (8.15)	287.2 (8.13)	286.9 (8.13)	288.2 (8.16)
20X20 FLOW RATE SCFM (SCMM)	45.9 (1.30)	45.7 (1.30)	46.7 (1.32)	46.6 (1.32)
GAS METER FLOW RATE SCFM (SCMM)	.85 (.02)	.81 (.02)	.85 (.02)	.82 (.02)
TOTAL FLOW SCF (SCM)	2817. (79.8)	4827. (136.7)	2814. (79.7)	4855. (137.5)
HC SAMPLE METER/RANGE/PPM (BAG)	11.8/ 3/ 118.45	75.7/ 2/ 75.72	90.2/ 2/ 90.22	73.7/ 2/ 73.72
HC BCKGRD METER/RANGE/PPM	.4/ 3/ 4.02	4.1/ 2/ 4.10	4.4/ 2/ 4.40	3.8/ 2/ 3.80
CO SAMPLE METER/RANGE/PPM	57.3/ 1/ 557.83	76.6/ 14/ 369.39	96.5/ 14/ 482.27	73.3/ 14/ 351.34
CO BCKGRD METER/RANGE/PPM	.0/ 1/ .00	.2/ 14/ .83	.2/ 14/ .83	.2/ 14/ .83
CO2 SAMPLE METER/RANGE/PCT	50.1/ 1/ .9058	69.4/ 11/ .5732	84.4/ 11/ .7817	67.2/ 11/ .5459
CO2 BCKGRD METER/RANGE/PCT	3.4/ 1/ .0558	10.4/ 11/ .0604	10.5/ 11/ .0610	10.5/ 11/ .0610
NOX SAMPLE METER/RANGE/PPM (BAG) (D)	37.6/ 3/ 94.00	47.8/ 2/ 48.01	92.6/ 2/ 92.71	48.3/ 2/ 48.50
NOX BCKGRD METER/RANGE/PPM	.1/ 3/ .25	.1/ 2/ .10	.2/ 2/ .21	.2/ 2/ .21
CH4 SAMPLE PPM (1.170)	5.89	4.15	4.84	4.39
CH4 BCKGRD PPM	2.25	2.16	2.17	2.14
DILUTION FACTOR	13.79	21.73	16.00	22.81
HC CONCENTRATION PPM	114.73	71.81	86.10	70.08
CO CONCENTRATION PPM	534.79	355.77	462.76	338.53
CO2 CONCENTRATION PCT	.8541	.5156	.7246	.4876
NOX CONCENTRATION PPM	93.76	47.91	92.52	48.31
CH4 CONCENTRATION PPM	3.81	2.08	2.81	2.34
NMHC CONCENTRATION PPM	110.27	69.37	82.81	67.34
HC MASS GRAMS	5.510	5.910	4.131	5.801
CO MASS GRAMS	49.675	56.622	42.934	54.193
CO2 MASS GRAMS	1247.61	1290.46	1057.21	1227.61
NOX MASS GRAMS	15.031	13.159	14.814	13.346
PM MASS MILLIGRAMS	10.4	7.5	5.5	7.7
CH4 MASS GRAMS	.202	.190	.149	.215
NMHC MASS GRAMS (FID)	5.297	5.709	3.973	5.575
FUEL MASS KG	.442	.460	.374	.438
FUEL ECONOMY MPG (L/100KM)	23.03 (10.22)	23.72 (9.92)	27.11 (8.68)	24.92 (9.44)

4-BAG COMPOSITE RESULTS

HC	G/MI	1.422	CH4	G/MI	.051
CO	G/MI	13.596	NMHC	G/MI	1.369
NOX	G/MI	3.790			
PM	MG/MI	2.0			
FUEL ECONOMY MPG (L/100KM)		24.83 (9.47)			

SOUTHWEST RESEARCH INSTITUTE - DEPARTMENT OF EMISSIONS RESEARCH

COMPUTER PROGRAM LDT 3.2-R 4-BAG EPA FTP VEHICLE EMISSION RESULTS PROJECT NO. 03-15812-00

VEHICLE NUMBER	7329	TEST RFAE15CAT-T1	GASOLINE EPA TIER EM-7580-F
VEHICLE MODEL	2011 TOYOTA CAMRY	DATE 10/19/2010 RUN	FUEL DENSITY 6.269 LB/GAL
ENGINE	2.5 L (153 CID)-4	DYNO 3 BAG CART 2	H .134 C .818 O .047 X .000
TRANSMISSION	A	ACTUAL ROAD LOAD 11.30 HP (8.43 KW)	FTP
ODOMETER	12494 MILES (20102 KM)	TEST WEIGHT 3625 LBS (1643 KG)	

BAROMETER 29.11 IN HG (739.4 MM HG) DRY BULB TEMPERATURE 71.0°F (21.7°C) NOX HUMIDITY C.F. 1.051

RELATIVE HUMIDITY 72.8 PCT.

BAG NUMBER	1	2	3	4
BAG DESCRIPTION	COLD TRANSIENT (0-505 SEC.)	STABILIZED (505-1372 SEC.)	HOT TRANSIENT (0- 505 SEC.)	HOT STABILIZED (505-1372 SEC.)
RUN TIME SECONDS	505.2	868.0	505.0	868.2
DRY/WET CORRECTION FACTOR, SAMP/BACK	.971/.981	.975/.981	.973/.981	.975/.981
MEASURED DISTANCE MILES (KM)	3.59 (5.78)	3.86 (6.21)	3.58 (5.76)	3.84 (6.18)
BLOWER FLOW RATE SCFM (SCMM)	287.3 (8.14)	286.6 (8.12)	285.9 (8.10)	288.0 (8.16)
20X20 FLOW RATE SCFM (SCMM)	46.5 (1.32)	46.2 (1.31)	46.9 (1.33)	46.9 (1.33)
GAS METER FLOW RATE SCFM (SCMM)	.85 (.02)	.82 (.02)	.85 (.02)	.82 (.02)
TOTAL FLOW SCF (SCM)	2818. (79.8)	4827. (136.7)	2808. (79.5)	4857. (137.6)
HC SAMPLE METER/RANGE/PPM (BAG)	8.6/ 2/ 8.60	3.7/ 2/ 3.70	3.8/ 2/ 3.80	3.5/ 2/ 3.50
HC BCKGRD METER/RANGE/PPM	4.0/ 2/ 4.00	4.0/ 2/ 4.00	3.4/ 2/ 3.40	3.5/ 2/ 3.50
CO SAMPLE METER/RANGE/PPM	16.7/ 12/ 16.15	.3/ 12/ .30	.2/ 12/ .20	.2/ 12/ .20
CO BCKGRD METER/RANGE/PPM	.2/ 12/ .20	.2/ 12/ .20	.1/ 12/ .10	.2/ 12/ .20
CO2 SAMPLE METER/RANGE/PCT	53.5/ 1/ .9738	32.7/ 1/ .5710	44.0/ 1/ .7859	32.0/ 1/ .5580
CO2 BCKGRD METER/RANGE/PCT	2.8/ 1/ .0459	3.1/ 1/ .0508	2.8/ 1/ .0459	2.9/ 1/ .0475
NOX SAMPLE METER/RANGE/PPM (BAG) (D)	5.0/ 1/ 1.25	1.2/ 1/ .30	2.2/ 1/ .55	1.6/ 1/ .40
NOX BCKGRD METER/RANGE/PPM	.8/ 1/ .20	.7/ 1/ .18	.8/ 1/ .20	.8/ 1/ .20
CH4 SAMPLE PPM (1.170)	2.65	1.90	2.07	1.90
CH4 BCKGRD PPM	1.97	1.95	2.00	1.95
DILUTION FACTOR	13.66	23.34	16.96	23.88
HC CONCENTRATION PPM	4.89	-.13	.60	.15
CO CONCENTRATION PPM	15.28	.10	.10	.01
CO2 CONCENTRATION PCT	.9313	.5224	.7428	.5125
NOX CONCENTRATION PPM	1.07	.13	.36	.21
CH4 CONCENTRATION PPM	.82	.04	.19	.04
NMHC CONCENTRATION PPM	3.93	-.18	.38	.10
HC MASS GRAMS	.238	.000	.029	.012
CO MASS GRAMS	1.419	.016	.009	.001
CO2 MASS GRAMS	1360.64	1307.57	1081.50	1290.80
NOX MASS GRAMS	.171	.036	.058	.058
PM MASS MILLIGRAMS	9.3	6.6	12.6	14.0
CH4 MASS GRAMS	.044	.004	.010	.004
NMHC MASS GRAMS (FID)	.191	.000	.018	.008
FUEL MASS KG	.455	.436	.361	.431
FUEL ECONOMY MPG (L/100KM)	22.43 (10.49)	25.16 (9.35)	28.20 (8.34)	25.35 (9.28)

4-BAG COMPOSITE RESULTS

HC	G/MI	.017	CH4	G/MI	.004
CO	G/MI	.084	NMHC	G/MI	.013
NOX	G/MI	.021			
PM	MG/MI	3.0			
FUEL ECONOMY MPG (L/100KM)		25.41 (9.26)			

SOUTHWEST RESEARCH INSTITUTE - DEPARTMENT OF EMISSIONS RESEARCH

COMPUTER PROGRAM LDT 3.2-R 4-BAG EPA FTP VEHICLE EMISSION RESULTS PROJECT NO. 03-15812-00

VEHICLE NUMBER	7329	TEST RFAE15CAT-T3	GASOLINE EPA TIER EM-7580-F
VEHICLE MODEL	2011 TOYOTA CAMRY	DATE 10/21/2010 RUN	FUEL DENSITY 6.269 LB/GAL
ENGINE	2.5 L (153 CID)-4	DYNO 3 BAG CART 2	H .134 C .818 O .047 X .000
TRANSMISSION	A	ACTUAL ROAD LOAD 11.30 HP (8.43 KW)	FTP
ODOMETER	12523 MILES (20149 KM)	TEST WEIGHT 3625 LBS (1643 KG)	

BAROMETER 29.15 IN HG (740.4 MM HG) DRY BULB TEMPERATURE 72.0°F (22.2°C) NOX HUMIDITY C.F. .993

RELATIVE HUMIDITY 61.0 PCT.

BAG NUMBER	1	2	3	4
BAG DESCRIPTION	COLD TRANSIENT (0-505 SEC.)	STABILIZED (505-1372 SEC.)	HOT TRANSIENT (0- 505 SEC.)	HOT STABILIZED (505-1372 SEC.)
RUN TIME SECONDS	505.1	868.4	503.8	867.8
DRY/WET CORRECTION FACTOR, SAMP/BACK	.974/.983	.978/.983	.976/.983	.978/.983
MEASURED DISTANCE MILES (KM)	3.59 (5.78)	3.87 (6.23)	3.59 (5.78)	3.86 (6.21)
BLOWER FLOW RATE SCFM (SCMM)	287.9 (8.15)	286.5 (8.11)	286.7 (8.12)	286.1 (8.10)
20X20 FLOW RATE SCFM (SCMM)	45.7 (1.29)	45.5 (1.29)	46.8 (1.32)	46.3 (1.31)
GAS METER FLOW RATE SCFM (SCMM)	.85 (.02)	.83 (.02)	.85 (.02)	.81 (.02)
TOTAL FLOW SCF (SCM)	2815. (79.7)	4817. (136.4)	2808. (79.5)	4820. (136.5)
HC SAMPLE METER/RANGE/PPM (BAG)	9.7/ 2/ 9.70	4.2/ 2/ 4.20	4.2/ 2/ 4.20	4.1/ 2/ 4.10
HC BCKGRD METER/RANGE/PPM	4.2/ 2/ 4.20	4.1/ 2/ 4.10	4.1/ 2/ 4.10	4.1/ 2/ 4.10
CO SAMPLE METER/RANGE/PPM	15.7/ 12/ 15.19	.9/ 12/ .89	.3/ 12/ .30	.4/ 12/ .39
CO BCKGRD METER/RANGE/PPM	.3/ 12/ .30	.5/ 12/ .49	.3/ 12/ .30	.4/ 12/ .39
CO2 SAMPLE METER/RANGE/PCT	52.2/ 1/ .9477	33.1/ 1/ .5785	43.8/ 1/ .7821	32.1/ 1/ .5599
CO2 BCKGRD METER/RANGE/PCT	2.9/ 1/ .0475	3.1/ 1/ .0508	3.0/ 1/ .0492	3.2/ 1/ .0525
NOX SAMPLE METER/RANGE/PPM (BAG) (D)	5.5/ 1/ 1.38	1.1/ 1/ .28	5.0/ 1/ 1.25	1.4/ 1/ .35
NOX BCKGRD METER/RANGE/PPM	.6/ 1/ .15	.5/ 1/ .13	.5/ 1/ .13	.5/ 1/ .13
CH4 SAMPLE PPM (1.170)	2.94	2.10	2.29	2.10
CH4 BCKGRD PPM	2.12	2.12	2.13	2.12
DILUTION FACTOR	14.04	23.03	17.04	23.80
HC CONCENTRATION PPM	5.80	.28	.34	.17
CO CONCENTRATION PPM	14.34	.40	.01	.01
CO2 CONCENTRATION PCT	.9036	.5299	.7358	.5096
NOX CONCENTRATION PPM	1.24	.16	1.13	.23
CH4 CONCENTRATION PPM	.97	.08	.29	.07
NMHC CONCENTRATION PPM	4.66	.19	.00	.09
HC MASS GRAMS	.282	.023	.017	.014
CO MASS GRAMS	1.331	.063	.001	.002
CO2 MASS GRAMS	1318.90	1323.45	1071.11	1273.79
NOX MASS GRAMS	.187	.040	.171	.060
PM MASS MILLIGRAMS	17.1	11.2	16.0	11.1
CH4 MASS GRAMS	.052	.007	.015	.007
NMHC MASS GRAMS (FID)	.227	.016	.000	.007
FUEL MASS KG	.441	.442	.357	.425
FUEL ECONOMY MPG (L/100KM)	23.14 (10.16)	24.91 (9.44)	28.56 (8.24)	25.82 (9.11)

4-BAG COMPOSITE RESULTS

HC	G/MI	.020	CH4	G/MI	.005
CO	G/MI	.081	NMHC	G/MI	.015
NOX	G/MI	.031			
PM	MG/MI	3.7			
FUEL ECONOMY MPG (L/100KM)		25.76 (9.13)			

SOUTHWEST RESEARCH INSTITUTE - DEPARTMENT OF EMISSIONS RESEARCH

COMPUTER PROGRAM LDT 3.2-R 4-BAG EPA FTP VEHICLE EMISSION RESULTS PROJECT NO. 03-15812-00

VEHICLE NUMBER	7329	TEST RFAE15CAT-T4	GASOLINE EPA TIER EM-7580-F
VEHICLE MODEL	2011 TOYOTA CAMRY	DATE 10/22/2010 RUN	FUEL DENSITY 6.269 LB/GAL
ENGINE	2.5 L (153 CID)-4	DYNO 3 BAG CART 2	H .134 C .818 O .047 X .000
TRANSMISSION	A	ACTUAL ROAD LOAD 11.30 HP (8.43 KW)	FTP
ODOMETER	12537 MILES (20172 KM)	TEST WEIGHT 3625 LBS (1643 KG)	

BAROMETER 29.07 IN HG (738.4 MM HG) DRY BULB TEMPERATURE 71.0°F (21.7°C) NOX HUMIDITY C.F. 1.051

RELATIVE HUMIDITY 72.8 PCT.

BAG NUMBER	1	2	3	4
BAG DESCRIPTION	COLD TRANSIENT (0-505 SEC.)	STABILIZED (505-1372 SEC.)	HOT TRANSIENT (0- 505 SEC.)	HOT STABILIZED (505-1372 SEC.)
RUN TIME SECONDS	505.0	868.6	505.2	868.2
DRY/WET CORRECTION FACTOR, SAMP/BACK	.971/.981	.975/.981	.973/.981	.975/.981
MEASURED DISTANCE MILES (KM)	3.59 (5.78)	3.87 (6.23)	3.58 (5.76)	3.87 (6.23)
BLOWER FLOW RATE SCFM (SCMM)	288.5 (8.17)	286.2 (8.11)	286.4 (8.11)	286.7 (8.12)
20X20 FLOW RATE SCFM (SCMM)	45.8 (1.30)	45.6 (1.29)	46.6 (1.32)	46.5 (1.32)
GAS METER FLOW RATE SCFM (SCMM)	.84 (.02)	.80 (.02)	.82 (.02)	.81 (.02)
TOTAL FLOW SCF (SCM)	2820. (79.9)	4815. (136.3)	2811. (79.6)	4833. (136.9)
HC SAMPLE METER/RANGE/PPM (BAG)	10.1/ 2/ 10.10	3.4/ 2/ 3.40	3.6/ 2/ 3.60	3.2/ 2/ 3.20
HC BCKGRD METER/RANGE/PPM	3.3/ 2/ 3.30	3.4/ 2/ 3.40	3.2/ 2/ 3.20	3.2/ 2/ 3.20
CO SAMPLE METER/RANGE/PPM	20.7/ 12/ 19.97	.3/ 12/ .30	.2/ 12/ .20	.3/ 12/ .30
CO BCKGRD METER/RANGE/PPM	.5/ 12/ .49	.2/ 12/ .20	.2/ 12/ .20	.2/ 12/ .20
CO2 SAMPLE METER/RANGE/PCT	52.4/ 1/ .9517	33.2/ 1/ .5804	43.9/ 1/ .7840	31.9/ 1/ .5562
CO2 BCKGRD METER/RANGE/PCT	2.9/ 1/ .0475	3.0/ 1/ .0492	2.9/ 1/ .0475	2.9/ 1/ .0475
NOX SAMPLE METER/RANGE/PPM (BAG) (D)	6.1/ 1/ 1.53	1.0/ 1/ .25	3.5/ 1/ .88	1.1/ 1/ .28
NOX BCKGRD METER/RANGE/PPM	.3/ 1/ .08	.4/ 1/ .10	.4/ 1/ .10	.4/ 1/ .10
CH4 SAMPLE PPM (1.170)	2.82	2.02	2.14	2.02
CH4 BCKGRD PPM	1.97	1.94	1.96	1.94
DILUTION FACTOR	13.97	22.96	17.00	23.96
HC CONCENTRATION PPM	7.04	.15	.59	.13
CO CONCENTRATION PPM	18.68	.10	.01	.10
CO2 CONCENTRATION PCT	.9076	.5334	.7393	.5107
NOX CONCENTRATION PPM	1.46	.15	.78	.18
CH4 CONCENTRATION PPM	.99	.16	.29	.16
NMHC CONCENTRATION PPM	5.88	-.04	.25	-.05
HC MASS GRAMS	.343	.012	.029	.011
CO MASS GRAMS	1.737	.016	.001	.016
CO2 MASS GRAMS	1327.16	1331.42	1077.55	1279.59
NOX MASS GRAMS	.234	.042	.125	.049
PM MASS MILLIGRAMS	.0	.0	.0	.0
CH4 MASS GRAMS	.053	.014	.015	.014
NMHC MASS GRAMS (FID)	.287	.000	.012	.000
FUEL MASS KG	.444	.444	.360	.427
FUEL ECONOMY MPG (L/100KM)	22.98 (10.24)	24.77 (9.50)	28.31 (8.31)	25.77 (9.13)

4-BAG COMPOSITE RESULTS

HC	G/MI	.024	CH4	G/MI	.006
CO	G/MI	.102	NMHC	G/MI	.017
NOX	G/MI	.029			
PM	MG/MI	.0			
FUEL ECONOMY MPG (L/100KM)		25.62 (9.18)			

SOUTHWEST RESEARCH INSTITUTE - DEPARTMENT OF EMISSIONS RESEARCH

COMPUTER PROGRAM LDT 3.2-R 4-BAG EPA FTP VEHICLE EMISSION RESULTS PROJECT NO. 03-15812-00

VEHICLE NUMBER	7329	TEST RFAE15-T1	GASOLINE EPA TIER EM-7580-F
VEHICLE MODEL	2011 TOYOTA CAMRY	DATE 10/25/2010 RUN	FUEL DENSITY 6.269 LB/GAL
ENGINE	2.5 L (153 CID)-4	DYNO 3 BAG CART 2	H .134 C .818 O .047 X .000
TRANSMISSION	A	ACTUAL ROAD LOAD 11.30 HP (8.43 KW)	FTP
ODOMETER	12592 MILES (20260 KM)	TEST WEIGHT 3625 LBS (1643 KG)	

BAROMETER 28.81 IN HG (731.8 MM HG) DRY BULB TEMPERATURE 72.0°F (22.2°C) NOX HUMIDITY C.F. 1.047

RELATIVE HUMIDITY 69.1 PCT.

BAG NUMBER	1	2	3	4
BAG DESCRIPTION	COLD TRANSIENT (0-505 SEC.)	STABILIZED (505-1372 SEC.)	HOT TRANSIENT (0- 505 SEC.)	HOT STABILIZED (505-1372 SEC.)
RUN TIME SECONDS	504.7	867.7	505.1	868.1
DRY/WET CORRECTION FACTOR, SAMP/BACK	.972/.981	.975/.981	.974/.981	.976/.981
MEASURED DISTANCE MILES (KM)	3.59 (5.78)	3.85 (6.19)	3.58 (5.76)	3.86 (6.21)
BLOWER FLOW RATE SCFM (SCMM)	283.4 (8.03)	282.6 (8.00)	282.9 (8.01)	282.6 (8.00)
20X20 FLOW RATE SCFM (SCMM)	45.0 (1.28)	45.2 (1.28)	45.7 (1.30)	45.6 (1.29)
GAS METER FLOW RATE SCFM (SCMM)	.85 (.02)	.81 (.02)	.85 (.02)	.80 (.02)
TOTAL FLOW SCF (SCM)	2770. (78.4)	4752. (134.6)	2774. (78.6)	4760. (134.8)
HC SAMPLE METER/RANGE/PPM (BAG)	11.5/ 3/ 115.44	70.8/ 2/ 70.82	82.0/ 2/ 82.02	69.7/ 2/ 69.72
HC BCKGRD METER/RANGE/PPM	.4/ 3/ 4.02	4.5/ 2/ 4.50	4.9/ 2/ 4.90	4.9/ 2/ 4.90
CO SAMPLE METER/RANGE/PPM	72.9/ 14/ 349.16	51.8/ 14/ 238.50	62.3/ 14/ 292.57	51.2/ 14/ 235.47
CO BCKGRD METER/RANGE/PPM	.0/ 14/ .00	.0/ 14/ .00	.1/ 14/ .42	.2/ 14/ .83
CO2 SAMPLE METER/RANGE/PCT	51.0/ 1/ .9237	31.4/ 1/ .5469	41.7/ 1/ .7415	30.7/ 1/ .5340
CO2 BCKGRD METER/RANGE/PCT	2.9/ 1/ .0475	2.9/ 1/ .0475	2.8/ 1/ .0459	2.7/ 1/ .0442
NOX SAMPLE METER/RANGE/PPM (BAG) (D)	39.9/ 3/ 99.74	50.8/ 2/ 50.98	39.9/ 3/ 99.74	55.3/ 2/ 55.45
NOX BCKGRD METER/RANGE/PPM	.0/ 3/ .00	.1/ 2/ .10	.1/ 3/ .25	.2/ 2/ .21
CH4 SAMPLE PPM (1.170)	5.13	3.97	4.25	3.97
CH4 BCKGRD PPM	2.15	2.17	2.16	2.17
DILUTION FACTOR	13.77	23.11	17.15	23.66
HC CONCENTRATION PPM	111.71	66.51	77.40	65.02
CO CONCENTRATION PPM	334.98	230.59	281.36	226.94
CO2 CONCENTRATION PCT	.8797	.5015	.6983	.4916
NOX CONCENTRATION PPM	99.74	50.88	99.51	55.25
CH4 CONCENTRATION PPM	3.14	1.90	2.22	1.89
NMHC CONCENTRATION PPM	108.04	64.29	74.81	62.81
HC MASS GRAMS	5.347	5.462	3.710	5.348
CO MASS GRAMS	30.591	36.130	25.729	35.616
CO2 MASS GRAMS	1263.31	1235.62	1004.21	1213.37
NOX MASS GRAMS	15.670	13.716	15.655	14.917
PM MASS MILLIGRAMS	12.9	6.4	8.7	2.0
CH4 MASS GRAMS	.164	.170	.116	.170
NMHC MASS GRAMS (FID)	5.171	5.280	3.586	5.166
FUEL MASS KG	.443	.437	.352	.429
FUEL ECONOMY MPG (L/100KM)	23.05 (10.21)	25.07 (9.38)	28.90 (8.14)	25.59 (9.19)

4-BAG COMPOSITE RESULTS

HC	G/MI	1.319	CH4	G/MI	.041
CO	G/MI	8.556	NMHC	G/MI	1.275
NOX	G/MI	4.041			
PM	MG/MI	1.9			
FUEL ECONOMY MPG (L/100KM)		25.78 (9.13)			

SOUTHWEST RESEARCH INSTITUTE - DEPARTMENT OF EMISSIONS RESEARCH

COMPUTER PROGRAM LDT 3.2-R 4-BAG EPA FTP VEHICLE EMISSION RESULTS PROJECT NO. 03-15812-00

VEHICLE NUMBER	7329	TEST RFAE15-T2	GASOLINE EPA TIER EM-7580-F
VEHICLE MODEL	2011 TOYOTA CAMRY	DATE 10/26/2010 RUN	FUEL DENSITY 6.269 LB/GAL
ENGINE	2.5 L (153 CID)-4	DYNO 3 BAG CART 2	H .134 C .818 O .047 X .000
TRANSMISSION	A	ACTUAL ROAD LOAD 11.30 HP (8.43 KW)	FTP
ODOMETER	12607 MILES (20284 KM)	TEST WEIGHT 3625 LBS (1643 KG)	

BAROMETER 28.85 IN HG (732.8 MM HG) DRY BULB TEMPERATURE 71.0°F (21.7°C) NOX HUMIDITY C.F. 1.006

RELATIVE HUMIDITY 64.6 PCT.

BAG NUMBER	1	2	3	4
BAG DESCRIPTION	COLD TRANSIENT (0-505 SEC.)	STABILIZED (505-1372 SEC.)	HOT TRANSIENT (0- 505 SEC.)	HOT STABILIZED (505-1372 SEC.)
RUN TIME SECONDS	505.0	868.5	505.0	868.1
DRY/WET CORRECTION FACTOR, SAMP/BACK	.974/.983	.977/.983	.975/.983	.978/.983
MEASURED DISTANCE MILES (KM)	3.57 (5.74)	3.85 (6.19)	3.58 (5.76)	3.86 (6.21)
BLOWER FLOW RATE SCFM (SCMM)	285.8 (8.09)	284.3 (8.05)	282.5 (8.00)	283.0 (8.02)
20X20 FLOW RATE SCFM (SCMM)	45.7 (1.29)	45.5 (1.29)	46.4 (1.31)	46.3 (1.31)
GAS METER FLOW RATE SCFM (SCMM)	.85 (.02)	.81 (.02)	.85 (.02)	.81 (.02)
TOTAL FLOW SCF (SCM)	2797. (79.2)	4786. (135.5)	2775. (78.6)	4776. (135.3)
HC SAMPLE METER/RANGE/PPM (BAG)	11.0/ 3/ 110.42	72.8/ 2/ 72.82	84.7/ 2/ 84.72	66.8/ 2/ 66.82
HC BCKGRD METER/RANGE/PPM	.4/ 3/ 4.02	4.2/ 2/ 4.20	4.8/ 2/ 4.80	4.4/ 2/ 4.40
CO SAMPLE METER/RANGE/PPM	72.2/ 14/ 345.36	49.7/ 14/ 227.92	61.3/ 14/ 287.34	49.2/ 14/ 225.41
CO BCKGRD METER/RANGE/PPM	.1/ 14/ .42	.1/ 14/ .42	.0/ 14/ .00	.0/ 14/ .00
CO2 SAMPLE METER/RANGE/PCT	50.6/ 1/ .9157	30.6/ 1/ .5321	41.7/ 1/ .7415	30.1/ 1/ .5229
CO2 BCKGRD METER/RANGE/PCT	2.9/ 1/ .0475	2.8/ 1/ .0459	2.8/ 1/ .0459	2.7/ 1/ .0442
NOX SAMPLE METER/RANGE/PPM (BAG) (D)	40.7/ 3/ 101.74	51.7/ 2/ 51.88	39.7/ 3/ 99.24	53.6/ 2/ 53.76
NOX BCKGRD METER/RANGE/PPM	.1/ 3/ .25	.3/ 2/ .31	.1/ 3/ .25	.2/ 2/ .21
CH4 SAMPLE PPM (1.170)	5.06	3.76	4.14	3.76
CH4 BCKGRD PPM	2.05	1.97	1.94	1.97
DILUTION FACTOR	13.89	23.75	17.15	24.19
HC CONCENTRATION PPM	106.69	68.79	80.20	62.60
CO CONCENTRATION PPM	331.52	220.37	277.12	218.38
CO2 CONCENTRATION PCT	.8716	.4882	.6983	.4805
NOX CONCENTRATION PPM	101.51	51.58	99.01	53.56
CH4 CONCENTRATION PPM	3.16	1.87	2.31	1.87
NMHC CONCENTRATION PPM	102.99	66.60	77.49	60.41
HC MASS GRAMS	5.157	5.689	3.846	5.166
CO MASS GRAMS	30.574	34.770	25.356	34.389
CO2 MASS GRAMS	1264.19	1211.34	1004.78	1189.97
NOX MASS GRAMS	15.464	13.443	14.964	13.933
PM MASS MILLIGRAMS	11.3	11.4	6.0	4.9
CH4 MASS GRAMS	.167	.169	.121	.169
NMHC MASS GRAMS (FID)	4.978	5.508	3.716	4.986
FUEL MASS KG	.443	.428	.352	.420
FUEL ECONOMY MPG (L/100KM)	22.91 (10.27)	25.57 (9.20)	28.88 (8.14)	26.12 (9.01)

4-BAG COMPOSITE RESULTS

HC	G/MI	1.319	CH4	G/MI	.042
CO	G/MI	8.364	NMHC	G/MI	1.274
NOX	G/MI	3.889			
PM	MG/MI	2.2			
FUEL ECONOMY MPG (L/100KM)		26.02 (9.04)			

SOUTHWEST RESEARCH INSTITUTE - DEPARTMENT OF EMISSIONS RESEARCH

COMPUTER PROGRAM LDT 3.2-R 4-BAG EPA FTP VEHICLE EMISSION RESULTS PROJECT NO. 03-15812-00

VEHICLE NUMBER	7329	TEST RFAE15-T3	GASOLINE EPA TIER EM-7580-F
VEHICLE MODEL	2011 TOYOTA CAMRY	DATE 10/27/2010 RUN	FUEL DENSITY 6.269 LB/GAL
ENGINE	2.5 L (153 CID)-4	DYNO 3 BAG CART 2	H .134 C .818 O .047 X .000
TRANSMISSION	A	ACTUAL ROAD LOAD 11.30 HP (8.43 KW)	FTP
ODOMETER	12621 MILES (20307 KM)	TEST WEIGHT 3625 LBS (1643 KG)	

BAROMETER 28.95 IN HG (735.3 MM HG) DRY BULB TEMPERATURE 71.2°F (21.8°C) NOX HUMIDITY C.F. 1.052

RELATIVE HUMIDITY 72.0 PCT.

BAG NUMBER	1	2	3	4
BAG DESCRIPTION	COLD TRANSIENT (0-505 SEC.)	STABILIZED (505-1372 SEC.)	HOT TRANSIENT (0- 505 SEC.)	HOT STABILIZED (505-1372 SEC.)
RUN TIME SECONDS	505.0	867.9	505.0	868.2
DRY/WET CORRECTION FACTOR, SAMP/BACK	.972/.981	.975/.981	.973/.981	.975/.981
MEASURED DISTANCE MILES (KM)	3.59 (5.78)	3.85 (6.19)	3.58 (5.76)	3.87 (6.23)
BLOWER FLOW RATE SCFM (SCMM)	282.3 (8.00)	285.3 (8.08)	285.7 (8.09)	284.1 (8.05)
20X20 FLOW RATE SCFM (SCMM)	46.3 (1.31)	46.1 (1.30)	46.4 (1.31)	46.5 (1.32)
GAS METER FLOW RATE SCFM (SCMM)	.85 (.02)	.82 (.02)	.84 (.02)	.82 (.02)
TOTAL FLOW SCF (SCM)	2773. (78.5)	4804. (136.1)	2802. (79.4)	4795. (135.8)
HC SAMPLE METER/RANGE/PPM (BAG)	11.4/ 3/ 114.43	71.7/ 2/ 71.72	85.3/ 2/ 85.32	69.9/ 2/ 69.92
HC BCKGRD METER/RANGE/PPM	.5/ 3/ 5.02	4.6/ 2/ 4.60	4.5/ 2/ 4.50	4.5/ 2/ 4.50
CO SAMPLE METER/RANGE/PPM	72.9/ 14/ 349.16	50.1/ 14/ 229.93	66.3/ 14/ 313.69	52.2/ 14/ 240.52
CO BCKGRD METER/RANGE/PPM	.0/ 14/ .00	.0/ 14/ .00	.1/ 14/ .42	.1/ 14/ .42
CO2 SAMPLE METER/RANGE/PCT	50.8/ 1/ .9197	31.2/ 1/ .5432	41.4/ 1/ .7357	30.3/ 1/ .5266
CO2 BCKGRD METER/RANGE/PCT	2.9/ 1/ .0475	2.9/ 1/ .0475	2.8/ 1/ .0459	2.8/ 1/ .0459
NOX SAMPLE METER/RANGE/PPM (BAG) (D)	40.3/ 3/ 100.74	50.4/ 2/ 50.59	39.3/ 3/ 98.24	51.9/ 2/ 52.07
NOX BCKGRD METER/RANGE/PPM	.1/ 3/ .25	.4/ 2/ .41	.1/ 3/ .25	.1/ 2/ .10
CH4 SAMPLE PPM (1.170)	4.75	3.78	4.36	3.78
CH4 BCKGRD PPM	2.03	2.08	2.04	2.08
DILUTION FACTOR	13.82	23.29	17.22	23.95
HC CONCENTRATION PPM	109.78	67.31	81.08	65.60
CO CONCENTRATION PPM	334.68	222.11	301.44	232.03
CO2 CONCENTRATION PCT	.8757	.4977	.6925	.4826
NOX CONCENTRATION PPM	100.51	50.19	98.01	51.98
CH4 CONCENTRATION PPM	2.87	1.79	2.43	1.79
NMHC CONCENTRATION PPM	106.42	65.22	78.23	63.51
HC MASS GRAMS	5.261	5.589	3.926	5.436
CO MASS GRAMS	30.604	35.182	27.849	36.682
CO2 MASS GRAMS	1259.22	1239.92	1006.14	1199.93
NOX MASS GRAMS	15.878	13.735	15.642	14.195
PM MASS MILLIGRAMS	12.0	6.5	6.7	9.7
CH4 MASS GRAMS	.150	.162	.129	.162
NMHC MASS GRAMS (FID)	5.100	5.415	3.788	5.263
FUEL MASS KG	.442	.438	.354	.425
FUEL ECONOMY MPG (L/100KM)	23.12 (10.17)	25.01 (9.41)	28.73 (8.19)	25.89 (9.09)

4-BAG COMPOSITE RESULTS

HC	G/MI	1.343	CH4	G/MI	.040
CO	G/MI	8.740	NMHC	G/MI	1.300
NOX	G/MI	3.995			
PM	MG/MI	2.3			
FUEL ECONOMY MPG (L/100KM)		25.84 (9.10)			

APPENDIX B

HYDROCARBON SPECIATION DATA

TABLE B-1. HYDROCARBON SPECIATION DATA FOR 211b
BASELINE FUEL WITH AFTERTREATMENT

	mg/mile			mg/mile			mg/mile		
	RFBASECAT-2	RFBASECAT-3	RFBASECAT-6	RFBASECAT-2	RFBASECAT-3	RFBASECAT-6	RFBASECAT-2	RFBASECAT-3	RFBASECAT-6
COMPOUND	C505	C505	C505	C867	C867	C867	H505	H505	H505
METHANE	10.6	12.5	8.9	0.5	0.5	3.1	1.9	2.0	9.5
ETHANE	3.5	4.1	4.0	0.1	ND	ND	0.1	0.1	0.2
ETHYLENE	9.1	10.9	11.7	ND	ND	ND	0.1	0.1	0.1
PROPANE	1.4	0.2	0.2	ND	ND	ND	ND	ND	Trace
PROPYLENE	6.9	8.3	9.1	ND	ND	ND	ND	ND	ND
ACETYLENE	3.4	3.9	4.8	ND	ND	ND	Trace	Trace	ND
PROPADIENE	ND	ND	ND	ND	ND	ND	ND	ND	ND
BUTANE	0.9	0.6	0.6	Trace	ND	ND	ND	ND	Trace
TRANS-2-BUTENE	0.5	0.6	0.7	ND	ND	ND	ND	ND	ND
1-BUTENE	ND	ND	ND	ND	ND	ND	ND	ND	ND
2-METHYLPROPENE (ISOBUTYLENE)	0.6	0.8	0.9	ND	ND	ND	ND	ND	ND
2,2-DIMETHYLPROPANE (NEOPENTANE)	0.1	0.1	0.1	ND	ND	ND	ND	ND	ND
PROPYNE	0.1	0.1	0.2	ND	ND	ND	ND	ND	ND
1,3-BUTADIENE	1.0	1.3	1.4	ND	ND	ND	ND	ND	ND
2-METHYLPROPANE (ISOBUTANE)	0.7	0.9	0.9	ND	ND	ND	ND	ND	ND
1-BUTYNE	ND	ND	ND	ND	ND	ND	ND	ND	ND
METHANOL	ND	ND	0.1	ND	ND	ND	ND	ND	ND
CIS-2-BUTENE	3.9	4.8	5.1	ND	ND	ND	ND	ND	ND
3-METHYL-1-BUTENE	0.1	0.1	0.1	ND	ND	ND	ND	ND	ND
ETHANOL	ND	ND	ND	ND	ND	ND	ND	ND	ND
2-METHYLBUTANE (ISOPENTANE)	8.1	6.6	14.5	ND	ND	ND	ND	0.1	0.1
2-BUTYNE	ND	ND	ND	ND	ND	ND	ND	ND	ND
1-PENTENE	ND	ND	ND	ND	ND	ND	ND	ND	ND
2-METHYL-1-BUTENE	ND	ND	ND	ND	ND	ND	ND	ND	ND
PENTANE	2.0	1.1	3.1	ND	ND	0.1	ND	ND	ND
UNIDENTIFIED C5 OLEFINS	ND	ND	ND	ND	ND	ND	ND	ND	ND
2-METHYL-1,3-BUTADIENE	ND	ND	ND	ND	ND	ND	ND	ND	ND
TRANS-2-PENTENE	0.2	0.2	0.3	ND	ND	ND	ND	ND	ND
3,3-DIMETHYL-1-BUTENE	ND	ND	ND	ND	ND	ND	ND	ND	ND
CIS-2-PENTENE	ND	ND	ND	ND	ND	ND	ND	ND	ND
2-METHYL-2-BUTENE	0.4	0.6	0.4	ND	ND	ND	ND	ND	ND
TERT-BUTANOL	ND	ND	ND	ND	ND	ND	ND	ND	ND
CYCLOPENTADIENE	0.4	0.5	0.4	ND	ND	ND	ND	ND	ND
2,2-DIMETHYLBUTANE	0.3	0.2	0.4	ND	ND	ND	ND	ND	ND
CYCLOPENTENE	ND	ND	ND	ND	ND	ND	ND	ND	ND
4-METHYL-1-PENTENE	ND	ND	ND	ND	ND	ND	ND	ND	ND
3-METHYL-1-PENTENE	ND	ND	ND	ND	ND	ND	ND	ND	ND
CYCLOPENTANE	0.1	0.1	0.2	ND	ND	ND	ND	ND	ND
2,3-DIMETHYLBUTANE	0.7	0.7	1.5	ND	ND	ND	ND	ND	ND
MTBE	ND	ND	ND	ND	ND	ND	ND	ND	ND
4-METHYL-CIS-2-PENTENE	ND	ND	ND	ND	ND	ND	ND	ND	ND
2-METHYLPENTANE	1.0	0.9	1.9	ND	0.5	0.2	ND	ND	ND
4-METHYL-TRANS-2-PENTENE	ND	ND	ND	ND	ND	ND	ND	ND	ND
3-METHYLPENTANE	0.6	0.4	1.1	Trace	Trace	ND	ND	ND	ND
2-METHYL-1-PENTENE	ND	ND	ND	ND	ND	ND	ND	ND	ND
1-HEXENE	ND	ND	ND	ND	ND	ND	ND	ND	ND
HEXANE	1.0	0.8	1.7	ND	ND	ND	ND	ND	ND
UNIDENTIFIED C6	ND	ND	ND	ND	ND	ND	ND	ND	ND
TRANS-3-HEXENE	ND	ND	ND	ND	ND	ND	ND	ND	ND

TABLE B-1 (CONT'D). HYDROCARBON SPECIATION DATA FOR 211b BASELINE FUEL WITH AFTERTREATMENT

	mg/mile			mg/mile			mg/mile		
	RFBASECAT-2	RFBASECAT-3	RFBASECAT-6	RFBASECAT-2	RFBASECAT-3	RFBASECAT-6	RFBASECAT-2	RFBASECAT-3	RFBASECAT-6
COMPOUND	C505	C505	C505	C867	C867	C867	H505	H505	H505
CIS-3-HEXENE	ND	ND	ND	ND	ND	ND	ND	ND	ND
DI-ISOPROPYL ETHER	ND	ND	ND	ND	ND	ND	ND	ND	ND
TRANS-2-HEXENE	ND	ND	ND	ND	ND	ND	ND	ND	ND
3-METHYL-TRANS-2-PENTENE	ND	ND	ND	ND	ND	ND	ND	ND	ND
2-METHYL-2-PENTENE	ND	ND	ND	ND	ND	ND	ND	ND	ND
3-METHYLCYCLOPENTENE	ND	ND	ND	ND	ND	ND	ND	ND	ND
CIS-2-HEXENE	ND	ND	ND	ND	ND	ND	ND	ND	ND
ETBE	ND	ND	ND	ND	ND	ND	ND	ND	ND
3-METHYL-CIS-2-PENTENE	ND	ND	ND	ND	ND	ND	ND	ND	ND
2,2-DIMETHYLPENTANE, NOTE A	0.3	0.3	0.5	ND	ND	ND	ND	ND	ND
METHYLCYCLOPENTANE, NOTE A	0.3	0.2	0.5	ND	ND	ND	ND	ND	ND
2,4-DIMETHYLPENTANE	0.7	0.6	1.3	ND	ND	ND	ND	ND	ND
2,2,3-TRIMETHYLBUTANE	ND	ND	ND	ND	ND	ND	ND	ND	ND
3,4-DIMETHYL-1-PENTENE	ND	ND	ND	ND	ND	ND	ND	ND	ND
1-METHYLCYCLOPENTENE	ND	ND	ND	ND	ND	ND	ND	ND	ND
BENZENE	5.9	6.5	6.0	ND	ND	ND	ND	0.1	ND
3-METHYL-1-HEXENE	ND	ND	ND	ND	ND	ND	ND	ND	ND
3,3-DIMETHYLPENTANE	ND	ND	ND	ND	ND	ND	ND	ND	ND
CYCLOHEXANE	0.4	0.4	0.8	ND	ND	ND	ND	ND	ND
2-METHYLHEXANE	0.9	0.8	1.5	ND	0.2	ND	Trace	0.1	ND
2,3-DIMETHYLPENTANE	ND	ND	ND	ND	ND	ND	ND	ND	ND
1,1-DIMETHYLCYCLOPENTANE	ND	ND	ND	ND	ND	ND	ND	ND	ND
TERT-AMYL METHYL ETHER	ND	ND	ND	ND	ND	ND	ND	ND	ND
CYCLOHEXENE	ND	ND	ND	ND	ND	ND	ND	ND	ND
3-METHYLHEXANE	0.3	0.1	0.4	ND	ND	ND	ND	ND	ND
CIS-1,3-DIMETHYLCYCLOPENTANE	ND	ND	ND	ND	ND	ND	ND	ND	ND
3-ETHYLPENTANE	0.1	0.1	0.1	ND	ND	ND	ND	ND	ND
TRANS-1,2-DIMETHYLCYCLOPENTANE	ND	ND	ND	ND	ND	ND	ND	ND	ND
TRANS-1,3-DIMETHYLCYCLOPENTANE	ND	ND	ND	ND	ND	ND	ND	ND	ND
1-HEPTENE	ND	ND	ND	ND	ND	ND	ND	ND	ND
2,2,4-TRIMETHYLPENTANE	7.7	7.4	14.3	ND	0.4	0.2	0.2	0.6	0.5
2-METHYL-1-HEXENE	ND	ND	ND	ND	ND	ND	ND	ND	ND
TRANS-3-HEPTENE	ND	ND	ND	ND	ND	ND	ND	ND	ND
HEPTANE	0.4	0.2	0.7	ND	ND	ND	ND	ND	ND
CIS-3-HEPTENE	ND	ND	ND	ND	ND	ND	ND	ND	ND
UNIDENTIFIED C7	ND	ND	ND	ND	ND	ND	ND	ND	ND
2-METHYL-2-HEXENE	ND	ND	ND	ND	ND	ND	ND	ND	ND
3-METHYL-TRANS-3-HEXENE	ND	ND	ND	ND	ND	ND	ND	ND	ND
TRANS-2-HEPTENE	ND	ND	ND	ND	ND	ND	ND	ND	ND
3-ETHYL-CIS-2-PENTENE	ND	ND	ND	ND	ND	ND	ND	ND	ND
2,4,4-TRIMETHYL-1-PENTENE	ND	ND	ND	ND	ND	ND	ND	ND	ND
2,3-DIMETHYL-2-PENTENE	ND	ND	ND	ND	ND	ND	ND	ND	ND
CIS-2-HEPTENE	ND	ND	ND	ND	ND	ND	ND	ND	ND
METHYLCYCLOHEXANE	0.6	0.5	1.0	ND	ND	ND	ND	ND	ND
CIS-1,2-DIMETHYLCYCLOPENTANE	ND	ND	ND	ND	ND	ND	ND	ND	ND
2,2-DIMETHYLHEXANE	ND	ND	ND	ND	ND	ND	ND	ND	ND
1,1,3-TRIMETHYLCYCLOPENTANE	ND	ND	ND	ND	ND	ND	ND	ND	ND
2,4,4-TRIMETHYL-2-PENTENE	ND	ND	ND	ND	ND	ND	ND	ND	ND

TABLE B-1 (CONT'D). HYDROCARBON SPECIATION DATA FOR 211b BASELINE FUEL WITH AFTERTREATMENT

	mg/mile			mg/mile			mg/mile		
	RFBASECAT-2	RFBASECAT-3	RFBASECAT-6	RFBASECAT-2	RFBASECAT-3	RFBASECAT-6	RFBASECAT-2	RFBASECAT-3	RFBASECAT-6
COMPOUND	C505	C505	C505	C867	C867	C867	H505	H505	H505
2,2,3-TRIMETHYLPENTANE	ND	ND	ND	ND	ND	ND	ND	ND	ND
2,5-DIMETHYLHEXANE	ND	ND	ND	ND	ND	ND	ND	ND	ND
ETHYLCYCLOPENTANE	ND	ND	ND	ND	ND	ND	ND	ND	ND
2,4-DIMETHYLHEXANE	1.8	1.8	3.0	ND	ND	ND	ND	ND	ND
1-TRANS-2-CIS-4-TRIMETHYLCYCLOPENTANE	ND	ND	ND	ND	ND	ND	ND	ND	ND
3,3-DIMETHYLHEXANE	ND	ND	ND	ND	ND	ND	ND	ND	ND
1-TRANS-2-CIS-3-TRIMETHYLCYCLOPENTANE	ND	ND	ND	ND	ND	ND	ND	ND	ND
2,3,4-TRIMETHYLPENTANE	2.3	2.2	4.0	ND	ND	ND	0.1	0.1	ND
2,3,3-TRIMETHYLPENTANE	1.9	1.8	3.0	ND	ND	ND	ND	ND	ND
TOLUENE	19.6	19.2	23.8	ND	0.1	ND	ND	ND	ND
2,3-DIMETHYLHEXANE	ND	ND	ND	ND	ND	ND	ND	ND	ND
1,1,2-TRIMETHYLCYCLOPENTANE	ND	ND	ND	ND	ND	ND	ND	ND	ND
2-METHYLHEPTANE	0.9	0.9	1.4	ND	ND	ND	ND	ND	ND
3,4-DIMETHYLHEXANE, NOTE B	ND	ND	ND	ND	ND	ND	ND	ND	ND
4-METHYLHEPTANE	ND	ND	ND	ND	ND	ND	ND	ND	ND
3-METHYLHEPTANE	ND	ND	ND	ND	ND	ND	ND	ND	ND
1-CIS,2-TRANS,3-TRIMETHYLCYCLOPENTANE	0.2	0.2	0.2	ND	ND	ND	ND	ND	ND
CIS-1,3-DIMETHYLCYCLOHEXANE	ND	ND	ND	ND	ND	ND	ND	ND	ND
TRANS-1,4-DIMETHYLCYCLOHEXANE	ND	ND	ND	ND	ND	ND	ND	ND	ND
3-ETHYLHEXANE	ND	ND	ND	ND	ND	ND	ND	ND	ND
2,2,5-TRIMETHYLHEXANE	0.9	1.0	1.4	ND	ND	ND	ND	ND	ND
TRANS-1-METHYL-3-ETHYLCYCLOPENTANE	ND	ND	ND	ND	ND	ND	ND	ND	ND
CIS-1-METHYL-3-ETHYLCYCLOPENTANE	ND	ND	ND	ND	ND	ND	ND	ND	ND
1,1-DIMETHYLCYCLOHEXANE	ND	ND	ND	ND	ND	ND	ND	ND	ND
TRANS-1-METHYL-2-ETHYLCYCLOPENTANE	ND	ND	ND	ND	ND	ND	ND	ND	ND
1-METHYL-1-ETHYL-CYCLOPENTANE	ND	ND	ND	ND	ND	ND	ND	ND	ND
2,4,4-TRIMETHYLHEXANE	ND	ND	ND	ND	ND	ND	ND	ND	ND
2,2,4-TRIMETHYLHEXANE	ND	ND	ND	ND	ND	ND	ND	ND	ND
TRANS-1,2-DIMETHYLCYCLOHEXANE	ND	ND	ND	ND	ND	ND	ND	ND	ND
1-OCTENE	ND	ND	ND	ND	ND	ND	ND	ND	ND
TRANS-4-OCTENE	ND	ND	ND	ND	ND	ND	ND	ND	ND
OCTANE	ND	ND	ND	ND	ND	ND	ND	ND	ND
UNIDENTIFIED C8	ND	ND	ND	ND	ND	ND	ND	ND	ND
TRANS-2-OCTENE	ND	ND	ND	ND	ND	ND	ND	ND	ND
TRANS-1,3-DIMETHYLCYCLOHEXANE, NOTE C	ND	ND	ND	ND	ND	ND	ND	ND	ND
CIS-2-OCTENE	ND	ND	ND	ND	ND	ND	ND	ND	ND
ISOPROPYLCYCLOPENTANE	ND	ND	ND	ND	ND	ND	ND	ND	ND
2,2-DIMETHYLHEPTANE	ND	ND	ND	ND	ND	ND	ND	ND	ND
2,3,5-TRIMETHYLHEXANE	ND	ND	ND	ND	ND	ND	ND	ND	ND
CIS-1-METHYL-2-ETHYLCYCLOPENTANE	ND	ND	ND	ND	ND	ND	ND	ND	ND
2,4-DIMETHYLHEPTANE	ND	ND	ND	ND	ND	ND	ND	ND	ND
4,4-DIMETHYLHEPTANE	ND	ND	ND	ND	ND	ND	ND	ND	ND
CIS-1,2-DIMETHYLCYCLOHEXANE	ND	ND	ND	ND	ND	ND	ND	ND	ND
ETHYLCYCLOHEXANE	ND	ND	ND	ND	ND	ND	ND	ND	ND
2,6-DIMETHYLHEPTANE, NOTE D	ND	ND	ND	ND	ND	ND	ND	ND	ND
1,1,3-TRIMETHYLCYCLOHEXANE	ND	ND	ND	ND	ND	ND	ND	ND	ND
2,5-DIMETHYLHEPTANE, NOTE E	ND	ND	ND	ND	ND	ND	ND	ND	ND
3,3-DIMETHYLHEPTANE	ND	ND	ND	ND	ND	ND	ND	ND	ND
3,5-DIMETHYLHEPTANE, NOTE E	ND	ND	ND	ND	ND	ND	ND	ND	ND

TABLE B-1 (CONT'D). HYDROCARBON SPECIATION DATA FOR 211b BASELINE FUEL WITH AFTERTREATMENT

	mg/mile			mg/mile			mg/mile		
	RFBASECAT-2	RFBASECAT-3	RFBASECAT-6	RFBASECAT-2	RFBASECAT-3	RFBASECAT-6	RFBASECAT-2	RFBASECAT-3	RFBASECAT-6
COMPOUND	C505	C505	C505	C867	C867	C867	H505	H505	H505
ETHYLBENZENE	1.5	0.9	0.6	ND	ND	ND	ND	ND	ND
2,3,4-TRIMETHYLHEXANE	ND	ND	ND	ND	ND	ND	ND	ND	ND
2,3-DIMETHYLHEPTANE	ND	ND	ND	ND	ND	ND	ND	ND	ND
m-& p-XYLENE	ND	ND	ND	ND	ND	ND	ND	ND	ND
4-METHYLOCTANE	ND	ND	ND	ND	ND	ND	ND	ND	ND
3,4-DIMETHYLHEPTANE	ND	ND	ND	ND	ND	ND	ND	ND	ND
4-ETHYLHEPTANE	ND	ND	ND	ND	ND	ND	ND	ND	ND
2-METHYLOCTANE	ND	ND	ND	ND	ND	ND	ND	ND	ND
3-METHYLOCTANE	ND	ND	ND	ND	ND	ND	ND	ND	ND
STYRENE	ND	ND	ND	ND	ND	ND	ND	ND	ND
o-XYLENE	ND	ND	ND	ND	ND	ND	ND	ND	ND
1-NONENE	ND	ND	ND	ND	ND	ND	ND	ND	ND
TRANS-3-NONENE	ND	ND	ND	ND	ND	ND	ND	ND	ND
CIS-3-NONENE	ND	ND	ND	ND	ND	ND	ND	ND	ND
NONANE	ND	ND	ND	ND	ND	ND	ND	ND	ND
TRANS-2-NONENE	ND	ND	ND	ND	ND	ND	ND	ND	ND
ISOPROPYLBENZENE (CUMENE)	ND	ND	ND	ND	ND	ND	ND	ND	ND
2,2-DIMETHYLOCTANE	ND	ND	ND	ND	ND	ND	ND	ND	ND
2,4-DIMETHYLOCTANE	ND	ND	ND	ND	ND	ND	ND	ND	ND
n-PROPYLBENZENE	ND	ND	ND	ND	ND	ND	ND	ND	ND
1-METHYL-3-ETHYLBENZENE	ND	ND	ND	ND	ND	ND	ND	ND	ND
1-METHYL-4-ETHYLBENZENE	ND	ND	ND	ND	ND	ND	ND	ND	ND
1,3,5-TRIMETHYLBENZENE	ND	ND	ND	ND	ND	ND	ND	ND	ND
1-METHYL-2-ETHYLBENZENE	ND	ND	ND	ND	ND	ND	ND	ND	ND
1,2,4-TRIMETHYLBENZENE	0.4	0.3	0.4	ND	ND	ND	ND	ND	ND
TERT-BUTYLBENZENE	ND	ND	ND	ND	ND	ND	ND	ND	ND
1-DECENE	ND	ND	ND	ND	ND	ND	ND	ND	ND
DECANE, NOTE F	ND	ND	ND	ND	ND	ND	ND	ND	ND
ISOBUTYLBENZENE, NOTE F	ND	ND	ND	ND	ND	ND	ND	ND	ND
1,3,-DIMETHYL-5-ETHYLBENZENE	ND	ND	ND	ND	ND	ND	ND	ND	ND
METHYLPROPYLBENZENE (sec butylbenzene)	ND	ND	ND	ND	ND	ND	ND	ND	ND
1-METHYL-3-ISOPROPYLBENZENE	ND	ND	ND	ND	ND	ND	ND	ND	ND
1,2,3-TRIMETHYLBENZENE	ND	ND	ND	ND	ND	ND	ND	ND	ND
1-METHYL-4-ISOPROPYLBENZENE	ND	ND	ND	ND	ND	ND	ND	ND	ND
INDAN	ND	ND	ND	ND	ND	ND	ND	ND	ND
1-METHYL-2-ISOPROPYLBENZENE	ND	ND	ND	ND	ND	ND	ND	ND	ND
1,3-DIETHYLBENZENE	ND	ND	ND	ND	ND	ND	ND	ND	ND
1,4-DIETHYLBENZENE	ND	ND	ND	ND	ND	ND	ND	ND	ND
1-METHYL-3-N-PROPYLBENZENE	ND	ND	ND	ND	ND	ND	ND	ND	ND
1-METHYL-4-N-PROPYLBENZENE, NOTE G	ND	ND	ND	ND	ND	ND	ND	ND	ND
1,2 DIETHYLBENZENE	ND	ND	ND	ND	ND	ND	ND	ND	ND
1-METHYL-2-N-PROPYLBENZENE	ND	ND	ND	ND	ND	ND	ND	ND	ND
1,4-DIMETHYL-2-ETHYLBENZENE	ND	ND	ND	ND	ND	ND	ND	ND	ND
1,3-DIMETHYL-4-ETHYLBENZENE	ND	ND	ND	ND	ND	ND	ND	ND	ND
1,2-DIMETHYL-4-ETHYLBENZENE	ND	ND	ND	ND	ND	ND	ND	ND	ND
1,3-DIMETHYL-2-ETHYLBENZENE	ND	ND	ND	ND	ND	ND	ND	ND	ND
UNDECANE	ND	ND	ND	ND	ND	ND	ND	ND	ND
1,2-DIMETHYL-3-ETHYLBENZENE	ND	ND	ND	ND	ND	ND	ND	ND	ND
1,2,4,5-TETRAMETHYLBENZENE	ND	ND	ND	ND	ND	ND	ND	ND	ND
2-METHYLBUTYLBENZENE (sec AMYLBENZENE)	ND	ND	ND	ND	ND	ND	ND	ND	ND

TABLE B-1 (CONT'D). HYDROCARBON SPECIATION DATA FOR 211b BASELINE FUEL WITH AFTERTREATMENT

	mg/mile			mg/mile			mg/mile		
	RFBASECAT-2	RFBASECAT-3	RFBASECAT-6	RFBASECAT-2	RFBASECAT-3	RFBASECAT-6	RFBASECAT-2	RFBASECAT-3	RFBASECAT-6
COMPOUND	C505	C505	C505	C867	C867	C867	H505	H505	H505
3,4 DIMETHYLCUMENE	ND	ND	ND	ND	ND	ND	ND	ND	ND
1,2,3,5-TETRAMETHYLBENZENE	ND	ND	ND	ND	ND	ND	ND	ND	ND
TERT-1-BUT-2-METHYLBENZENE	ND	ND	ND	ND	ND	ND	ND	ND	ND
1,2,3,4-TETRAMETHYLBENZENE	ND	ND	ND	ND	ND	ND	ND	ND	ND
N-PENT-BENZENE	ND	ND	ND	ND	ND	ND	ND	ND	ND
TERT-1-BUT-3,5-DIMETHYLBENZENE	ND	ND	ND	ND	ND	ND	ND	ND	ND
TERT-1-BUTYL-4-ETHYLBENZENE	ND	ND	ND	ND	ND	ND	ND	ND	ND
NAPHTHALENE	ND	ND	ND	ND	ND	ND	ND	ND	ND
DODECANE	ND	ND	ND	ND	ND	ND	ND	ND	ND
1,3,5-TRIETHYLBENZENE	ND	ND	ND	ND	ND	ND	ND	ND	ND
1,2,4-TRIETHYLBENZENE	ND	ND	ND	ND	ND	ND	ND	ND	ND
HEXYLBENZENE	ND	ND	ND	ND	ND	ND	ND	ND	ND
UNIDENTIFIED C9-C12+	0.1	ND	ND	ND	ND	ND	ND	ND	ND
FORMALDEHYDE	1.0	1.1	1.2	0.1	0.1	0.1	Trace	Trace	Trace
ACETALDEHYDE	0.5	0.5	0.6	Trace	Trace	Trace	Trace	Trace	Trace
ACROLEIN	0.2	0.2	0.2	Trace	Trace	ND	Trace	ND	ND
ACETONE	0.4	ND	0.4	Trace	ND	Trace	Trace	Trace	Trace
PROPIONALDEHYDE	0.2	0.2	0.2	Trace	ND	ND	Trace	ND	ND
CROTONALDEHYDE	ND	ND	0.1	ND	ND	ND	ND	ND	ND
METHACROLEIN	0.2	0.1	0.2	Trace	Trace	ND	Trace	ND	ND
N-/ISOBUTYRALDEHYDE	0.1	Trace	Trace	ND	Trace	ND	ND	ND	ND
METHYL ETHYL KETONE	ND	ND	Trace	ND	ND	ND	ND	ND	ND
BENZALDEHYDE	0.3	0.4	0.4	Trace	Trace	ND	Trace	Trace	ND
ISOVALERALDEHYDE	Trace	ND	Trace	Trace	ND	Trace	ND	ND	Trace
VALERALDEHYDE	ND	ND	ND	ND	ND	ND	ND	ND	Trace
O-TOLUALDEHYDE	ND	Trace	ND	ND	ND	ND	ND	ND	0.1
M/P-TOLUALDEHYDE	Trace	0.1	Trace	ND	ND	ND	ND	ND	ND
METHYL ISOBUTYL KETONE	ND	ND	ND	ND	ND	ND	ND	ND	ND
HEXANALDEHYDE	ND	ND	ND	ND	ND	ND	ND	ND	ND
DIMETHYLBENZALDEHYDE	Trace	ND	ND	Trace	ND	ND	Trace	ND	ND
SUMMED SPECIATED VALUES	107.3	109.4	142.2	0.7	1.9	3.8	2.5	3.2	10.6

A - 2,2-Dimethylpentane and methylcyclopentane co-elute. GC peak area split equally between the two compounds.

B - 3-Methyl-3-ethyl-pentane co-elutes with reported compound. Not reported separately.

C - Cis-1,4-Dimethylcyclohexane co-elutes with reported compound. Not reported separately.

D - Propylcyclopentane co-elutes with reported compound. Not reported separately.

E - 2,5-Dimethylheptane and 3,5-dimethylheptane co-elute. GC peak area split equally between the two compounds.

F - Decane and isobutylbenzene co-elute. GC peak area split equally between the two compounds.

G - n-Butylbenzene co-elutes with reported compound. Not reported separately.

TABLE B-2. HYDROCARBON SPECIATION DATA FOR 211b BASELINE FUEL WITHOUT AFTERTREATMENT

	mg/mile			mg/mile			mg/mile		
	RFABASE-1	RFABASE-2	RFABASE-4	RFABASE-1	RFABASE-2	RFABASE-4	RFABASE-1	RFABASE-2	RFABASE-4
COMPOUND	C505	C505	C505	C867	C867	C867	H505	H505	H505
METHANE	49.6	52.7	52.7	52.9	50.4	52.7	37.4	36.7	38.7
ETHANE	17.9	19.7	19.7	17.2	16.1	15.9	12.0	11.7	13.4
ETHYLENE	103.9	111.7	110.5	108.2	100.7	98.6	74.5	72.4	81.6
PROPANE	1.4	3.0	1.6	1.8	1.1	1.7	1.1	0.6	1.2
PROPYLENE	96.5	103.7	102.7	90.7	85.0	82.3	66.9	64.8	72.9
ACETYLENE	66.5	73.4	72.3	71.8	67.2	68.5	48.5	47.1	55.7
PROPADIENE	ND	ND	ND	ND	ND	ND	ND	ND	ND
BUTANE	8.4	9.8	9.2	10.9	10.9	10.9	7.1	7.0	8.4
TRANS-2-BUTENE	8.7	9.3	9.3	8.3	7.8	7.5	6.0	5.9	6.7
1-BUTENE	9.9	10.6	10.5	9.5	8.7	8.4	6.9	6.7	7.4
2-METHYLPROPENE (ISOBUTYLENE)	63.7	69.0	69.0	61.5	58.8	57.0	44.5	44.1	50.2
2,2-DIMETHYLPROPANE (NEOPENTANE)	84.4	100.5	93.6	101.3	95.4	98.2	68.2	65.4	79.4
PROPYNE	1.0	1.3	1.1	1.2	1.1	1.1	0.8	0.8	0.9
1,3-BUTADIENE	12.0	16.4	15.8	11.5	12.1	11.5	6.5	8.6	9.7
2-METHYLPROPANE (ISOBUTANE)	8.3	9.4	5.4	6.8	5.9	5.7	5.3	5.2	5.5
1-BUTYNE	ND	ND	ND	ND	ND	ND	ND	ND	ND
METHANOL	7.9	9.7	7.6	5.1	8.2	6.6	5.3	5.5	5.5
CIS-2-BUTENE	6.1	6.6	6.6	5.8	5.5	5.3	4.2	4.2	4.6
3-METHYL-1-BUTENE	0.6	3.6	0.7	3.5	2.1	2.0	2.5	0.4	1.7
ETHANOL	0.6	0.6	0.6	0.9	0.6	0.6	0.4	0.8	0.5
2-METHYLBUTANE (ISOPENTANE)	10.0	11.4	10.8	8.7	8.1	7.9	6.7	6.6	7.1
2-BUTYNE	1.4	1.6	1.4	1.3	2.3	2.2	1.4	1.4	0.9
1-PENTENE	0.3	1.4	0.3	0.2	0.5	0.5	0.3	0.3	0.1
2-METHYL-1-BUTENE	6.5	7.7	6.6	5.9	8.1	7.5	4.6	4.6	5.4
PENTANE	17.9	23.9	18.4	19.1	23.2	23.6	13.2	12.9	17.2
UNIDENTIFIED C5 OLEFINS	ND	ND	ND	ND	ND	ND	ND	ND	ND
2-METHYL-1,3-BUTADIENE	2.9	6.7	5.2	3.1	5.5	5.3	1.2	1.8	1.9
TRANS-2-PENTENE	1.4	1.7	1.5	1.2	1.7	1.5	1.0	1.1	1.1
3,3-DIMETHYL-1-BUTENE	0.8	1.0	0.8	0.7	0.9	0.9	0.5	0.6	0.6
CIS-2-PENTENE	0.8	1.0	0.8	0.7	0.9	0.9	0.5	0.6	0.6
2-METHYL-2-BUTENE	4.3	7.3	7.2	7.0	8.2	8.0	2.3	1.9	2.9
TERT-BUTANOL	ND	ND	ND	ND	ND	ND	ND	ND	ND
CYCLOPENTADIENE	0.1	0.2	0.2	0.2	0.3	0.4	ND	ND	ND
2,2-DIMETHYLBUTANE	1.7	2.6	1.9	1.8	2.4	2.4	1.2	1.2	1.7
CYCLOPENTENE	0.7	0.8	0.7	0.9	0.9	0.9	0.4	0.5	0.6
4-METHYL-1-PENTENE	ND	ND	ND	ND	ND	ND	ND	ND	ND
3-METHYL-1-PENTENE	2.5	3.3	2.4	1.6	2.2	2.2	0.6	1.5	1.9
CYCLOPENTANE	1.9	2.3	1.8	1.9	2.3	2.2	1.4	1.3	1.5
2,3-DIMETHYLBUTANE	7.1	9.6	7.1	7.5	8.9	8.7	1.3	4.9	6.6
MTBE	ND	ND	ND	ND	ND	ND	ND	ND	ND
4-METHYL-CIS-2-PENTENE	ND	ND	ND	ND	ND	ND	ND	ND	ND
2-METHYLPENTANE	9.2	12.6	0.8	9.5	11.3	11.4	6.4	6.2	8.7
4-METHYL-TRANS-2-PENTENE	0.6	0.8	0.6	0.6	0.6	0.5	0.4	0.4	0.5
3-METHYLPENTANE	5.3	7.2	5.2	5.8	6.8	6.4	4.1	3.8	4.9
2-METHYL-1-PENTENE	1.2	1.1	0.6	0.6	0.4	0.7	0.7	0.3	0.6
1-HEXENE	0.5	0.4	0.6	0.6	0.4	0.7	0.5	0.3	0.6
HEXANE	8.0	11.1	8.2	8.3	9.9	9.9	5.6	5.4	7.5
UNIDENTIFIED C6	3.7	5.1	4.3	1.9	1.9	3.5	7.3	2.0	3.3
TRANS-3-HEXENE	ND	ND	ND	ND	ND	ND	ND	ND	ND

TABLE B-2 (CONT'D). HYDROCARBON SPECIATION DATA FOR 211b BASELINE FUEL WITHOUT AFTERTREATMENT

	mg/mile			mg/mile			mg/mile		
	RFABASE-1	RFABASE-2	RFABASE-4	RFABASE-1	RFABASE-2	RFABASE-4	RFABASE-1	RFABASE-2	RFABASE-4
COMPOUND	C505	C505	C505	C867	C867	C867	H505	H505	H505
CIS-3-HEXENE	0.1	0.2	0.1	ND	ND	0.1	ND	ND	ND
DI-ISOPROPYL ETHER	ND	ND	ND	ND	ND	ND	ND	ND	ND
TRANS-2-HEXENE	0.2	0.3	0.2	0.2	0.2	0.3	0.1	0.1	0.2
3-METHYL-TRANS-2-PENTENE	0.6	0.8	0.6	0.6	0.7	0.7	0.4	0.4	0.6
2-METHYL-2-PENTENE	0.9	1.0	0.9	0.8	0.9	1.0	0.5	0.5	0.7
3-METHYLCYCLOPENTENE	ND	ND	ND	ND	ND	ND	ND	ND	ND
CIS-2-HEXENE	0.1	0.4	0.1	0.1	0.4	0.4	ND	ND	ND
ETBE	ND	ND	ND	ND	ND	ND	ND	ND	ND
3-METHYL-CIS-2-PENTENE	0.3	0.5	0.4	0.4	0.4	0.4	0.2	0.2	0.4
2,2-DIMETHYLPENTANE, NOTE A	2.5	3.4	2.5	2.4	3.0	3.0	1.7	1.6	2.3
METHYLCYCLOPENTANE, NOTE A	2.4	3.3	2.4	2.3	2.9	3.0	1.6	1.6	2.3
2,4-DIMETHYLPENTANE	6.3	8.6	6.4	1.3	7.8	8.2	4.4	4.3	6.0
2,2,3-TRIMETHYLBUTANE	0.7	0.9	0.4	6.5	0.5	1.0	0.5	0.5	0.6
3,4-DIMETHYL-1-PENTENE	0.5	0.5	0.4	0.3	0.4	0.6	0.2	0.3	0.6
1-METHYLCYCLOPENTENE	0.1	5.0	0.3	0.2	4.4	0.3	0.2	3.0	0.2
BENZENE	34.2	54.2	52.6	36.8	47.9	44.5	24.8	35.1	36.8
3-METHYL-1-HEXENE	ND	ND	ND	ND	ND	ND	ND	ND	ND
3,3-DIMETHYLPENTANE	0.4	0.3	0.3	0.4	0.4	0.4	0.2	0.2	0.3
CYCLOHEXANE	3.0	3.2	4.6	3.5	4.0	4.3	2.4	3.0	3.5
2-METHYLHEXANE	7.9	7.8	7.9	7.7	9.8	9.8	5.4	5.3	7.4
2,3-DIMETHYLPENTANE	0.6	1.0	0.7	0.4	0.6	0.6	0.3	0.3	0.5
1,1-DIMETHYLCYCLOPENTANE	ND	ND	ND	ND	ND	ND	ND	ND	ND
TERT-AMYL METHYL ETHER	ND	ND	ND	ND	ND	ND	ND	ND	ND
CYCLOHEXENE	1.1	1.0	0.9	0.8	1.1	1.0	0.6	0.7	0.8
3-METHYLHEXANE	2.5	3.2	2.5	2.3	3.0	3.1	1.6	1.6	2.3
CIS-1,3-DIMETHYLCYCLOPENTANE	0.5	0.6	0.5	0.3	0.5	0.5	0.3	0.3	0.4
3-ETHYLPENTANE	0.6	0.8	0.6	0.5	0.7	0.7	0.4	0.4	0.5
TRANS-1,2-DIMETHYLCYCLOPENTANE	ND	ND	ND	ND	ND	ND	ND	ND	ND
TRANS-1,3-DIMETHYLCYCLOPENTANE	0.6	0.8	0.6	0.6	0.7	0.7	0.4	0.4	0.5
1-HEPTENE	ND	ND	ND	ND	ND	ND	ND	ND	ND
2,2,4-TRIMETHYLPENTANE	86.1	114.3	89.1	88.4	109.8	109.6	61.0	59.0	82.4
2-METHYL-1-HEXENE	ND	0.2	ND	ND	ND	ND	ND	ND	ND
TRANS-3-HEPTENE	ND	ND	ND	ND	ND	ND	ND	ND	ND
HEPTANE	3.7	4.8	3.8	3.7	4.1	4.1	2.4	2.1	3.2
CIS-3-HEPTENE	ND	ND	ND	ND	ND	ND	ND	ND	ND
UNIDENTIFIED C7	3.0	1.8	2.5	0.8	1.8	1.8	1.1	1.1	1.8
2-METHYL-2-HEXENE	ND	0.3	0.1	ND	ND	ND	ND	ND	ND
3-METHYL-TRANS-3-HEXENE	ND	ND	ND	ND	ND	ND	ND	ND	ND
TRANS-2-HEPTENE	ND	0.1	0.1	ND	ND	ND	ND	ND	ND
3-ETHYL-CIS-2-PENTENE	ND	ND	ND	ND	ND	ND	ND	ND	ND
2,4,4-TRIMETHYL-1-PENTENE	0.2	0.3	0.3	0.2	0.3	0.4	0.2	0.2	0.2
2,3-DIMETHYL-2-PENTENE	ND	ND	ND	ND	ND	ND	ND	ND	ND
CIS-2-HEPTENE	0.4	0.6	0.6	0.4	0.6	0.6	0.3	0.3	0.3
METHYLCYCLOHEXANE	5.6	7.6	6.0	5.6	7.0	7.1	3.9	3.7	5.3
CIS-1,2-DIMETHYLCYCLOPENTANE	ND	ND	ND	ND	ND	ND	ND	ND	ND
2,2-DIMETHYLHEXANE	0.4	0.4	0.4	0.4	0.5	0.5	0.3	0.2	0.3
1,1,3-TRIMETHYLCYCLOPENTANE	ND	ND	0.2	ND	ND	ND	ND	ND	ND
2,4,4-TRIMETHYL-2-PENTENE	0.4	0.1	0.3	0.5	0.6	0.5	0.2	0.2	0.3

TABLE B-2 (CONT'D). HYDROCARBON SPECIATION DATA FOR 211b BASELINE FUEL WITHOUT AFTERTREATMENT

	mg/mile			mg/mile			mg/mile		
	RFABASE-1	RFABASE-2	RFABASE-4	RFABASE-1	RFABASE-2	RFABASE-4	RFABASE-1	RFABASE-2	RFABASE-4
COMPOUND	C505	C505	C505	C867	C867	C867	H505	H505	H505
2,2,3-TRIMETHYLPENTANE	9.6	11.9	10.0	11.1	13.2	13.3	7.7	7.4	8.9
2,5-DIMETHYLHEXANE	ND	ND	ND	ND	ND	ND	ND	ND	ND
ETHYLCYCLOPENTANE	ND	ND	ND	ND	ND	ND	ND	ND	ND
2,4-DIMETHYLHEXANE	10.8	15.1	11.5	9.9	13.3	13.4	6.8	6.6	10.6
1-TRANS-2-CIS-4-TRIMETHYLCYCLOPENTANE	0.3	0.4	0.2	0.2	0.5	0.4	0.2	0.2	0.3
3,3-DIMETHYLHEXANE	ND	ND	ND	ND	ND	ND	ND	ND	ND
1-TRANS-2-CIS-3-TRIMETHYLCYCLOPENTANE	0.3	0.1	0.1	ND	ND	0.2	ND	ND	ND
2,3,4-TRIMETHYLPENTANE	27.9	36.8	29.1	29.5	36.9	37.3	20.4	19.5	27.8
2,3,3-TRIMETHYLPENTANE	14.8	9.0	22.3	18.9	7.3	22.4	12.6	4.6	18.0
TOLUENE	221.6	236.0	293.9	237.2	207.4	249.8	162.0	195.6	205.5
2,3-DIMETHYLHEXANE	11.2	20.8	17.4	13.2	17.9	16.3	8.8	0.4	13.6
1,1,2-TRIMETHYLCYCLOPENTANE	ND	ND	ND	ND	ND	ND	ND	ND	ND
2-METHYLHEPTANE	1.0	1.3	1.0	0.9	1.2	1.3	0.7	0.6	0.9
3,4-DIMETHYLHEXANE, NOTE B	1.6	1.8	1.5	1.3	1.9	2.0	1.1	0.9	1.5
4-METHYLHEPTANE	ND	ND	ND	ND	ND	ND	ND	ND	ND
3-METHYLHEPTANE	0.9	1.0	0.8	0.7	1.0	1.0	0.6	0.4	0.8
1-CIS,2-TRANS,3-TRIMETHYLCYCLOPENTANE	0.8	0.9	0.7	0.6	0.8	0.9	0.5	0.3	0.7
CIS-1,3-DIMETHYLCYCLOHEXANE	ND	ND	ND	ND	ND	ND	ND	ND	ND
TRANS-1,4-DIMETHYLCYCLOHEXANE	0.3	0.3	0.3	0.2	0.3	0.4	0.2	0.2	0.2
3-ETHYLHEXANE	ND	ND	ND	ND	ND	ND	ND	ND	ND
2,2,5-TRIMETHYLHEXANE	11.1	14.0	11.4	11.7	14.5	14.7	8.2	7.7	10.9
TRANS-1-METHYL-3-ETHYLCYCLOPENTANE	ND	ND	ND	ND	ND	ND	ND	ND	ND
CIS-1-METHYL-3-ETHYLCYCLOPENTANE	ND	ND	ND	ND	ND	ND	ND	ND	ND
1,1-DIMETHYLCYCLOHEXANE	ND	ND	ND	ND	ND	ND	ND	ND	ND
TRANS-1-METHYL-2-ETHYLCYCLOPENTANE	ND	ND	ND	ND	ND	ND	ND	ND	ND
1-METHYL-1-ETHYL-CYCLOPENTANE	0.7	1.1	0.9	0.8	1.1	1.3	0.7	0.6	1.0
2,4,4-TRIMETHYLHEXANE	ND	ND	ND	ND	ND	ND	ND	ND	ND
2,2,4-TRIMETHYLHEXANE	ND	ND	ND	ND	ND	ND	ND	ND	ND
TRANS-1,2-DIMETHYLCYCLOHEXANE	0.3	0.3	0.3	0.3	0.3	0.4	0.2	0.2	0.2
1-OCTENE	ND	ND	ND	ND	ND	ND	ND	ND	ND
TRANS-4-OCTENE	ND	ND	ND	ND	ND	ND	ND	ND	ND
OCTANE	1.1	1.4	1.1	1.0	1.4	1.4	0.8	0.7	1.0
UNIDENTIFIED C8	0.3	0.3	0.1	ND	ND	0.4	ND	ND	0.2
TRANS-2-OCTENE	0.2	0.2	0.2	0.3	0.2	0.2	0.1	ND	0.2
TRANS-1,3-DIMETHYLCYCLOHEXANE, NOTE C	0.3	0.4	0.3	0.3	0.4	0.4	0.2	0.2	0.3
CIS-2-OCTENE	0.2	0.2	0.1	ND	ND	0.2	ND	ND	0.2
ISOPROPYLCYCLOPENTANE	0.4	0.5	0.4	0.6	0.5	0.5	0.3	0.2	0.4
2,2-DIMETHYLHEPTANE	ND	ND	ND	ND	ND	ND	ND	ND	ND
2,3,5-TRIMETHYLHEXANE	1.7	2.1	1.8	1.8	2.3	2.3	1.3	1.2	1.7
CIS-1-METHYL-2-ETHYLCYCLOPENTANE	ND	ND	ND	ND	ND	ND	ND	ND	ND
2,4-DIMETHYLHEPTANE	0.3	0.4	0.4	0.3	0.4	0.4	0.2	0.4	0.3
4,4-DIMETHYLHEPTANE	ND	ND	ND	ND	ND	ND	ND	ND	ND
CIS-1,2-DIMETHYLCYCLOHEXANE	0.4	0.6	0.5	0.7	0.3	0.3	0.5	1.0	0.3
ETHYLCYCLOHEXANE	0.2	0.3	ND	ND	ND	ND	ND	ND	0.1
2,6-DIMETHYLHEPTANE, NOTE D	ND	ND	ND	ND	ND	ND	ND	ND	ND
1,1,3-TRIMETHYLCYCLOHEXANE	ND	ND	ND	ND	ND	ND	ND	ND	ND
2,5-DIMETHYLHEPTANE, NOTE E	0.4	0.6	0.5	0.4	0.4	0.4	0.4	0.7	0.4
3,3-DIMETHYLHEPTANE	ND	ND	ND	ND	ND	ND	ND	ND	ND
3,5-DIMETHYLHEPTANE, NOTE E	0.4	0.6	0.5	0.4	0.4	0.4	0.4	0.7	0.4

TABLE B-2 (CONT'D). HYDROCARBON SPECIATION DATA FOR 211b BASELINE FUEL WITHOUT AFTERTREATMENT

	mg/mile			mg/mile			mg/mile		
	RFABASE-1	RFABASE-2	RFABASE-4	RFABASE-1	RFABASE-2	RFABASE-4	RFABASE-1	RFABASE-2	RFABASE-4
COMPOUND	C505	C505	C505	C867	C867	C867	H505	H505	H505
ETHYLBENZENE	15.2	20.5	16.6	12.5	15.5	16.0	8.7	10.5	12.3
2,3,4-TRIMETHYLHEXANE	ND	ND	ND	ND	ND	ND	ND	ND	ND
2,3-DIMETHYLHEPTANE	ND	ND	ND	ND	ND	ND	ND	ND	ND
m-& p-XYLENE	15.9	21.7	18.5	13.8	17.5	18.8	9.4	11.9	14.5
4-METHYLOCTANE	ND	ND	ND	ND	ND	ND	ND	ND	ND
3,4-DIMETHYLHEPTANE	ND	ND	ND	ND	ND	ND	ND	ND	ND
4-ETHYLHEPTANE	ND	ND	ND	ND	ND	ND	ND	ND	ND
2-METHYLOCTANE	0.4	0.6	0.5	0.2	0.5	0.6	0.2	0.2	0.4
3-METHYLOCTANE	0.2	0.2	0.2	0.1	0.3	0.3	0.1	0.1	0.2
STYRENE	5.4	1.0	8.3	4.9	7.9	7.7	1.5	1.8	3.6
o-XYLENE	11.3	15.4	13.2	10.8	13.8	14.9	7.4	7.8	11.2
1-NONENE	0.7	1.0	0.8	0.9	1.1	1.3	0.6	0.6	0.8
TRANS-3-NONENE	ND	ND	ND	ND	ND	ND	ND	ND	ND
CIS-3-NONENE	ND	ND	ND	ND	ND	ND	ND	ND	ND
NONANE	0.9	1.3	1.0	0.9	1.2	1.3	0.7	0.7	1.0
TRANS-2-NONENE	ND	ND	ND	ND	ND	ND	ND	ND	ND
ISOPROPYLBENZENE (CUMENE)	2.2	2.9	2.4	2.3	3.0	3.1	1.6	1.6	2.4
2,2-DIMETHYLOCTANE	0.8	1.0	0.8	0.8	1.1	1.1	0.7	0.2	0.8
2,4-DIMETHYLOCTANE	ND	ND	ND	0.2	ND	ND	ND	ND	ND
n-PROPYLBENZENE	10.0	13.7	11.4	10.6	14.1	15.0	7.4	7.8	11.3
1-METHYL-3-ETHYLBENZENE	36.3	50.6	43.0	37.4	49.2	53.3	25.9	27.8	40.1
1-METHYL-4-ETHYLBENZENE	16.1	21.8	18.9	16.3	21.7	23.5	11.1	11.7	17.3
1,3,5-TRIMETHYLBENZENE	17.9	25.2	21.4	18.2	24.2	26.5	12.3	13.3	19.7
1-METHYL-2-ETHYLBENZENE	14.8	19.9	16.7	18.2	21.8	22.6	12.0	12.5	15.8
1,2,4-TRIMETHYLBENZENE	52.1	84.9	70.9	35.9	70.0	80.5	34.0	37.6	57.9
TERT-BUTYLBENZENE	ND	ND	ND	ND	ND	ND	ND	ND	ND
1-DECENE	ND	ND	ND	ND	ND	ND	ND	ND	ND
DECANE, NOTE F	0.3	1.3	1.3	ND	0.6	0.8	0.2	0.2	1.0
ISOBUTYLBENZENE, NOTE F	0.2	1.2	1.3	ND	0.6	0.7	0.2	0.2	1.0
1,3,-DIMETHYL-5-ETHYLBENZENE	ND	ND	ND	ND	ND	ND	ND	ND	ND
METHYLPROPYLBENZENE (sec butylbenzene)	ND	ND	ND	0.1	0.1	2.0	ND	ND	ND
1-METHYL-3-ISOPROPYLBENZENE	3.8	1.2	3.7	8.4	7.0	5.9	5.5	5.8	3.3
1,2,3-TRIMETHYLBENZENE	3.1	4.0	ND	5.6	4.7	5.4	3.1	3.1	3.1
1-METHYL-4-ISOPROPYLBENZENE	ND	ND	ND	ND	ND	ND	ND	ND	ND
INDAN	ND	ND	ND	ND	ND	ND	ND	ND	ND
1-METHYL-2-ISOPROPYLBENZENE	1.7	4.7	4.1	2.7	2.6	2.6	1.9	1.6	2.3
1,3-DIETHYLBENZENE	ND	4.7	3.9	ND	ND	ND	ND	ND	ND
1,4-DIETHYLBENZENE	2.2	2.4	2.0	2.4	4.5	4.5	1.8	1.7	3.8
1-METHYL-3-N-PROPYLBENZENE	ND	ND	ND	ND	ND	ND	ND	ND	ND
1-METHYL-4-N-PROPYLBENZENE, NOTE G	ND	ND	ND	ND	ND	ND	ND	ND	ND
1,2 DIETHYLBENZENE	ND	ND	ND	ND	0.3	ND	1.2	ND	5.3
1-METHYL-2-N-PROPYLBENZENE	2.4	4.1	3.5	4.3	4.7	3.1	2.8	2.7	3.2
1,4-DIMETHYL-2-ETHYLBENZENE	1.0	4.3	3.7	1.4	1.7	1.5	1.3	1.0	1.5
1,3-DIMETHYL-4-ETHYLBENZENE	ND	ND	ND	ND	ND	ND	ND	ND	ND
1,2-DIMETHYL-4-ETHYLBENZENE	0.9	4.8	4.0	0.5	3.3	1.4	0.4	1.0	1.0
1,3-DIMETHYL-2-ETHYLBENZENE	1.4	0.2	1.1	ND	ND	ND	ND	ND	ND
UNDECANE	1.5	0.2	1.1	ND	ND	ND	ND	ND	ND
1,2-DIMETHYL-3-ETHYLBENZENE	ND	ND	ND	ND	ND	ND	ND	ND	ND
1,2,4,5-TETRAMETHYLBENZENE	ND	ND	ND	ND	ND	ND	ND	ND	ND
2-METHYLBUTYLBENZENE (sec AMYLBENZENE)	ND	ND	ND	ND	ND	ND	ND	ND	ND

TABLE B-2 (CONT'D). HYDROCARBON SPECIATION DATA FOR 211b BASELINE FUEL WITHOUT AFTERTREATMENT

	mg/mile			mg/mile			mg/mile		
	RFABASE-1	RFABASE-2	RFABASE-4	RFABASE-1	RFABASE-2	RFABASE-4	RFABASE-1	RFABASE-2	RFABASE-4
COMPOUND	C505	C505	C505	C867	C867	C867	H505	H505	H505
3,4 DIMETHYLCUMENE	ND	ND	ND	ND	ND	ND	ND	ND	ND
1,2,3,5-TETRAMETHYLBENZENE	ND	ND	ND	ND	ND	ND	ND	ND	ND
TERT-1-BUT-2-METHYLBENZENE	ND	ND	ND	ND	ND	ND	ND	ND	ND
1,2,3,4-TETRAMETHYLBENZENE	ND	ND	ND	ND	ND	ND	ND	ND	ND
N-PENT-BENZENE	ND	ND	ND	ND	ND	ND	ND	ND	ND
TERT-1-BUT-3,5-DIMETHYLBENZENE	ND	ND	ND	ND	ND	ND	ND	ND	ND
TERT-1-BUTYL-4-ETHYLBENZENE	ND	ND	ND	ND	ND	ND	ND	ND	ND
NAPHTHALENE	ND	ND	ND	ND	ND	ND	ND	ND	ND
DODECANE	ND	ND	ND	ND	ND	ND	ND	ND	ND
1,3,5-TRIETHYLBENZENE	ND	ND	ND	ND	ND	ND	ND	ND	ND
1,2,4-TRIETHYLBENZENE	ND	ND	ND	ND	ND	ND	ND	ND	ND
HEXYLBENZENE	ND	ND	ND	ND	ND	ND	ND	ND	ND
UNIDENTIFIED C9-C12+	18.9	33.0	30.3	23.4	23.0	30.9	3.1	5.8	12.8
FORMALDEHYDE	56.4	54.6	58.9	56.4	54.4	59.0	41.4	38.5	39.9
ACETALDEHYDE	10.8	10.5	11.4	10.8	10.4	11.1	8.1	7.5	7.9
ACROLEIN	5.0	4.3	5.1	4.5	4.0	3.8	3.3	2.6	2.8
ACETONE	6.3	6.2	6.7	6.7	6.9	6.8	5.3	4.9	5.2
PROPIONALDEHYDE	4.4	5.0	4.7	4.4	5.1	4.7	3.5	3.6	3.4
CROTONALDEHYDE	2.9	3.4	3.3	3.2	3.4	3.2	2.5	2.3	2.4
METHACROLEIN	4.1	3.5	4.3	3.7	3.3	3.6	2.6	2.2	2.5
N-/ISOBUTYRALDEHYDE	0.9	1.0	1.2	1.1	1.2	1.1	0.7	0.7	0.8
METHYL ETHYL KETONE	0.6	0.7	0.8	0.5	0.5	0.8	0.3	0.3	0.4
BENZALDEHYDE	18.4	18.3	19.6	18.9	17.2	18.4	13.8	12.5	13.5
ISOVALERALDEHYDE	0.7	0.8	0.9	0.7	0.7	0.7	0.7	0.6	0.7
VALERALDEHYDE	0.1	0.4	0.4	0.3	0.2	0.1	0.3	0.3	0.3
O-TOLUALDEHYDE	1.5	1.8	1.9	1.5	4.4	1.7	1.1	1.0	1.2
M/P-TOLUALDEHYDE	6.3	6.6	5.9	6.3	2.1	5.6	4.7	3.8	4.2
METHYL ISOBUTYL KETONE	ND	ND	ND	ND	ND	ND	ND	ND	ND
HEXANALDEHYDE	0.1	0.3	0.3	0.2	1.3	0.3	0.2	1.1	0.1
DIMETHYLBENZALDEHYDE	4.7	6.1	6.2	4.7	4.9	6.1	3.6	3.8	3.5
SUMMED SPECIATED VALUES	1482.2	1789.4	1708.9	1525.5	1619.5	1706.0	1057.0	1079.4	1324.1

A - 2,2-Dimethylpentane and methylcyclopentane co-elute. GC peak area split equally between the two compounds.
B - 3-Methyl-3-ethyl-pentane co-elutes with reported compound. Not reported separately.
C - Cis-1,4-Dimethylcyclohexane co-elutes with reported compound. Not reported separately.
D - Propylcyclopentane co-elutes with reported compound. Not reported separately.
E - 2,5-Dimethylheptane and 3,5-dimethylheptane co-elute. GC peak area split equally between the two compounds.
F - Decane and isobutylbenzene co-elute. GC peak area split equally between the two compounds.
G - n-Butylbenzene co-elutes with reported compound. Not reported separately.

TABLE B-3. HYDROCARBON SPECIATION DATA FOR E10 WITH AFTERTREATMENT

	mg/mile			mg/mile			mg/mile		
	ADDCAT-1	ADDCAT-2	ADDCAT-3	ADDCAT-1	ADDCAT-2	ADDCAT-3	ADDCAT-1	ADDCAT-2	ADDCAT-3
COMPOUND	C505	C505	C505	C867	C867	C867	H505	H505	H505
METHANE	12.6	10.3	11.2	0.5	1.0	0.8	2.8	3.4	2.5
ETHANE	3.0	1.7	1.8	ND	ND	ND	0.1	0.2	0.1
ETHYLENE	6.3	3.4	3.8	Trace	ND	Trace	ND	Trace	Trace
PROPANE	1.1	0.1	0.1	ND	ND	ND	ND	ND	Trace
PROPYLENE	3.9	2.3	2.5	ND	ND	ND	ND	ND	ND
ACETYLENE	1.9	1.0	1.0	ND	ND	Trace	Trace	Trace	Trace
PROPADIENE	ND	ND	ND	ND	ND	ND	ND	ND	ND
BUTANE	0.1	0.3	0.3	ND	ND	Trace	Trace	Trace	Trace
TRANS-2-BUTENE	0.3	0.2	0.2	ND	ND	ND	ND	ND	ND
1-BUTENE	ND	ND	ND	ND	ND	ND	ND	ND	ND
2-METHYLPROPENE (ISOBUTYLENE)	2.4	1.4	1.6	ND	ND	ND	ND	ND	ND
2,2-DIMETHYLPROPANE (NEOPENTANE)	0.2	0.1	0.1	ND	ND	ND	ND	ND	ND
PROPYNE	ND	ND	ND	ND	ND	ND	ND	ND	ND
1,3-BUTADIENE	0.5	0.2	0.2	ND	ND	ND	ND	ND	ND
2-METHYLPROPANE (ISOBUTANE)	Trace	Trace	Trace	ND	ND	ND	ND	ND	ND
1-BUTYNE	ND	ND	ND	ND	ND	ND	ND	ND	ND
METHANOL	ND	ND	ND	ND	ND	ND	ND	ND	ND
CIS-2-BUTENE	0.2	0.1	0.2	ND	ND	ND	ND	ND	ND
3-METHYL-1-BUTENE	ND	ND	ND	ND	ND	ND	ND	ND	ND
ETHANOL	1.2	0.8	1.3	ND	ND	ND	ND	ND	ND
2-METHYLBUTANE (ISOPENTANE)	5.3	4.5	4.7	ND	ND	ND	0.3	0.4	0.3
2-BUTYNE	ND	ND	ND	ND	ND	ND	ND	ND	ND
1-PENTENE	ND	ND	ND	ND	ND	ND	ND	ND	ND
2-METHYL-1-BUTENE	ND	ND	ND	ND	ND	ND	ND	ND	ND
PENTANE	1.1	1.1	0.7	0.1	ND	ND	ND	Trace	ND
UNIDENTIFIED C5 OLEFINS	ND	ND	ND	ND	ND	ND	ND	ND	ND
2-METHYL-1,3-BUTADIENE	ND	ND	ND	ND	ND	ND	ND	ND	ND
TRANS-2-PENTENE	ND	ND	ND	ND	ND	ND	ND	ND	ND
3,3-DIMETHYL-1-BUTENE	ND	ND	ND	ND	ND	ND	ND	ND	ND
CIS-2-PENTENE	ND	ND	ND	ND	ND	ND	ND	ND	ND
2-METHYL-2-BUTENE	0.2	0.2	0.1	0.1	0.2	0.2	ND	ND	ND
TERT-BUTANOL	ND	ND	ND	ND	ND	ND	ND	ND	ND
CYCLOPENTADIENE	ND	ND	ND	ND	ND	ND	ND	ND	ND
2,2-DIMETHYLBUTANE	0.1	0.2	0.1	ND	ND	ND	ND	ND	ND
CYCLOPENTENE	ND	ND	ND	ND	ND	ND	ND	ND	ND
4-METHYL-1-PENTENE	ND	ND	ND	ND	ND	ND	ND	ND	ND
3-METHYL-1-PENTENE	ND	ND	ND	ND	ND	ND	ND	ND	ND
CYCLOPENTANE	0.1	0.1	0.1	ND	ND	ND	ND	ND	ND
2,3-DIMETHYLBUTANE	0.5	0.4	0.4	ND	ND	ND	ND	Trace	ND
MTBE	ND	ND	ND	ND	ND	ND	ND	ND	ND
4-METHYL-CIS-2-PENTENE	ND	ND	ND	ND	ND	ND	ND	ND	ND
2-METHYLPENTANE	0.6	0.5	0.4	ND	Trace	ND	0.1	ND	ND
4-METHYL-TRANS-2-PENTENE	ND	ND	ND	ND	ND	ND	ND	ND	ND
3-METHYLPENTANE	0.4	0.4	0.4	ND	ND	ND	ND	0.1	ND
2-METHYL-1-PENTENE	ND	ND	ND	ND	ND	ND	ND	ND	ND
1-HEXENE	ND	ND	ND	ND	ND	ND	ND	ND	ND
HEXANE	0.6	0.6	0.3	ND	0.2	ND	ND	ND	ND
UNIDENTIFIED C6	ND	ND	ND	ND	ND	ND	ND	ND	ND
TRANS-3-HEXENE	ND	ND	ND	ND	ND	ND	ND	ND	ND

TABLE B-3 (CONT'D). HYDROCARBON SPECIATION DATA FOR E10 WITH AFTERTREATMENT

	mg/mile			mg/mile			mg/mile		
	ADDCAT-1	ADDCAT-2	ADDCAT-3	ADDCAT-1	ADDCAT-2	ADDCAT-3	ADDCAT-1	ADDCAT-2	ADDCAT-3
COMPOUND	C505	C505	C505	C867	C867	C867	H505	H505	H505
CIS-3-HEXENE	ND	ND	ND	ND	ND	ND	ND	ND	ND
DI-ISOPROPYL ETHER	ND	ND	ND	ND	ND	ND	ND	ND	ND
TRANS-2-HEXENE	ND	ND	ND	ND	ND	ND	ND	ND	ND
3-METHYL-TRANS-2-PENTENE	ND	ND	ND	ND	ND	ND	ND	ND	ND
2-METHYL-2-PENTENE	ND	ND	ND	ND	ND	ND	ND	ND	ND
3-METHYLCYCLOPENTENE	ND	ND	ND	ND	ND	ND	ND	ND	ND
CIS-2-HEXENE	ND	ND	ND	ND	ND	ND	ND	ND	ND
ETBE	ND	ND	ND	ND	ND	ND	ND	ND	ND
3-METHYL-CIS-2-PENTENE	ND	ND	ND	ND	ND	ND	ND	ND	ND
2,2-DIMETHYLPENTANE, NOTE A	0.2	0.1	0.1	ND	ND	ND	ND	Trace	ND
METHYLCYCLOPENTANE, NOTE A	0.2	0.1	0.1	ND	ND	ND	ND	Trace	ND
2,4-DIMETHYLPENTANE	0.4	0.4	0.4	ND	ND	ND	ND	ND	ND
2,2,3-TRIMETHYLBUTANE	ND	ND	ND	ND	ND	ND	ND	ND	ND
3,4-DIMETHYL-1-PENTENE	ND	ND	ND	ND	ND	ND	ND	ND	ND
1-METHYLCYCLOPENTENE	ND	ND	ND	ND	ND	ND	ND	ND	ND
BENZENE	4.5	2.2	2.4	ND	ND	ND	ND	ND	ND
3-METHYL-1-HEXENE	ND	ND	ND	ND	ND	ND	ND	ND	ND
3,3-DIMETHYLPENTANE	ND	ND	ND	ND	ND	ND	ND	ND	ND
CYCLOHEXANE	0.3	0.2	0.2	ND	ND	ND	ND	ND	ND
2-METHYLHEXANE	0.6	0.5	0.4	ND	Trace	ND	ND	ND	ND
2,3-DIMETHYLPENTANE	ND	ND	ND	ND	ND	ND	ND	ND	ND
1,1-DIMETHYLCYCLOPENTANE	ND	ND	ND	ND	ND	ND	ND	ND	ND
TERT-AMYL METHYL ETHER	ND	ND	ND	ND	ND	ND	ND	ND	ND
CYCLOHEXENE	ND	ND	ND	ND	ND	ND	ND	ND	ND
3-METHYLHEXANE	0.2	0.1	0.1	ND	ND	ND	ND	ND	ND
CIS-1,3-DIMETHYLCYCLOPENTANE	ND	ND	ND	ND	ND	ND	ND	ND	ND
3-ETHYLPENTANE	ND	ND	ND	ND	ND	ND	ND	ND	ND
TRANS-1,2-DIMETHYLCYCLOPENTANE	ND	ND	ND	ND	ND	ND	ND	ND	ND
TRANS-1,3-DIMETHYLCYCLOPENTANE	ND	ND	ND	ND	ND	ND	ND	ND	ND
1-HEPTENE	ND	ND	ND	ND	ND	ND	ND	ND	ND
2,2,4-TRIMETHYLPENTANE	5.4	5.0	4.1	ND	Trace	ND	0.4	0.4	0.4
2-METHYL-1-HEXENE	ND	ND	ND	ND	ND	ND	ND	ND	ND
TRANS-3-HEPTENE	ND	ND	ND	ND	ND	ND	ND	ND	ND
HEPTANE	0.3	0.2	0.2	ND	ND	ND	ND	ND	ND
CIS-3-HEPTENE	ND	ND	ND	ND	ND	ND	ND	ND	ND
UNIDENTIFIED C7	ND	ND	ND	ND	ND	ND	ND	ND	ND
2-METHYL-2-HEXENE	ND	ND	ND	ND	ND	ND	ND	ND	ND
3-METHYL-TRANS-3-HEXENE	ND	ND	ND	ND	ND	ND	ND	ND	ND
TRANS-2-HEPTENE	ND	ND	ND	ND	ND	ND	ND	ND	ND
3-ETHYL-CIS-2-PENTENE	ND	ND	ND	ND	ND	ND	ND	ND	ND
2,4,4-TRIMETHYL-1-PENTENE	ND	ND	ND	ND	ND	ND	ND	ND	ND
2,3-DIMETHYL-2-PENTENE	ND	ND	ND	ND	ND	ND	ND	ND	ND
CIS-2-HEPTENE	ND	ND	ND	ND	ND	ND	ND	ND	ND
METHYLCYCLOHEXANE	0.4	0.3	0.3	ND	ND	ND	ND	ND	ND
CIS-1,2-DIMETHYLCYCLOPENTANE	ND	ND	ND	ND	ND	ND	ND	ND	ND
2,2-DIMETHYLHEXANE	ND	ND	ND	ND	ND	ND	ND	ND	ND
1,1,3-TRIMETHYLCYCLOPENTANE	ND	ND	ND	ND	ND	ND	ND	ND	ND
2,4,4-TRIMETHYL-2-PENTENE	ND	ND	ND	ND	ND	ND	ND	ND	ND

TABLE B-3 (CONT'D). HYDROCARBON SPECIATION DATA FOR E10 WITH AFTERTREATMENT

	mg/mile			mg/mile			mg/mile		
	ADDCAT-1	ADDCAT-2	ADDCAT-3	ADDCAT-1	ADDCAT-2	ADDCAT-3	ADDCAT-1	ADDCAT-2	ADDCAT-3
COMPOUND	C505	C505	C505	C867	C867	C867	H505	H505	H505
2,2,3-TRIMETHYLPENTANE	ND	ND	ND	ND	ND	ND	ND	ND	ND
2,5-DIMETHYLHEXANE	ND	ND	ND	ND	ND	ND	ND	ND	ND
ETHYLCYCLOPENTANE	ND	ND	ND	ND	ND	ND	ND	ND	ND
2,4-DIMETHYLHEXANE	1.2	1.0	0.8	ND	ND	ND	ND	ND	ND
1-TRANS-2-CIS-4-TRIMETHYLCYCLOPENTANE	ND	ND	ND	ND	ND	ND	ND	ND	ND
3,3-DIMETHYLHEXANE	ND	ND	ND	ND	ND	ND	ND	ND	ND
1-TRANS-2-CIS-3-TRIMETHYLCYCLOPENTANE	ND	ND	ND	ND	ND	ND	ND	ND	ND
2,3,4-TRIMETHYLPENTANE	1.7	1.4	1.3	ND	ND	ND	0.2	0.1	0.2
2,3,3-TRIMETHYLPENTANE	1.9	1.6	1.5	ND	ND	ND	ND	ND	ND
TOLUENE	11.3	6.5	7.0	ND	ND	ND	ND	ND	ND
2,3-DIMETHYLHEXANE	ND	ND	ND	ND	ND	ND	ND	ND	ND
1,1,2-TRIMETHYLCYCLOPENTANE	ND	ND	ND	ND	ND	ND	ND	ND	ND
2-METHYLHEPTANE	ND	ND	ND	ND	ND	ND	ND	ND	ND
3,4-DIMETHYLHEXANE, NOTE B	ND	ND	ND	ND	ND	ND	ND	ND	ND
4-METHYLHEPTANE	ND	ND	ND	ND	ND	ND	ND	ND	ND
3-METHYLHEPTANE	ND	ND	ND	ND	ND	ND	ND	ND	ND
1-CIS,2-TRANS,3-TRIMETHYLCYCLOPENTANE	ND	ND	ND	ND	ND	ND	ND	ND	ND
CIS-1,3-DIMETHYLCYCLOHEXANE	ND	ND	ND	ND	ND	ND	ND	ND	ND
TRANS-1,4-DIMETHYLCYCLOHEXANE	ND	ND	ND	ND	ND	ND	ND	ND	ND
3-ETHYLHEXANE	ND	ND	ND	ND	ND	ND	ND	ND	ND
2,2,5-TRIMETHYLHEXANE	0.6	0.5	0.1	ND	ND	ND	ND	ND	ND
TRANS-1-METHYL-3-ETHYLCYCLOPENTANE	ND	ND	ND	ND	ND	ND	ND	ND	ND
CIS-1-METHYL-3-ETHYLCYCLOPENTANE	ND	ND	ND	ND	ND	ND	ND	ND	ND
1,1-DIMETHYLCYCLOHEXANE	ND	ND	ND	ND	ND	ND	ND	ND	ND
TRANS-1-METHYL-2-ETHYLCYCLOPENTANE	ND	ND	ND	ND	ND	ND	ND	ND	ND
1-METHYL-1-ETHYL-CYCLOPENTANE	ND	ND	ND	ND	ND	ND	ND	ND	ND
2,4,4-TRIMETHYLHEXANE	ND	ND	ND	ND	ND	ND	ND	ND	ND
2,2,4-TRIMETHYLHEXANE	ND	ND	ND	ND	ND	ND	ND	ND	ND
TRANS-1,2-DIMETHYLCYCLOHEXANE	ND	ND	ND	ND	ND	ND	ND	ND	ND
1-OCTENE	ND	ND	ND	ND	ND	ND	ND	ND	ND
TRANS-4-OCTENE	ND	ND	ND	ND	ND	ND	ND	ND	ND
OCTANE	ND	ND	ND	ND	ND	ND	ND	ND	ND
UNIDENTIFIED C8	ND	ND	ND	ND	ND	ND	ND	ND	ND
TRANS-2-OCTENE	ND	ND	ND	ND	ND	ND	ND	ND	ND
TRANS-1,3-DIMETHYLCYCLOHEXANE, NOTE C	ND	ND	ND	ND	ND	ND	ND	ND	ND
CIS-2-OCTENE	ND	ND	ND	ND	ND	ND	ND	ND	ND
ISOPROPYLCYCLOPENTANE	ND	ND	ND	ND	ND	ND	ND	ND	ND
2,2-DIMETHYLHEPTANE	ND	ND	ND	ND	ND	ND	ND	ND	ND
2,3,5-TRIMETHYLHEXANE	ND	ND	ND	ND	ND	ND	ND	ND	ND
CIS-1-METHYL-2-ETHYLCYCLOPENTANE	ND	ND	ND	ND	ND	ND	ND	ND	ND
2,4-DIMETHYLHEPTANE	ND	ND	ND	ND	ND	ND	ND	ND	ND
4,4-DIMETHYLHEPTANE	ND	ND	ND	ND	ND	ND	ND	ND	ND
CIS-1,2-DIMETHYLCYCLOHEXANE	ND	ND	ND	ND	ND	ND	ND	ND	ND
ETHYLCYCLOHEXANE	ND	ND	ND	ND	ND	ND	ND	ND	ND
2,6-DIMETHYLHEPTANE, NOTE D	ND	ND	ND	ND	ND	ND	ND	ND	ND
1,1,3-TRIMETHYLCYCLOHEXANE	ND	ND	ND	ND	ND	ND	ND	ND	ND
2,5-DIMETHYLHEPTANE, NOTE E	ND	ND	ND	ND	ND	ND	ND	ND	ND
3,3-DIMETHYLHEPTANE	ND	ND	ND	ND	ND	ND	ND	ND	ND
3,5-DIMETHYLHEPTANE, NOTE E	ND	ND	ND	ND	ND	ND	ND	ND	ND

TABLE B-3 (CONT'D). HYDROCARBON SPECIATION DATA FOR E10 WITH AFTERTREATMENT

	mg/mile			mg/mile			mg/mile		
	ADDCAT-1	ADDCAT-2	ADDCAT-3	ADDCAT-1	ADDCAT-2	ADDCAT-3	ADDCAT-1	ADDCAT-2	ADDCAT-3
COMPOUND	C505	C505	C505	C867	C867	C867	H505	H505	H505
ETHYLBENZENE	ND	ND	ND	ND	ND	ND	ND	ND	ND
2,3,4-TRIMETHYLHEXANE	ND	ND	ND	ND	ND	ND	ND	ND	ND
2,3-DIMETHYLHEPTANE	ND	ND	ND	ND	ND	ND	ND	ND	ND
m-& p-XYLENE	ND	ND	ND	ND	ND	ND	ND	ND	ND
4-METHYLOCTANE	ND	ND	ND	ND	ND	ND	ND	ND	ND
3,4-DIMETHYLHEPTANE	ND	ND	ND	ND	ND	ND	ND	ND	ND
4-ETHYLHEPTANE	ND	ND	ND	ND	ND	ND	ND	ND	ND
2-METHYLOCTANE	ND	ND	ND	ND	ND	ND	ND	ND	ND
3-METHYLOCTANE	ND	ND	ND	ND	ND	ND	ND	ND	ND
STYRENE	ND	ND	ND	ND	ND	ND	ND	ND	ND
o-XYLENE	ND	ND	ND	ND	ND	ND	ND	ND	ND
1-NONENE	ND	ND	ND	ND	ND	ND	ND	ND	ND
TRANS-3-NONENE	ND	ND	ND	ND	ND	ND	ND	ND	ND
CIS-3-NONENE	ND	ND	ND	ND	ND	ND	ND	ND	ND
NONANE	ND	ND	ND	ND	ND	ND	ND	ND	ND
TRANS-2-NONENE	ND	ND	ND	ND	ND	ND	ND	ND	ND
ISOPROPYLBENZENE (CUMENE)	ND	ND	ND	ND	ND	ND	ND	ND	ND
2,2-DIMETHYLOCTANE	ND	ND	ND	ND	ND	ND	ND	ND	ND
2,4-DIMETHYLOCTANE	ND	ND	ND	ND	ND	ND	ND	ND	ND
n-PROPYLBENZENE	ND	ND	ND	ND	ND	ND	ND	ND	ND
1-METHYL-3-ETHYLBENZENE	ND	ND	ND	ND	ND	ND	ND	ND	ND
1-METHYL-4-ETHYLBENZENE	ND	ND	ND	ND	ND	ND	ND	ND	ND
1,3,5-TRIMETHYLBENZENE	ND	ND	ND	ND	ND	ND	ND	ND	ND
1-METHYL-2-ETHYLBENZENE	ND	ND	ND	ND	ND	ND	ND	ND	ND
1,2,4-TRIMETHYLBENZENE	ND	ND	ND	ND	ND	ND	ND	ND	ND
TERT-BUTYLBENZENE	ND	ND	ND	ND	ND	ND	ND	ND	ND
1-DECENE	ND	ND	ND	ND	ND	ND	ND	ND	ND
DECANE, NOTE F	ND	ND	ND	ND	ND	ND	ND	ND	ND
ISOBUTYLBENZENE, NOTE F	ND	ND	ND	ND	ND	ND	ND	ND	ND
1,3,-DIMETHYL-5-ETHYLBENZENE	ND	ND	ND	ND	ND	ND	ND	ND	ND
METHYLPROPYLBENZENE (sec butylbenzene)	ND	ND	ND	ND	ND	ND	ND	ND	ND
1-METHYL-3-ISOPROPYLBENZENE	ND	ND	ND	ND	ND	ND	ND	ND	ND
1,2,3-TRIMETHYLBENZENE	ND	ND	ND	ND	ND	ND	ND	ND	ND
1-METHYL-4-ISOPROPYLBENZENE	ND	ND	ND	ND	ND	ND	ND	ND	ND
INDAN	ND	ND	ND	ND	ND	ND	ND	ND	ND
1-METHYL-2-ISOPROPYLBENZENE	ND	ND	ND	ND	ND	ND	ND	ND	ND
1,3-DIETHYLBENZENE	ND	ND	ND	ND	ND	ND	ND	ND	ND
1,4-DIETHYLBENZENE	ND	ND	ND	ND	ND	ND	ND	ND	ND
1-METHYL-3-N-PROPYLBENZENE	ND	ND	ND	ND	ND	ND	ND	ND	ND
1-METHYL-4-N-PROPYLBENZENE, NOTE G	ND	ND	ND	ND	ND	ND	ND	ND	ND
1,2 DIETHYLBENZENE	ND	ND	ND	ND	ND	ND	ND	ND	ND
1-METHYL-2-N-PROPYLBENZENE	ND	ND	ND	ND	ND	ND	ND	ND	ND
1,4-DIMETHYL-2-ETHYLBENZENE	ND	ND	ND	ND	ND	ND	ND	ND	ND
1,3-DIMETHYL-4-ETHYLBENZENE	ND	ND	ND	ND	ND	ND	ND	ND	ND
1,2-DIMETHYL-4-ETHYLBENZENE	ND	ND	ND	ND	ND	ND	ND	ND	ND
1,3-DIMETHYL-2-ETHYLBENZENE	ND	ND	ND	ND	ND	ND	ND	ND	ND
UNDECANE	ND	ND	ND	ND	ND	ND	ND	ND	ND
1,2-DIMETHYL-3-ETHYLBENZENE	ND	ND	ND	ND	ND	ND	ND	ND	ND
1,2,4,5-TETRAMETHYLBENZENE	ND	ND	ND	ND	ND	ND	ND	ND	ND
2-METHYLBUTYLBENZENE (sec AMYLBENZENE)	ND	ND	ND	ND	ND	ND	ND	ND	ND

TABLE B-3 (CONT'D). HYDROCARBON SPECIATION DATA FOR E10 WITH AFTERTREATMENT

	mg/mile			mg/mile			mg/mile		
	ADDCAT-1	ADDCAT-2	ADDCAT-3	ADDCAT-1	ADDCAT-2	ADDCAT-3	ADDCAT-1	ADDCAT-2	ADDCAT-3
COMPOUND	C505	C505	C505	C867	C867	C867	H505	H505	H505
3,4 DIMETHYLCUMENE	ND	ND	ND	ND	ND	ND	ND	ND	ND
1,2,3,5-TETRAMETHYLBENZENE	ND	ND	ND	ND	ND	ND	ND	ND	ND
TERT-1-BUT-2-METHYLBENZENE	ND	ND	ND	ND	ND	ND	ND	ND	ND
1,2,3,4-TETRAMETHYLBENZENE	ND	ND	ND	ND	ND	ND	ND	ND	ND
N-PENT-BENZENE	ND	ND	ND	ND	ND	ND	ND	ND	ND
TERT-1-BUT-3,5-DIMETHYLBENZENE	ND	ND	ND	ND	ND	ND	ND	ND	ND
TERT-1-BUTYL-4-ETHYLBENZENE	ND	ND	ND	ND	ND	ND	ND	ND	ND
NAPHTHALENE	ND	ND	ND	ND	ND	ND	ND	ND	ND
DODECANE	ND	ND	ND	ND	ND	ND	ND	ND	ND
1,3,5-TRIETHYLBENZENE	ND	ND	ND	ND	ND	ND	ND	ND	ND
1,2,4-TRIETHYLBENZENE	ND	ND	ND	ND	ND	ND	ND	ND	ND
HEXYLBENZENE	ND	ND	ND	ND	ND	ND	ND	ND	ND
UNIDENTIFIED C9-C12+	ND	ND	ND	ND	ND	ND	ND	ND	ND
FORMALDEHYDE	0.9	0.4	0.4	0.1	0.1	0.1	Trace	0.1	Trace
ACETALDEHYDE	1.4	0.8	0.9	Trace	Trace	Trace	Trace	Trace	Trace
ACROLEIN	0.2	0.1	0.1	Trace	ND	ND	Trace	Trace	ND
ACETONE	0.3	0.2	0.2	Trace	Trace	Trace	0.1	Trace	Trace
PROPIONALDEHYDE	0.1	0.1	0.1	ND	Trace	Trace	ND	ND	Trace
CROTONALDEHYDE	ND	ND	Trace	ND	ND	Trace	ND	ND	ND
METHACROLEIN	0.2	0.2	0.1	Trace	0.1	ND	Trace	Trace	Trace
N-/ISOBUTYRALDEHYDE	ND	ND	ND	ND	ND	ND	ND	ND	ND
METHYL ETHYL KETONE	ND	ND	Trace	ND	ND	Trace	ND	ND	ND
BENZALDEHYDE	0.3	0.1	0.2	Trace	Trace	Trace	Trace	Trace	0.1
ISOVALERALDEHYDE	ND	ND	ND	ND	ND	ND	ND	Trace	ND
VALERALDEHYDE	ND	ND	ND	ND	Trace	ND	ND	0.1	Trace
O-TOLUALDEHYDE	ND	ND	ND	Trace	ND	ND	ND	ND	ND
M/P-TOLUALDEHYDE	0.1	ND	ND	ND	ND	ND	ND	ND	ND
METHYL ISOBUTYL KETONE	ND	ND	ND	ND	ND	ND	ND	ND	ND
HEXANALDEHYDE	ND	ND	ND	ND	ND	ND	ND	ND	ND
DIMETHYLBENZALDEHYDE	ND	ND	ND	ND	ND	ND	ND	ND	ND
SUMMED SPECIATED VALUES	75.1	51.8	52.1	0.8	1.5	1.1	4.0	4.6	3.5

A - 2,2-Dimethylpentane and methylcyclopentane co-elute. GC peak area split equally between the two compounds.
B - 3-Methyl-3-ethyl-pentane co-elutes with reported compound. Not reported separately.
C - Cis-1,4-Dimethylcyclohexane co-elutes with reported compound. Not reported separately.
D - Propylcyclopentane co-elutes with reported compound. Not reported separately.
E - 2,5-Dimethylheptane and 3,5-dimethylheptane co-elute. GC peak area split equally between the two compounds.
F - Decane and isobutylbenzene co-elute. GC peak area split equally between the two compounds.
G - n-Butylbenzene co-elutes with reported compound. Not reported separately.

TABLE B-4. HYDROCARBON SPECIATION DATA FOR E10 WITHOUT AFTERTREATMENT

	mg/mile			mg/mile			mg/mile		
	RFAE10ADD-1	RFAE10ADD-2	RFAE10ADD-3	RFAE10ADD-1	RFAE10ADD-2	RFAE10ADD-3	RFAE10ADD-1	RFAE10ADD-2	RFAE10ADD-3
COMPOUND	C505	C505	C505	C867	C867	C867	H505	H505	H505
METHANE	50.7	105.6	56.3	53.9	14.0	49.4	38.2	11.2	41.6
ETHANE	17.0	17.1	16.9	15.1	15.0	14.4	11.2	10.8	10.6
ETHYLENE	99.9	100.4	99.1	98.4	97.6	94.1	71.4	70.1	69.0
PROPANE	1.3	2.9	2.6	1.6	1.3	1.3	1.1	0.9	0.8
PROPYLENE	86.9	85.1	83.9	75.0	73.8	71.4	59.2	57.4	56.5
ACETYLENE	65.6	68.3	67.6	68.1	69.8	67.2	48.5	48.9	48.0
PROPADIENE	ND	ND	ND	ND	ND	ND	ND	ND	ND
BUTANE	3.4	3.8	3.5	4.0	5.6	3.3	2.7	3.5	2.5
TRANS-2-BUTENE	8.0	7.7	7.6	6.8	6.7	6.5	5.4	5.2	5.2
1-BUTENE	8.7	8.4	8.2	7.6	7.3	7.0	5.9	5.7	5.6
2-METHYLPROPENE (ISOBUTYLENE)	59.1	57.6	56.9	52.1	51.3	49.9	40.6	39.3	38.9
2,2-DIMETHYLPROPANE (NEOPENTANE)	79.8	79.9	79.5	86.5	88.4	84.5	63.2	63.6	60.8
PROPYNE	ND	Trace	Trace	ND	Trace	ND	ND	ND	ND
1,3-BUTADIENE	12.7	11.9	12.1	10.5	9.9	9.7	7.5	6.4	7.0
2-METHYLPROPANE (ISOBUTANE)	7.5	6.9	7.3	6.0	4.0	5.8	4.8	4.1	4.5
1-BUTYNE	ND	ND	ND	ND	ND	ND	ND	ND	ND
METHANOL	8.6	8.9	8.6	8.9	9.3	8.7	5.8	6.0	6.1
CIS-2-BUTENE	5.6	5.4	5.4	4.8	4.7	4.6	3.8	3.7	3.6
3-METHYL-1-BUTENE	ND	ND	ND	ND	ND	ND	ND	ND	ND
ETHANOL	71.9	72.0	72.6	70.5	65.8	66.2	51.4	50.7	51.9
2-METHYLBUTANE (ISOPENTANE)	8.9	9.0	8.9	7.5	7.3	7.2	6.0	5.7	5.6
2-BUTYNE	1.5	0.2	1.4	1.7	1.9	1.8	0.9	0.9	1.0
1-PENTENE	4.3	1.0	2.0	3.3	3.4	3.3	2.1	2.4	1.9
2-METHYL-1-BUTENE	7.1	5.1	6.7	7.1	6.8	6.7	4.9	4.8	5.3
PENTANE	20.4	18.7	20.4	22.5	21.8	22.5	16.5	15.7	15.9
UNIDENTIFIED C5 OLEFINS	ND	ND	ND	ND	ND	ND	ND	ND	ND
2-METHYL-1,3-BUTADIENE	6.4	4.4	4.2	5.6	5.0	4.5	1.5	1.7	1.5
TRANS-2-PENTENE	1.9	1.3	1.9	1.8	1.8	1.7	1.4	1.3	1.3
3,3-DIMETHYL-1-BUTENE	1.0	0.7	1.0	1.1	1.1	1.1	0.7	0.7	0.6
CIS-2-PENTENE	1.0	0.7	1.0	1.1	1.0	1.0	0.8	0.7	0.6
2-METHYL-2-BUTENE	8.7	6.0	5.6	7.6	8.4	6.9	0.2	0.7	3.7
TERT-BUTANOL	ND	ND	ND	ND	ND	ND	ND	ND	ND
CYCLOPENTADIENE	0.5	0.2	0.2	0.6	1.0	0.2	0.1	0.1	0.1
2,2-DIMETHYLBUTANE	2.2	2.1	2.0	2.4	2.3	2.1	1.6	1.5	1.4
CYCLOPENTENE	0.7	0.7	0.7	0.9	0.8	0.8	0.6	0.5	0.6
4-METHYL-1-PENTENE	ND	ND	ND	ND	ND	ND	ND	ND	ND
3-METHYL-1-PENTENE	2.7	2.5	2.4	2.3	2.0	2.2	1.8	1.6	1.8
CYCLOPENTANE	1.9	1.8	1.8	1.9	2.0	1.9	1.4	1.4	1.4
2,3-DIMETHYLBUTANE	7.9	7.6	7.8	8.5	8.1	8.2	6.1	5.8	6.0
MTBE	ND	ND	ND	ND	ND	ND	ND	ND	ND
4-METHYL-CIS-2-PENTENE	ND	ND	ND	ND	ND	ND	ND	ND	ND
2-METHYLPENTANE	10.4	10.1	10.3	11.2	10.4	10.6	8.1	7.6	7.8
4-METHYL-TRANS-2-PENTENE	0.7	0.7	0.7	0.7	0.7	0.6	0.5	0.5	0.5
3-METHYLPENTANE	6.0	5.7	5.8	6.8	5.7	6.1	4.6	4.3	4.4
2-METHYL-1-PENTENE	0.4	0.5	0.6	0.7	0.6	0.7	0.9	0.4	0.4
1-HEXENE	1.0	0.5	0.6	0.7	0.6	0.7	0.4	0.4	0.4
HEXANE	9.1	8.9	9.1	9.6	9.2	9.4	6.9	6.6	6.7
UNIDENTIFIED C6	4.1	4.1	3.4	3.9	3.1	2.5	2.9	2.9	2.9
TRANS-3-HEXENE	ND	ND	ND	ND	ND	ND	ND	ND	ND

TABLE B-4 (CONT'D). HYDROCARBON SPECIATION DATA FOR E10 WITHOUT AFTERTREATMENT

	mg/mile			mg/mile			mg/mile		
	RFAE10ADD-1	RFAE10ADD-2	RFAE10ADD-3	RFAE10ADD-1	RFAE10ADD-2	RFAE10ADD-3	RFAE10ADD-1	RFAE10ADD-2	RFAE10ADD-3
COMPOUND	C505	C505	C505	C867	C867	C867	H505	H505	H505
CIS-3-HEXENE	0.2	0.1	0.1	ND	ND	ND	ND	ND	ND
DI-ISOPROPYL ETHER	ND	ND	ND	ND	ND	ND	ND	ND	ND
TRANS-2-HEXENE	0.3	0.2	0.2	0.3	0.2	0.2	0.1	0.1	0.1
3-METHYL-TRANS-2-PENTENE	0.4	0.7	0.7	0.7	0.6	0.7	0.4	0.5	0.5
2-METHYL-2-PENTENE	0.5	0.8	0.8	0.9	0.8	0.8	0.5	0.5	0.6
3-METHYLCYCLOPENTENE	ND	ND	ND	ND	ND	ND	ND	ND	ND
CIS-2-HEXENE	0.4	0.3	0.1	0.4	0.3	0.2	ND	ND	ND
ETBE	ND	ND	ND	ND	ND	ND	ND	ND	ND
3-METHYL-CIS-2-PENTENE	0.5	0.5	0.4	0.4	0.4	0.4	0.2	0.2	0.2
2,2-DIMETHYLPENTANE, NOTE A	2.8	2.7	2.8	2.8	2.8	2.8	2.1	2.0	2.0
METHYLCYCLOPENTANE, NOTE A	2.7	2.7	2.8	2.7	2.7	2.8	2.1	2.0	2.0
2,4-DIMETHYLPENTANE	7.3	7.1	7.3	7.6	7.6	7.6	5.5	5.3	5.5
2,2,3-TRIMETHYLBUTANE	0.8	0.7	0.7	0.4	0.5	0.7	0.6	0.6	0.6
3,4-DIMETHYL-1-PENTENE	0.6	0.5	0.4	0.4	0.3	0.5	0.5	0.2	0.2
1-METHYLCYCLOPENTENE	0.1	0.2	0.3	0.2	0.4	0.3	0.2	0.2	0.2
BENZENE	39.1	43.2	45.6	45.2	51.6	42.2	34.6	35.1	33.6
3-METHYL-1-HEXENE	ND	ND	ND	ND	ND	ND	ND	ND	ND
3,3-DIMETHYLPENTANE	0.4	0.3	0.3	0.4	0.3	0.4	0.2	0.2	0.2
CYCLOHEXANE	3.7	4.0	4.1	4.3	5.0	3.8	3.3	3.4	3.0
2-METHYLHEXANE	9.1	8.7	8.8	9.4	9.5	9.3	6.8	6.4	6.6
2,3-DIMETHYLPENTANE	0.9	0.8	0.8	0.6	0.7	0.7	0.4	0.4	0.4
1,1-DIMETHYLCYCLOPENTANE	ND	ND	ND	ND	ND	ND	ND	ND	ND
TERT-AMYL METHYL ETHER	ND	ND	ND	ND	ND	ND	ND	ND	ND
CYCLOHEXENE	1.0	0.9	0.9	1.0	1.1	1.0	0.7	0.7	0.7
3-METHYLHEXANE	2.9	2.7	2.8	2.9	3.1	3.0	2.1	2.0	2.1
CIS-1,3-DIMETHYLCYCLOPENTANE	0.6	0.5	0.5	0.5	0.7	0.6	0.4	0.3	0.3
3-ETHYLPENTANE	0.8	0.7	0.7	0.7	0.8	0.8	0.5	0.5	0.5
TRANS-1,2-DIMETHYLCYCLOPENTANE	ND	ND	ND	ND	ND	ND	ND	ND	ND
TRANS-1,3-DIMETHYLCYCLOPENTANE	0.8	0.7	0.7	0.8	0.8	0.8	0.5	0.5	0.5
1-HEPTENE	ND	ND	ND	ND	ND	ND	ND	ND	ND
2,2,4-TRIMETHYLPENTANE	100.2	96.8	98.8	106.1	105.0	105.1	75.4	73.5	74.9
2-METHYL-1-HEXENE	ND	0.1	0.1	ND	ND	ND	ND	ND	ND
TRANS-3-HEPTENE	ND	ND	ND	ND	ND	ND	ND	ND	ND
HEPTANE	4.3	3.9	4.0	4.4	10.6	7.0	3.0	2.8	2.9
CIS-3-HEPTENE	ND	ND	ND	ND	ND	ND	ND	ND	ND
UNIDENTIFIED C7	3.0	2.6	1.0	1.5	1.6	1.6	1.9	1.6	1.6
2-METHYL-2-HEXENE	ND	0.1	ND	ND	ND	ND	ND	ND	ND
3-METHYL-TRANS-3-HEXENE	ND	ND	ND	ND	ND	ND	ND	ND	ND
TRANS-2-HEPTENE	0.2	0.1	0.1	0.1	ND	ND	ND	ND	ND
3-ETHYL-CIS-2-PENTENE	ND	ND	ND	ND	ND	ND	ND	ND	ND
2,4,4-TRIMETHYL-1-PENTENE	0.3	0.2	0.2	0.3	0.4	0.4	0.2	0.2	0.2
2,3-DIMETHYL-2-PENTENE	ND	ND	ND	ND	ND	ND	ND	ND	ND
CIS-2-HEPTENE	0.6	0.5	0.5	0.6	0.6	0.6	0.2	0.3	0.3
METHYLCYCLOHEXANE	6.5	6.4	6.5	6.8	6.7	6.5	4.8	4.6	4.6
CIS-1,2-DIMETHYLCYCLOPENTANE	ND	ND	ND	ND	ND	ND	ND	ND	ND
2,2-DIMETHYLHEXANE	0.4	0.4	0.4	0.5	0.5	0.4	0.3	0.3	0.3
1,1,3-TRIMETHYLCYCLOPENTANE	ND	ND	ND	ND	ND	ND	ND	ND	ND
2,4,4-TRIMETHYL-2-PENTENE	ND	ND	0.5	0.5	0.2	0.2	0.3	0.2	0.1

TABLE B-4 (CONT'D). HYDROCARBON SPECIATION DATA FOR E10 WITHOUT AFTERTREATMENT

	mg/mile			mg/mile			mg/mile		
	RFAE10ADD-1	RFAE10ADD-2	RFAE10ADD-3	RFAE10ADD-1	RFAE10ADD-2	RFAE10ADD-3	RFAE10ADD-1	RFAE10ADD-2	RFAE10ADD-3
COMPOUND	C505	C505	C505	C867	C867	C867	H505	H505	H505
2,2,3-TRIMETHYLPENTANE	10.8	10.6	11.3	13.3	12.8	13.1	8.7	8.5	9.0
2,5-DIMETHYLHEXANE	ND	ND	ND	ND	ND	ND	ND	ND	ND
ETHYLCYCLOPENTANE	ND	ND	ND	ND	ND	ND	ND	ND	ND
2,4-DIMETHYLHEXANE	13.0	12.6	12.6	12.2	12.0	12.2	9.4	9.1	9.0
1-TRANS-2-CIS-4-TRIMETHYLCYCLOPENTANE	0.4	0.4	0.4	0.4	0.4	0.4	0.3	0.3	0.3
3,3-DIMETHYLHEXANE	ND	ND	ND	ND	ND	ND	ND	ND	ND
1-TRANS-2-CIS-3-TRIMETHYLCYCLOPENTANE	ND	ND	ND	ND	ND	ND	ND	ND	ND
2,3,4-TRIMETHYLPENTANE	32.7	31.5	32.4	35.7	35.2	35.5	25.2	24.5	24.7
2,3,3-TRIMETHYLPENTANE	18.4	19.4	19.8	23.0	26.5	21.0	17.2	17.6	16.0
TOLUENE	225.7	230.4	250.8	243.1	264.5	225.1	188.2	185.6	180.6
2,3-DIMETHYLHEXANE	ND	ND	13.7	ND	ND	14.7	ND	ND	10.9
1,1,2-TRIMETHYLCYCLOPENTANE	ND	ND	ND	ND	ND	ND	ND	ND	ND
2-METHYLHEPTANE	1.1	1.2	1.2	1.2	1.3	1.3	0.9	0.9	0.9
3,4-DIMETHYLHEXANE, NOTE B	1.6	1.7	1.8	1.8	1.7	1.9	1.4	1.4	1.4
4-METHYLHEPTANE	ND	ND	ND	ND	ND	ND	ND	ND	ND
3-METHYLHEPTANE	0.9	0.9	1.1	0.9	0.9	1.0	0.7	0.8	0.8
1-CIS,2-TRANS,3-TRIMETHYLCYCLOPENTANE	0.8	0.8	0.9	0.8	0.8	0.9	0.6	0.6	0.7
CIS-1,3-DIMETHYLCYCLOHEXANE	ND	ND	ND	ND	ND	ND	ND	ND	ND
TRANS-1,4-DIMETHYLCYCLOHEXANE	0.3	0.3	0.4	0.3	0.4	0.4	0.2	0.2	0.2
3-ETHYLHEXANE	ND	ND	ND	ND	ND	ND	ND	ND	ND
2,2,5-TRIMETHYLHEXANE	12.8	12.4	12.9	14.1	14.0	14.4	10.0	9.7	9.9
TRANS-1-METHYL-3-ETHYLCYCLOPENTANE	ND	ND	ND	ND	ND	ND	ND	ND	ND
CIS-1-METHYL-3-ETHYLCYCLOPENTANE	ND	ND	ND	ND	ND	ND	ND	ND	ND
1,1-DIMETHYLCYCLOHEXANE	ND	ND	ND	ND	ND	ND	ND	ND	ND
TRANS-1-METHYL-2-ETHYLCYCLOPENTANE	ND	ND	ND	ND	ND	ND	ND	ND	ND
1-METHYL-1-ETHYL-CYCLOPENTANE	0.7	0.7	0.7	0.9	0.9	0.9	0.7	0.6	0.7
2,4,4-TRIMETHYLHEXANE	ND	ND	ND	ND	ND	ND	ND	ND	ND
2,2,4-TRIMETHYLHEXANE	ND	ND	ND	ND	ND	ND	ND	ND	ND
TRANS-1,2-DIMETHYLCYCLOHEXANE	0.3	0.3	0.3	0.3	0.4	0.4	0.2	0.2	0.2
1-OCTENE	ND	ND	ND	ND	ND	ND	ND	ND	ND
TRANS-4-OCTENE	ND	ND	ND	ND	ND	ND	ND	ND	ND
OCTANE	1.2	1.2	1.2	1.3	1.3	1.4	0.9	0.9	0.9
UNIDENTIFIED C8	ND	ND	ND	ND	0.4	ND	ND	0.2	ND
TRANS-2-OCTENE	0.1	0.1	0.1	0.2	0.2	0.2	0.1	0.1	0.1
TRANS-1,3-DIMETHYLCYCLOHEXANE, NOTE C	0.3	0.2	0.2	0.3	0.3	0.3	0.2	0.2	0.2
CIS-2-OCTENE	ND	ND	ND	ND	ND	ND	ND	ND	ND
ISOPROPYLCYCLOPENTANE	0.4	0.4	0.4	0.4	0.5	0.4	0.3	0.3	0.3
2,2-DIMETHYLHEPTANE	ND	ND	ND	ND	ND	ND	ND	ND	ND
2,3,5-TRIMETHYLHEXANE	2.0	1.9	1.9	2.2	2.1	2.2	1.5	1.5	1.5
CIS-1-METHYL-2-ETHYLCYCLOPENTANE	ND	0.1	ND	ND	ND	ND	ND	ND	ND
2,4-DIMETHYLHEPTANE	0.4	0.4	0.3	0.4	0.3	0.3	0.3	0.3	0.3
4,4-DIMETHYLHEPTANE	ND	ND	ND	ND	ND	ND	ND	ND	ND
CIS-1,2-DIMETHYLCYCLOHEXANE	0.6	0.5	0.5	0.8	0.3	0.9	0.6	0.2	0.2
ETHYLCYCLOHEXANE	0.3	0.3	0.3	ND	ND	ND	ND	ND	ND
2,6-DIMETHYLHEPTANE, NOTE D	ND	ND	ND	ND	ND	ND	ND	ND	ND
1,1,3-TRIMETHYLCYCLOHEXANE	ND	ND	ND	ND	ND	ND	ND	ND	ND
2,5-DIMETHYLHEPTANE, NOTE E	0.6	0.5	0.5	0.6	0.4	0.6	0.2	0.4	0.4
3,3-DIMETHYLHEPTANE	ND	ND	ND	ND	ND	ND	ND	ND	ND
3,5-DIMETHYLHEPTANE, NOTE E	0.6	0.5	0.5	0.6	0.4	0.6	0.2	0.4	0.4

TABLE B-4 (CONT'D). HYDROCARBON SPECIATION DATA FOR E10 WITHOUT AFTERTREATMENT

	mg/mile			mg/mile			mg/mile		
	RFAE10ADD-1	RFAE10ADD-2	RFAE10ADD-3	RFAE10ADD-1	RFAE10ADD-2	RFAE10ADD-3	RFAE10ADD-1	RFAE10ADD-2	RFAE10ADD-3
COMPOUND	C505	C505	C505	C867	C867	C867	H505	H505	H505
ETHYLBENZENE	16.7	16.2	16.9	14.7	13.4	14.3	10.8	10.1	10.9
2,3,4-TRIMETHYLHEXANE	ND	ND	ND	ND	ND	ND	ND	ND	ND
2,3-DIMETHYLHEPTANE	ND	ND	ND	ND	ND	ND	ND	ND	ND
m-& p-XYLENE	19.3	18.8	19.5	17.6	16.4	17.3	12.5	12.3	13.3
4-METHYLOCTANE	ND	ND	ND	ND	ND	ND	ND	ND	ND
3,4-DIMETHYLHEPTANE	ND	ND	ND	ND	ND	ND	ND	ND	ND
4-ETHYLHEPTANE	ND	ND	ND	ND	ND	ND	ND	ND	ND
2-METHYLOCTANE	0.5	0.5	0.5	0.4	0.5	0.5	0.4	0.5	0.4
3-METHYLOCTANE	0.2	0.2	0.2	0.2	0.3	0.3	0.2	0.2	0.2
STYRENE	9.2	8.1	6.7	8.3	7.0	5.9	2.2	3.0	1.8
o-XYLENE	13.9	13.2	13.9	13.7	12.6	13.5	9.4	9.5	10.0
1-NONENE	0.9	0.9	0.9	1.0	1.0	1.0	0.7	0.9	0.7
TRANS-3-NONENE	ND	ND	ND	ND	ND	ND	ND	ND	ND
CIS-3-NONENE	ND	ND	ND	ND	ND	ND	ND	ND	ND
NONANE	1.0	1.1	1.1	1.3	1.2	1.2	0.9	1.0	0.9
TRANS-2-NONENE	ND	ND	ND	ND	ND	ND	ND	ND	ND
ISOPROPYLBENZENE (CUMENE)	0.8	2.5	2.6	0.8	2.7	2.8	0.7	2.0	2.1
2,2-DIMETHYLOCTANE	0.2	0.9	0.8	0.2	0.9	0.8	0.1	0.7	0.6
2,4-DIMETHYLOCTANE	ND	ND	ND	ND	ND	ND	ND	ND	ND
n-PROPYLBENZENE	12.4	11.7	12.4	13.4	12.5	13.1	9.4	9.3	9.9
1-METHYL-3-ETHYLBENZENE	47.0	43.6	45.8	49.6	44.4	47.7	34.2	33.2	35.9
1-METHYL-4-ETHYLBENZENE	20.0	18.5	19.9	20.9	19.7	20.3	14.4	14.0	15.4
1,3,5-TRIMETHYLBENZENE	23.7	21.8	23.0	24.6	21.9	23.3	16.6	16.4	17.8
1-METHYL-2-ETHYLBENZENE	18.7	17.3	18.2	22.9	19.1	21.7	14.1	13.3	14.7
1,2,4-TRIMETHYLBENZENE	77.9	72.9	71.3	72.3	63.7	66.0	45.9	46.6	51.1
TERT-BUTYLBENZENE	ND	ND	ND	ND	ND	ND	ND	ND	ND
1-DECENE	ND	ND	ND	ND	ND	ND	ND	ND	ND
DECANE, NOTE F	1.2	0.5	1.6	1.1	0.6	0.2	0.4	0.3	0.5
ISOBUTYLBENZENE, NOTE F	1.1	0.4	1.5	1.0	0.6	0.2	0.4	0.3	0.5
1,3,-DIMETHYL-5-ETHYLBENZENE	ND	ND	ND	ND	ND	ND	ND	ND	ND
METHYLPROPYLBENZENE (sec butylbenzene)	ND	ND	ND	1.1	1.1	0.9	2.7	1.0	4.5
1-METHYL-3-ISOPROPYLBENZENE	15.0	14.4	10.9	8.7	5.8	6.2	4.9	4.6	5.0
1,2,3-TRIMETHYLBENZENE	4.2	1.0	3.7	5.3	6.1	5.1	3.0	4.4	3.7
1-METHYL-4-ISOPROPYLBENZENE	ND	ND	ND	ND	ND	ND	ND	ND	ND
INDAN	ND	ND	ND	ND	ND	ND	ND	ND	ND
1-METHYL-2-ISOPROPYLBENZENE	ND	ND	ND	0.4	ND	ND	ND	ND	ND
1,3-DIETHYLBENZENE	4.1	3.6	3.8	4.2	2.5	3.0	2.8	2.1	2.8
1,4-DIETHYLBENZENE	7.8	3.9	5.2	4.8	2.8	1.0	3.6	2.9	3.9
1-METHYL-3-N-PROPYLBENZENE	ND	ND	ND	ND	ND	ND	ND	ND	ND
1-METHYL-4-N-PROPYLBENZENE, NOTE G	ND	ND	ND	ND	ND	ND	ND	ND	ND
1,2 DIETHYLBENZENE	ND	ND	ND	ND	ND	ND	ND	ND	ND
1-METHYL-2-N-PROPYLBENZENE	ND	ND	ND	ND	ND	ND	ND	ND	ND
1,4-DIMETHYL-2-ETHYLBENZENE	ND	ND	ND	ND	ND	ND	ND	ND	ND
1,3-DIMETHYL-4-ETHYLBENZENE	ND	ND	ND	ND	ND	ND	ND	ND	ND
1,2-DIMETHYL-4-ETHYLBENZENE	4.1	2.3	2.0	0.7	1.6	0.6	0.8	1.0	0.7
1,3-DIMETHYL-2-ETHYLBENZENE	ND	ND	ND	ND	ND	ND	ND	ND	ND
UNDECANE	ND	ND	ND	ND	ND	ND	ND	ND	ND
1,2-DIMETHYL-3-ETHYLBENZENE	ND	ND	ND	ND	ND	ND	ND	ND	ND
1,2,4,5-TETRAMETHYLBENZENE	ND	ND	ND	ND	ND	ND	ND	ND	ND
2-METHYLBUTYLBENZENE (sec AMYLBENZENE)	ND	ND	ND	ND	ND	ND	ND	ND	ND

TABLE B-4 (CONT'D). HYDROCARBON SPECIATION DATA FOR E10 WITHOUT AFTERTREATMENT

	mg/mile			mg/mile			mg/mile		
	RFAE10ADD-1	RFAE10ADD-2	RFAE10ADD-3	RFAE10ADD-1	RFAE10ADD-2	RFAE10ADD-3	RFAE10ADD-1	RFAE10ADD-2	RFAE10ADD-3
COMPOUND	C505	C505	C505	C867	C867	C867	H505	H505	H505
3,4 DIMETHYLCUMENE	ND	ND	ND	ND	ND	ND	ND	ND	ND
1,2,3,5-TETRAMETHYLBENZENE	ND	ND	ND	ND	ND	ND	ND	ND	ND
TERT-1-BUT-2-METHYLBENZENE	ND	ND	ND	ND	ND	ND	ND	ND	ND
1,2,3,4-TETRAMETHYLBENZENE	ND	ND	ND	ND	ND	ND	ND	ND	ND
N-PENT-BENZENE	ND	ND	ND	ND	ND	ND	ND	ND	ND
TERT-1-BUT-3,5-DIMETHYLBENZENE	ND	ND	ND	ND	ND	ND	ND	ND	ND
TERT-1-BUTYL-4-ETHYLBENZENE	ND	ND	ND	ND	ND	ND	ND	ND	ND
NAPHTHALENE	ND	ND	ND	ND	ND	ND	ND	ND	ND
DODECANE	ND	ND	ND	ND	ND	ND	ND	ND	ND
1,3,5-TRIETHYLBENZENE	ND	ND	ND	ND	ND	ND	ND	ND	ND
1,2,4-TRIETHYLBENZENE	ND	ND	ND	ND	ND	ND	ND	ND	ND
HEXYLBENZENE	ND	ND	ND	ND	ND	ND	ND	ND	ND
UNIDENTIFIED C9-C12+	27.1	22.8	28.1	21.2	26.4	20.1	16.9	21.1	15.2
FORMALDEHYDE	42.8	33.9	50.7	51.7	51.4	48.1	35.1	27.2	38.5
ACETALDEHYDE	19.3	15.7	22.2	21.6	21.5	21.1	15.0	11.8	16.2
ACROLEIN	2.5	3.8	3.4	2.6	3.0	3.0	1.7	1.9	2.2
ACETONE	5.2	5.4	5.9	6.7	7.7	6.4	4.8	5.1	5.1
PROPIONALDEHYDE	3.7	2.4	2.8	4.2	3.2	2.7	3.1	3.5	2.3
CROTONALDEHYDE	1.9	0.5	1.0	0.7	0.7	0.8	0.4	0.5	1.1
METHACROLEIN	2.9	Trace	3.5	3.2	3.0	2.7	2.3	1.7	3.2
N-/ISOBUTYRALDEHYDE	0.8	0.6	0.9	1.1	1.0	0.7	0.7	0.5	1.1
METHYL ETHYL KETONE	0.6	0.5	0.7	0.8	0.8	0.7	0.6	0.4	1.0
BENZALDEHYDE	12.4	10.4	14.4	14.7	14.9	14.7	10.3	8.5	11.6
ISOVALERALDEHYDE	ND	ND	ND	ND	Trace	ND	0.5	ND	ND
VALERALDEHYDE	ND	ND	ND	0.5	ND	ND	0.3	ND	ND
O-TOLUALDEHYDE	ND	1.2	1.8	ND	2.2	2.1	2.1	1.1	1.4
M/P-TOLUALDEHYDE	8.8	3.4	4.8	8.5	5.1	5.0	4.5	2.9	3.9
METHYL ISOBUTYL KETONE	ND	ND	ND	ND	ND	ND	ND	ND	ND
HEXANALDEHYDE	ND	Trace	Trace	Trace	Trace	0.1	ND	0.1	Trace
DIMETHYLBENZALDEHYDE	4.3	2.7	3.9	5.0	4.2	4.1	3.6	2.8	3.7
SUMMED SPECIATED VALUES	1644.9	1631.6	1673.7	1669.1	1626.5	1597.3	1200.4	1143.4	1212.8

A - 2,2-Dimethylpentane and methylcyclopentane co-elute. GC peak area split equally between the two compounds.
B - 3-Methyl-3-ethyl-pentane co-elutes with reported compound. Not reported separately.
C - Cis-1,4-Dimethylcyclohexane co-elutes with reported compound. Not reported separately.
D - Propylcyclopentane co-elutes with reported compound. Not reported separately.
E - 2,5-Dimethylheptane and 3,5-dimethylheptane co-elute. GC peak area split equally between the two compounds.
F - Decane and isobutylbenzene co-elute. GC peak area split equally between the two compounds.
G - n-Butylbenzene co-elutes with reported compound. Not reported separately.

TABLE B-5. HYDROCARBON SPECIATION DATA FOR E15 WITH AFTERTREATMENT

	mg/mile			mg/mile			mg/mile		
	RFAE15CAT-1	RFAE15CAT-3	RFAE15CAT-4	RFAE15CAT-1	RFAE15CAT-3	RFAE15CAT-4	RFAE15CAT-1	RFAE15CAT-3	RFAE15CAT-4
COMPOUND	C505	C505	C505	C867	C867	C867	H505	H505	H505
METHANE	12.3	14.5	14.8	1.0	1.8	3.6	2.8	4.2	4.2
ETHANE	1.6	2.9	2.8	ND	Trace	Trace	0.1	0.2	0.3
ETHYLENE	3.4	6.5	7.5	Trace	Trace	ND	Trace	Trace	ND
PROPANE	1.3	0.2	0.1	ND	ND	ND	ND	ND	ND
PROPYLENE	2.0	3.6	4.4	ND	ND	ND	ND	ND	ND
ACETYLENE	0.9	1.9	2.2	ND	ND	ND	ND	Trace	Trace
PROPADIENE	ND	ND	ND	ND	ND	ND	ND	ND	ND
BUTANE	0.2	0.3	0.2	Trace	ND	ND	ND	ND	ND
TRANS-2-BUTENE	0.2	0.3	0.4	ND	ND	ND	ND	ND	ND
1-BUTENE	ND	ND	ND	ND	ND	ND	ND	ND	ND
2-METHYLPROPENE (ISOBUTYLENE)	1.3	2.2	2.6	ND	ND	ND	ND	ND	ND
2,2-DIMETHYLPROPANE (NEOPENTANE)	4.9	5.2	4.9	ND	0.2	ND	ND	0.1	Trace
PROPYNE	ND	ND	ND	ND	ND	ND	ND	ND	ND
1,3-BUTADIENE	0.2	0.5	0.6	ND	ND	ND	ND	ND	ND
2-METHYLPROPANE (ISOBUTANE)	0.1	0.2	0.3	ND	ND	ND	ND	ND	ND
1-BUTYNE	ND	ND	ND	ND	ND	ND	ND	ND	ND
METHANOL	ND	ND	0.3	ND	ND	0.2	ND	ND	ND
CIS-2-BUTENE	0.2	0.2	0.3	ND	ND	ND	ND	ND	ND
3-METHYL-1-BUTENE	ND	ND	ND	ND	ND	ND	ND	ND	ND
ETHANOL	1.8	2.9	4.4	0.1	ND	0.1	ND	ND	ND
2-METHYLBUTANE (ISOPENTANE)	0.1	0.2	0.3	ND	ND	ND	ND	ND	ND
2-BUTYNE	ND	ND	ND	ND	ND	ND	ND	ND	ND
1-PENTENE	ND	ND	ND	ND	ND	ND	ND	ND	ND
2-METHYL-1-BUTENE	ND	ND	ND	ND	ND	ND	ND	ND	ND
PENTANE	1.1	1.4	1.5	0.6	0.4	0.4	0.1	0.5	0.1
UNIDENTIFIED C5 OLEFINS	ND	ND	ND	ND	ND	ND	ND	ND	ND
2-METHYL-1,3-BUTADIENE	ND	ND	ND	ND	ND	ND	ND	ND	ND
TRANS-2-PENTENE	ND	ND	ND	ND	ND	ND	ND	ND	ND
3,3-DIMETHYL-1-BUTENE	ND	ND	ND	ND	ND	ND	ND	ND	ND
CIS-2-PENTENE	ND	ND	ND	ND	ND	ND	ND	ND	ND
2-METHYL-2-BUTENE	0.3	0.3	0.4	ND	ND	ND	ND	ND	ND
TERT-BUTANOL	ND	ND	ND	ND	ND	ND	ND	ND	ND
CYCLOPENTADIENE	ND	ND	ND	ND	ND	ND	ND	ND	ND
2,2-DIMETHYLBUTANE	0.2	0.2	0.2	ND	ND	ND	ND	ND	ND
CYCLOPENTENE	ND	ND	ND	ND	ND	ND	ND	ND	ND
4-METHYL-1-PENTENE	ND	ND	ND	ND	ND	ND	ND	ND	ND
3-METHYL-1-PENTENE	ND	ND	ND	ND	ND	ND	ND	ND	ND
CYCLOPENTANE	ND	ND	ND	ND	ND	ND	ND	ND	ND
2,3-DIMETHYLBUTANE	0.5	0.5	0.6	ND	Trace	Trace	ND	Trace	Trace
MTBE	ND	ND	ND	ND	ND	ND	ND	ND	ND
4-METHYL-CIS-2-PENTENE	ND	ND	ND	ND	ND	ND	ND	ND	ND
2-METHYLPENTANE	0.6	0.7	0.8	0.1	ND	Trace	0.1	0.1	0.1
4-METHYL-TRANS-2-PENTENE	ND	ND	ND	ND	ND	ND	ND	ND	ND
3-METHYLPENTANE	0.4	0.6	0.4	0.3	0.3	ND	0.4	0.1	0.1
2-METHYL-1-PENTENE	ND	ND	ND	ND	ND	ND	ND	ND	ND
1-HEXENE	ND	ND	ND	ND	ND	ND	ND	ND	ND
HEXANE	0.5	0.6	0.7	ND	0.1	Trace	ND	Trace	Trace
UNIDENTIFIED C6	ND	ND	ND	ND	ND	ND	ND	ND	ND
TRANS-3-HEXENE	ND	ND	ND	ND	ND	ND	ND	ND	ND

TABLE B-5 (CONT'D). HYDROCARBON SPECIATION DATA FOR E15 WITH AFTERTREATMENT

	mg/mile			mg/mile			mg/mile		
	RFAE15CAT-1	RFAE15CAT-3	RFAE15CAT-4	RFAE15CAT-1	RFAE15CAT-3	RFAE15CAT-4	RFAE15CAT-1	RFAE15CAT-3	RFAE15CAT-4
COMPOUND	C505	C505	C505	C867	C867	C867	H505	H505	H505
CIS-3-HEXENE	ND	ND	ND	ND	ND	ND	ND	ND	ND
DI-ISOPROPYL ETHER	ND	ND	ND	ND	ND	ND	ND	ND	ND
TRANS-2-HEXENE	ND	ND	ND	ND	ND	ND	ND	ND	ND
3-METHYL-TRANS-2-PENTENE	ND	ND	ND	ND	ND	ND	ND	ND	ND
2-METHYL-2-PENTENE	ND	ND	ND	ND	ND	ND	ND	ND	ND
3-METHYLCYCLOPENTENE	ND	ND	ND	ND	ND	ND	ND	ND	ND
CIS-2-HEXENE	ND	ND	ND	ND	ND	ND	ND	ND	ND
ETBE	ND	ND	ND	ND	ND	ND	ND	ND	ND
3-METHYL-CIS-2-PENTENE	ND	ND	ND	ND	ND	ND	ND	ND	ND
2,2-DIMETHYLPENTANE, NOTE A	0.1	0.2	0.2	ND	Trace	Trace	ND	Trace	Trace
METHYLCYCLOPENTANE, NOTE A	0.1	0.2	0.2	ND	Trace	Trace	ND	Trace	Trace
2,4-DIMETHYLPENTANE	0.5	0.6	0.6	ND	ND	ND	ND	0.1	0.1
2,2,3-TRIMETHYLBUTANE	ND	ND	ND	ND	ND	ND	ND	ND	ND
3,4-DIMETHYL-1-PENTENE	ND	ND	ND	ND	ND	ND	ND	ND	ND
1-METHYLCYCLOPENTENE	ND	ND	ND	ND	ND	ND	ND	ND	ND
BENZENE	2.7	4.3	5.0	ND	ND	ND	0.1	ND	ND
3-METHYL-1-HEXENE	ND	ND	ND	ND	ND	ND	ND	ND	ND
3,3-DIMETHYLPENTANE	ND	ND	ND	ND	ND	ND	ND	ND	ND
CYCLOHEXANE	0.3	0.2	0.3	ND	Trace	ND	ND	Trace	ND
2-METHYLHEXANE	0.4	0.6	0.7	0.7	ND	0.3	0.3	0.2	0.2
2,3-DIMETHYLPENTANE	ND	ND	ND	ND	ND	ND	ND	ND	ND
1,1-DIMETHYLCYCLOPENTANE	ND	ND	ND	ND	ND	ND	ND	ND	ND
TERT-AMYL METHYL ETHER	ND	ND	ND	ND	ND	ND	ND	ND	ND
CYCLOHEXENE	ND	ND	ND	ND	ND	ND	ND	ND	ND
3-METHYLHEXANE	0.2	0.3	0.3	ND	ND	ND	ND	ND	ND
CIS-1,3-DIMETHYLCYCLOPENTANE	ND	ND	ND	ND	ND	ND	ND	ND	ND
3-ETHYLPENTANE	ND	ND	ND	ND	ND	ND	ND	ND	ND
TRANS-1,2-DIMETHYLCYCLOPENTANE	ND	ND	ND	ND	ND	ND	ND	ND	ND
TRANS-1,3-DIMETHYLCYCLOPENTANE	ND	ND	ND	ND	ND	ND	ND	ND	ND
1-HEPTENE	ND	ND	ND	ND	ND	ND	ND	ND	ND
2,2,4-TRIMETHYLPENTANE	5.5	6.1	6.6	1.5	0.1	0.1	0.2	0.9	0.4
2-METHYL-1-HEXENE	ND	ND	ND	ND	ND	ND	ND	ND	ND
TRANS-3-HEPTENE	ND	ND	ND	ND	ND	ND	ND	ND	ND
HEPTANE	1.7	0.3	0.3	ND	ND	ND	ND	2.1	1.2
CIS-3-HEPTENE	ND	ND	ND	ND	ND	ND	ND	ND	ND
UNIDENTIFIED C7	ND	ND	ND	ND	ND	ND	ND	ND	ND
2-METHYL-2-HEXENE	ND	ND	ND	ND	ND	ND	ND	ND	ND
3-METHYL-TRANS-3-HEXENE	ND	ND	ND	ND	ND	ND	ND	ND	ND
TRANS-2-HEPTENE	ND	ND	ND	ND	ND	ND	ND	ND	ND
3-ETHYL-CIS-2-PENTENE	ND	ND	ND	ND	ND	ND	ND	ND	ND
2,4,4-TRIMETHYL-1-PENTENE	ND	ND	ND	ND	ND	ND	ND	ND	ND
2,3-DIMETHYL-2-PENTENE	ND	ND	ND	ND	ND	ND	ND	ND	ND
CIS-2-HEPTENE	ND	ND	ND	ND	ND	ND	ND	ND	ND
METHYLCYCLOHEXANE	0.4	0.4	0.5	ND	ND	ND	ND	ND	ND
CIS-1,2-DIMETHYLCYCLOPENTANE	ND	ND	ND	ND	ND	ND	ND	ND	ND
2,2-DIMETHYLHEXANE	ND	ND	ND	ND	ND	ND	ND	ND	ND
1,1,3-TRIMETHYLCYCLOPENTANE	ND	ND	ND	ND	ND	ND	ND	ND	ND
2,4,4-TRIMETHYL-2-PENTENE	ND	ND	ND	ND	ND	ND	ND	ND	ND

TABLE B-5 (CONT'D). HYDROCARBON SPECIATION DATA FOR E15 WITH AFTERTREATMENT

	mg/mile			mg/mile			mg/mile		
	RFAE15CAT-1	RFAE15CAT-3	RFAE15CAT-4	RFAE15CAT-1	RFAE15CAT-3	RFAE15CAT-4	RFAE15CAT-1	RFAE15CAT-3	RFAE15CAT-4
COMPOUND	C505	C505	C505	C867	C867	C867	H505	H505	H505
2,2,3-TRIMETHYLPENTANE	ND	ND	ND	ND	ND	ND	ND	ND	ND
2,5-DIMETHYLHEXANE	ND	ND	ND	ND	ND	ND	ND	ND	ND
ETHYLCYCLOPENTANE	ND	ND	ND	ND	ND	ND	ND	ND	ND
2,4-DIMETHYLHEXANE	0.6	1.5	1.7	ND	ND	ND	ND	ND	ND
1-TRANS-2-CIS-4-TRIMETHYLCYCLOPENTANE	ND	ND	ND	ND	ND	ND	ND	ND	ND
3,3-DIMETHYLHEXANE	ND	ND	ND	ND	ND	ND	ND	ND	ND
1-TRANS-2-CIS-3-TRIMETHYLCYCLOPENTANE	ND	ND	ND	ND	ND	ND	ND	ND	ND
2,3,4-TRIMETHYLPENTANE	1.7	2.0	2.2	0.1	0.2	0.2	0.1	0.4	0.2
2,3,3-TRIMETHYLPENTANE	1.5	1.5	1.7	ND	ND	ND	0.2	0.6	0.2
TOLUENE	6.5	10.9	13.1	ND	ND	ND	1.6	1.5	0.1
2,3-DIMETHYLHEXANE	ND	ND	ND	ND	ND	ND	ND	ND	ND
1,1,2-TRIMETHYLCYCLOPENTANE	ND	ND	ND	ND	ND	ND	ND	ND	ND
2-METHYLHEPTANE	ND	ND	ND	ND	ND	ND	ND	ND	ND
3,4-DIMETHYLHEXANE, NOTE B	ND	ND	ND	ND	ND	ND	ND	ND	ND
4-METHYLHEPTANE	ND	ND	ND	ND	ND	ND	ND	ND	ND
3-METHYLHEPTANE	ND	ND	ND	ND	ND	ND	ND	ND	ND
1-CIS,2-TRANS,3-TRIMETHYLCYCLOPENTANE	ND	ND	ND	ND	ND	ND	ND	ND	ND
CIS-1,3-DIMETHYLCYCLOHEXANE	ND	ND	ND	ND	ND	ND	ND	ND	ND
TRANS-1,4-DIMETHYLCYCLOHEXANE	ND	ND	ND	ND	ND	ND	ND	ND	ND
3-ETHYLHEXANE	ND	ND	ND	ND	ND	ND	ND	ND	ND
2,2,5-TRIMETHYLHEXANE	0.8	0.8	0.9	ND	ND	ND	ND	ND	ND
TRANS-1-METHYL-3-ETHYLCYCLOPENTANE	ND	ND	ND	ND	ND	ND	ND	ND	ND
CIS-1-METHYL-3-ETHYLCYCLOPENTANE	ND	ND	ND	ND	ND	ND	ND	ND	ND
1,1-DIMETHYLCYCLOHEXANE	ND	ND	ND	ND	ND	ND	ND	ND	ND
TRANS-1-METHYL-2-ETHYLCYCLOPENTANE	ND	ND	ND	ND	ND	ND	ND	ND	ND
1-METHYL-1-ETHYL-CYCLOPENTANE	ND	ND	ND	ND	ND	ND	ND	ND	ND
2,4,4-TRIMETHYLHEXANE	ND	ND	ND	ND	ND	ND	ND	ND	ND
2,2,4-TRIMETHYLHEXANE	ND	ND	ND	ND	ND	ND	ND	ND	ND
TRANS-1,2-DIMETHYLCYCLOHEXANE	ND	ND	ND	ND	ND	ND	ND	ND	ND
1-OCTENE	ND	ND	ND	ND	ND	ND	ND	ND	ND
TRANS-4-OCTENE	ND	ND	ND	ND	ND	ND	ND	ND	ND
OCTANE	ND	ND	ND	ND	ND	ND	ND	ND	ND
UNIDENTIFIED C8	ND	ND	ND	ND	ND	ND	ND	ND	ND
TRANS-2-OCTENE	ND	ND	ND	ND	ND	ND	ND	ND	ND
TRANS-1,3-DIMETHYLCYCLOHEXANE, NOTE C	ND	ND	ND	ND	ND	ND	ND	ND	ND
CIS-2-OCTENE	ND	ND	ND	ND	ND	ND	ND	ND	ND
ISOPROPYLCYCLOPENTANE	ND	ND	ND	ND	ND	ND	ND	ND	ND
2,2-DIMETHYLHEPTANE	ND	ND	ND	ND	ND	ND	ND	ND	ND
2,3,5-TRIMETHYLHEXANE	ND	ND	ND	ND	ND	ND	ND	ND	ND
CIS-1-METHYL-2-ETHYLCYCLOPENTANE	ND	ND	ND	ND	ND	ND	ND	ND	ND
2,4-DIMETHYLHEPTANE	ND	ND	ND	ND	ND	ND	ND	ND	ND
4,4-DIMETHYLHEPTANE	ND	ND	ND	ND	ND	ND	ND	ND	ND
CIS-1,2-DIMETHYLCYCLOHEXANE	ND	ND	ND	ND	ND	ND	ND	ND	ND
ETHYLCYCLOHEXANE	ND	ND	ND	ND	ND	ND	ND	ND	ND
2,6-DIMETHYLHEPTANE, NOTE D	ND	ND	ND	ND	ND	ND	ND	ND	ND
1,1,3-TRIMETHYLCYCLOHEXANE	ND	ND	ND	ND	ND	ND	ND	ND	ND
2,5-DIMETHYLHEPTANE, NOTE E	ND	ND	ND	ND	ND	ND	ND	ND	ND
3,3-DIMETHYLHEPTANE	ND	ND	ND	ND	ND	ND	ND	ND	ND
3,5-DIMETHYLHEPTANE, NOTE E	ND	ND	ND	ND	ND	ND	ND	ND	ND

TABLE B-5 (CONT'D). HYDROCARBON SPECIATION DATA FOR E15 WITH AFTERTREATMENT

	mg/mile			mg/mile			mg/mile		
	RFAE15CAT-1	RFAE15CAT-3	RFAE15CAT-4	RFAE15CAT-1	RFAE15CAT-3	RFAE15CAT-4	RFAE15CAT-1	RFAE15CAT-3	RFAE15CAT-4
COMPOUND	C505	C505	C505	C867	C867	C867	H505	H505	H505
ETHYLBENZENE	0.4	0.8	0.1	ND	ND	ND	ND	ND	ND
2,3,4-TRIMETHYLHEXANE	ND	ND	ND	ND	ND	ND	ND	ND	ND
2,3-DIMETHYLHEPTANE	ND	ND	ND	ND	ND	ND	ND	ND	ND
m-& p-XYLENE	ND	ND	ND	ND	ND	ND	ND	ND	ND
4-METHYLOCTANE	ND	ND	ND	ND	ND	ND	ND	ND	ND
3,4-DIMETHYLHEPTANE	ND	ND	ND	ND	ND	ND	ND	ND	ND
4-ETHYLHEPTANE	ND	ND	ND	ND	ND	ND	ND	ND	ND
2-METHYLOCTANE	ND	ND	ND	ND	ND	ND	ND	ND	ND
3-METHYLOCTANE	ND	ND	ND	ND	ND	ND	ND	ND	ND
STYRENE	ND	ND	ND	ND	ND	ND	ND	ND	ND
o-XYLENE	ND	ND	ND	ND	ND	ND	ND	ND	ND
1-NONENE	ND	ND	ND	ND	ND	ND	ND	ND	ND
TRANS-3-NONENE	ND	ND	ND	ND	ND	ND	ND	ND	ND
CIS-3-NONENE	ND	ND	ND	ND	ND	ND	ND	ND	ND
NONANE	ND	ND	ND	ND	ND	ND	ND	ND	ND
TRANS-2-NONENE	ND	ND	ND	ND	ND	ND	ND	ND	ND
ISOPROPYLBENZENE (CUMENE)	ND	ND	ND	ND	ND	ND	ND	ND	ND
2,2-DIMETHYLOCTANE	ND	ND	ND	ND	ND	ND	ND	ND	ND
2,4-DIMETHYLOCTANE	ND	ND	ND	ND	ND	ND	ND	ND	ND
n-PROPYLBENZENE	ND	ND	ND	ND	ND	ND	ND	ND	ND
1-METHYL-3-ETHYLBENZENE	ND	ND	ND	ND	ND	ND	ND	ND	ND
1-METHYL-4-ETHYLBENZENE	ND	ND	ND	ND	ND	ND	ND	ND	ND
1,3,5-TRIMETHYLBENZENE	ND	ND	ND	ND	ND	ND	ND	ND	ND
1-METHYL-2-ETHYLBENZENE	ND	ND	ND	ND	ND	ND	ND	ND	ND
1,2,4-TRIMETHYLBENZENE	ND	ND	ND	ND	ND	ND	ND	ND	ND
TERT-BUTYLBENZENE	ND	ND	ND	ND	ND	ND	ND	ND	ND
1-DECENE	ND	ND	ND	ND	ND	ND	ND	ND	ND
DECANE, NOTE F	ND	ND	ND	ND	ND	ND	ND	ND	ND
ISOBUTYLBENZENE, NOTE F	ND	ND	ND	ND	ND	ND	ND	ND	ND
1,3,-DIMETHYL-5-ETHYLBENZENE	ND	ND	ND	ND	ND	ND	ND	ND	ND
METHYLPROPYLBENZENE (sec butylbenzene)	ND	ND	ND	ND	ND	ND	ND	ND	ND
1-METHYL-3-ISOPROPYLBENZENE	ND	ND	ND	ND	ND	ND	ND	ND	ND
1,2,3-TRIMETHYLBENZENE	ND	ND	ND	ND	ND	ND	ND	ND	ND
1-METHYL-4-ISOPROPYLBENZENE	ND	ND	ND	ND	ND	ND	ND	ND	ND
INDAN	ND	ND	ND	ND	ND	ND	ND	ND	ND
1-METHYL-2-ISOPROPYLBENZENE	ND	ND	ND	ND	ND	ND	ND	ND	ND
1,3-DIETHYLBENZENE	ND	ND	ND	ND	ND	ND	ND	ND	ND
1,4-DIETHYLBENZENE	ND	ND	ND	ND	ND	ND	ND	ND	ND
1-METHYL-3-N-PROPYLBENZENE	ND	ND	ND	ND	ND	ND	ND	ND	ND
1-METHYL-4-N-PROPYLBENZENE, NOTE G	ND	ND	ND	ND	ND	ND	ND	ND	ND
1,2 DIETHYLBENZENE	ND	ND	ND	ND	ND	ND	ND	ND	ND
1-METHYL-2-N-PROPYLBENZENE	ND	ND	ND	ND	ND	ND	ND	ND	ND
1,4-DIMETHYL-2-ETHYLBENZENE	ND	ND	ND	ND	ND	ND	ND	ND	ND
1,3-DIMETHYL-4-ETHYLBENZENE	ND	ND	ND	ND	ND	ND	ND	ND	ND
1,2-DIMETHYL-4-ETHYLBENZENE	ND	ND	ND	ND	ND	ND	ND	ND	ND
1,3-DIMETHYL-2-ETHYLBENZENE	ND	ND	ND	ND	ND	ND	ND	ND	ND
UNDECANE	ND	ND	ND	ND	ND	ND	ND	ND	ND
1,2-DIMETHYL-3-ETHYLBENZENE	ND	ND	ND	ND	ND	ND	ND	ND	ND
1,2,4,5-TETRAMETHYLBENZENE	ND	ND	ND	ND	ND	ND	ND	ND	ND
2-METHYLBUTYLBENZENE (sec AMYLBENZENE)	ND	ND	ND	ND	ND	ND	ND	ND	ND

TABLE B-5 (CONT'D). HYDROCARBON SPECIATION DATA FOR E15 WITH AFTERTREATMENT

	mg/mile			mg/mile			mg/mile		
	RFAE15CAT-1	RFAE15CAT-3	RFAE15CAT-4	RFAE15CAT-1	RFAE15CAT-3	RFAE15CAT-4	RFAE15CAT-1	RFAE15CAT-3	RFAE15CAT-4
COMPOUND	C505	C505	C505	C867	C867	C867	H505	H505	H505
3,4 DIMETHYLCUMENE	ND	ND	ND	ND	ND	ND	ND	ND	ND
1,2,3,5-TETRAMETHYLBENZENE	ND	ND	ND	ND	ND	ND	ND	ND	ND
TERT-1-BUT-2-METHYLBENZENE	ND	ND	ND	ND	ND	ND	ND	ND	ND
1,2,3,4-TETRAMETHYLBENZENE	ND	ND	ND	ND	ND	ND	ND	ND	ND
N-PENT-BENZENE	ND	ND	ND	ND	ND	ND	ND	ND	ND
TERT-1-BUT-3,5-DIMETHYLBENZENE	ND	ND	ND	ND	ND	ND	ND	ND	ND
TERT-1-BUTYL-4-ETHYLBENZENE	ND	ND	ND	ND	ND	ND	ND	ND	ND
NAPHTHALENE	ND	ND	ND	ND	ND	ND	ND	ND	ND
DODECANE	ND	ND	ND	ND	ND	ND	ND	ND	ND
1,3,5-TRIETHYLBENZENE	ND	ND	ND	ND	ND	ND	ND	ND	ND
1,2,4-TRIETHYLBENZENE	ND	ND	ND	ND	ND	ND	ND	ND	ND
HEXYLBENZENE	ND	ND	ND	ND	ND	ND	ND	ND	ND
UNIDENTIFIED C9-C12+	ND	ND	ND	ND	ND	ND	ND	ND	ND
FORMALDEHYDE	0.5	0.9	0.9	0.1	0.1	0.1	ND	0.1	Trace
ACETALDEHYDE	1.4	1.9	2.2	Trace	Trace	Trace	ND	Trace	Trace
ACROLEIN	0.1	0.1	0.1	Trace	ND	ND	ND	ND	Trace
ACETONE	0.3	0.2	0.2	Trace	Trace	Trace	ND	Trace	Trace
PROPIONALDEHYDE	0.1	0.1	0.1	Trace	ND	ND	ND	ND	ND
CROTONALDEHYDE	Trace	ND	Trace	ND	ND	ND	ND	ND	ND
METHACROLEIN	0.1	ND	0.1	ND	ND	Trace	ND	ND	ND
N-/ISOBUTYRALDEHYDE	ND	ND	Trace	ND	ND	Trace	ND	ND	ND
METHYL ETHYL KETONE	ND	ND	Trace	ND	ND	ND	ND	ND	ND
BENZALDEHYDE	0.2	0.2	0.3	Trace	ND	ND	ND	ND	ND
ISOVALERALDEHYDE	ND	Trace	ND	ND	Trace	ND	ND	Trace	ND
VALERALDEHYDE	ND	ND	ND	ND	ND	ND	ND	ND	ND
O-TOLUALDEHYDE	ND	Trace	ND	Trace	ND	ND	ND	ND	ND
M/P-TOLUALDEHYDE	ND	ND	ND	ND	ND	ND	ND	ND	ND
METHYL ISOBUTYL KETONE	ND	ND	ND	ND	ND	ND	ND	ND	ND
HEXANALDEHYDE	ND	ND	ND	ND	ND	ND	ND	ND	ND
DIMETHYLBENZALDEHYDE	ND	ND	ND	ND	ND	ND	ND	ND	ND
SUMMED SPECIATED VALUES	60.3	80.3	88.9	4.5	3.3	5.0	5.9	11.1	7.0

A - 2,2-Dimethylpentane and methylcyclopentane co-elute. GC peak area split equally between the two compounds.
B - 3-Methyl-3-ethyl-pentane co-elutes with reported compound. Not reported separately.
C - Cis-1,4-Dimethylcyclohexane co-elutes with reported compound. Not reported separately.
D - Propylcyclopentane co-elutes with reported compound. Not reported separately.
E - 2,5-Dimethylheptane and 3,5-dimethylheptane co-elute. GC peak area split equally between the two compounds.
F - Decane and isobutylbenzene co-elute. GC peak area split equally between the two compounds.
G - n-Butylbenzene co-elutes with reported compound. Not reported separately.

TABLE B-6. HYDROCARBON SPECIATION DATA FOR E15 WITHOUT AFTERTREATMENT

	mg/mile			mg/mile			mg/mile		
	RFAE15-1	RFAE15-2	RFAE15-3	RFAE15-1	RFAE15-2	RFAE15-3	RFAE15-1	RFAE15-2	RFAE15-3
COMPOUND	C505	C505	C505	C867	C867	C867	H505	H505	H505
METHANE	45.7	46.8	41.8	44.2	43.9	42.1	32.4	33.8	36.0
ETHANE	15.9	16.2	14.0	13.8	12.7	12.9	9.7	10.4	10.0
ETHYLENE	103.2	104.5	90.8	96.8	91.7	90.0	68.4	73.5	69.0
PROPANE	1.1	1.1	1.0	1.4	1.0	1.2	0.7	0.9	0.8
PROPYLENE	83.7	84.5	74.0	71.7	68.4	67.0	53.3	57.4	53.7
ACETYLENE	54.4	62.8	54.8	60.0	57.3	56.0	42.0	45.5	43.4
PROPADIENE	ND	ND	ND	ND	ND	ND	ND	ND	ND
BUTANE	6.5	2.6	2.5	3.2	3.0	2.8	1.9	2.2	2.2
TRANS-2-BUTENE	7.6	7.6	6.7	6.6	6.3	6.1	4.8	5.1	4.8
1-BUTENE	8.7	8.7	7.6	7.5	7.3	6.9	5.5	5.9	5.4
2-METHYLPROPENE (ISOBUTYLENE)	56.7	56.8	49.6	49.8	47.2	46.3	36.1	38.8	36.3
2,2-DIMETHYLPROPANE (NEOPENTANE)	70.0	67.8	63.3	74.9	71.1	68.4	48.9	53.4	51.5
PROPYNE	Trace	ND	ND	Trace	ND	ND	Trace	ND	ND
1,3-BUTADIENE	13.6	13.1	10.3	10.9	10.2	9.2	7.5	7.3	5.6
2-METHYLPROPANE (ISOBUTANE)	7.1	7.8	6.8	5.4	5.2	5.3	4.5	4.8	5.0
1-BUTYNE	ND	ND	ND	ND	ND	ND	ND	ND	ND
METHANOL	11.3	12.2	12.7	11.8	11.7	9.9	8.2	8.7	9.1
CIS-2-BUTENE	5.3	5.3	4.7	4.7	4.4	4.3	3.4	3.7	3.4
3-METHYL-1-BUTENE	ND	ND	ND	ND	ND	ND	ND	ND	ND
ETHANOL	85.2	84.2	85.0	78.6	78.2	74.4	56.1	56.0	56.9
2-METHYLBUTANE (ISOPENTANE)	9.1	9.3	8.1	7.3	6.9	6.8	5.7	6.2	5.8
2-BUTYNE	2.3	1.2	0.5	0.3	0.2	0.3	0.7	0.9	1.0
1-PENTENE	3.2	3.4	2.9	1.2	1.3	1.0	0.9	1.6	1.4
2-METHYL-1-BUTENE	6.5	5.8	5.4	5.0	5.1	5.0	3.6	4.6	4.9
PENTANE	17.0	14.7	16.8	16.9	18.4	17.8	11.4	12.7	13.5
UNIDENTIFIED C5 OLEFINS	ND	ND	ND	ND	ND	ND	ND	ND	ND
2-METHYL-1,3-BUTADIENE	6.4	5.4	4.4	4.5	4.6	3.8	1.8	2.1	1.6
TRANS-2-PENTENE	1.6	1.6	1.3	1.3	1.4	1.4	0.9	1.2	1.2
3,3-DIMETHYL-1-BUTENE	1.0	1.0	0.8	0.7	0.8	0.7	0.5	0.7	0.7
CIS-2-PENTENE	0.8	0.8	0.7	0.7	0.7	0.7	0.5	0.6	0.6
2-METHYL-2-BUTENE	8.7	7.1	5.5	7.9	5.9	5.1	3.8	0.8	1.5
TERT-BUTANOL	ND	ND	ND	ND	ND	ND	ND	ND	ND
CYCLOPENTADIENE	0.4	0.4	0.2	0.7	0.3	0.2	ND	0.1	ND
2,2-DIMETHYLBUTANE	2.0	1.6	1.9	2.0	2.1	1.9	1.3	1.2	1.2
CYCLOPENTENE	0.7	0.6	0.7	0.7	0.7	0.8	0.5	0.5	0.5
4-METHYL-1-PENTENE	ND	ND	ND	ND	ND	ND	ND	ND	ND
3-METHYL-1-PENTENE	3.6	3.0	3.6	2.4	2.8	2.6	1.8	2.1	2.1
CYCLOPENTANE	1.6	1.4	1.6	2.0	2.0	2.0	1.2	1.2	1.2
2,3-DIMETHYLBUTANE	6.8	5.7	7.0	7.0	7.5	7.3	4.7	4.8	5.2
MTBE	ND	ND	ND	ND	ND	ND	ND	ND	ND
4-METHYL-CIS-2-PENTENE	ND	ND	ND	ND	ND	ND	ND	ND	ND
2-METHYLPENTANE	9.0	7.4	9.1	8.9	9.5	9.3	6.0	6.1	6.6
4-METHYL-TRANS-2-PENTENE	0.7	0.6	0.7	0.6	0.6	0.6	0.4	0.4	0.4
3-METHYLPENTANE	5.1	4.5	5.4	5.2	5.9	5.4	3.3	3.7	3.8
2-METHYL-1-PENTENE	0.4	0.6	0.5	0.7	0.8	0.4	0.3	0.4	0.4
1-HEXENE	0.4	0.6	0.5	0.7	0.8	0.4	0.3	0.4	0.4
HEXANE	7.8	6.6	8.0	8.0	8.6	8.2	5.3	5.4	5.8
UNIDENTIFIED C6	4.3	3.8	4.9	2.2	2.7	3.1	2.1	2.6	2.3
TRANS-3-HEXENE	ND	ND	ND	ND	ND	ND	ND	ND	ND

TABLE B-6 (CONT'D). HYDROCARBON SPECIATION DATA FOR E15 WITHOUT AFTERTREATMENT

	mg/mile			mg/mile			mg/mile		
	RFAE15-1	RFAE15-2	RFAE15-3	RFAE15-1	RFAE15-2	RFAE15-3	RFAE15-1	RFAE15-2	RFAE15-3
COMPOUND	C505	C505	C505	C867	C867	C867	H505	H505	H505
CIS-3-HEXENE	0.1	ND	0.1	ND	0.2	ND	ND	ND	ND
DI-ISOPROPYL ETHER	ND	ND	ND	ND	ND	ND	ND	ND	ND
TRANS-2-HEXENE	0.3	0.2	0.3	0.3	0.3	0.2	0.1	0.1	0.1
3-METHYL-TRANS-2-PENTENE	0.7	0.5	0.7	0.6	0.7	0.7	0.4	0.4	0.4
2-METHYL-2-PENTENE	0.9	0.8	0.9	0.9	0.9	0.8	0.6	0.6	0.6
3-METHYLCYCLOPENTENE	ND	ND	ND	ND	ND	ND	ND	ND	ND
CIS-2-HEXENE	0.1	0.1	0.1	0.4	ND	0.1	ND	ND	ND
ETBE	ND	ND	ND	ND	ND	ND	ND	ND	ND
3-METHYL-CIS-2-PENTENE	0.5	0.4	0.4	0.4	0.4	0.4	0.2	0.2	0.2
2,2-DIMETHYLPENTANE, NOTE A	2.4	2.1	2.5	2.5	2.6	2.6	1.6	1.7	1.8
METHYLCYCLOPENTANE, NOTE A	2.4	2.0	2.4	2.4	2.6	2.5	1.6	1.6	1.8
2,4-DIMETHYLPENTANE	6.4	5.4	6.3	6.8	7.0	6.6	4.4	4.4	4.7
2,2,3-TRIMETHYLBUTANE	0.8	0.7	0.6	1.0	0.9	0.8	0.6	0.5	0.6
3,4-DIMETHYL-1-PENTENE	0.4	0.7	0.4	0.4	0.5	0.6	0.2	0.5	0.4
1-METHYLCYCLOPENTENE	0.1	0.1	0.8	0.3	0.2	0.3	0.2	0.1	0.2
BENZENE	44.2	39.4	44.6	36.1	31.3	42.0	26.9	24.7	31.4
3-METHYL-1-HEXENE	ND	ND	ND	ND	ND	ND	ND	ND	ND
3,3-DIMETHYLPENTANE	1.9	1.9	1.9	1.8	2.2	2.1	1.5	1.4	1.4
CYCLOHEXANE	3.8	3.3	3.8	3.4	3.0	3.7	2.3	2.1	2.6
2-METHYLHEXANE	7.6	6.8	7.9	8.1	8.6	8.4	5.4	5.5	5.8
2,3-DIMETHYLPENTANE	0.9	0.7	0.9	0.6	0.6	0.6	0.4	0.5	0.5
1,1-DIMETHYLCYCLOPENTANE	ND	ND	ND	ND	ND	ND	ND	ND	ND
TERT-AMYL METHYL ETHER	ND	ND	ND	ND	ND	ND	ND	ND	ND
CYCLOHEXENE	0.9	0.9	0.9	1.0	1.0	1.0	0.7	0.6	0.6
3-METHYLHEXANE	2.4	2.2	2.4	2.6	2.6	2.6	1.7	1.7	1.8
CIS-1,3-DIMETHYLCYCLOPENTANE	0.5	0.5	0.5	0.5	0.5	0.5	0.3	0.3	0.3
3-ETHYLPENTANE	0.7	0.6	0.7	0.7	0.7	0.7	0.5	0.5	0.5
TRANS-1,2-DIMETHYLCYCLOPENTANE	ND	ND	ND	ND	ND	ND	ND	ND	ND
TRANS-1,3-DIMETHYLCYCLOPENTANE	0.6	0.5	0.6	0.6	0.7	0.6	0.4	0.4	0.4
1-HEPTENE	ND	ND	ND	ND	ND	ND	ND	ND	ND
2,2,4-TRIMETHYLPENTANE	88.1	73.7	88.1	93.1	97.1	92.9	61.3	61.6	65.4
2-METHYL-1-HEXENE	ND	ND	0.1	ND	ND	ND	ND	ND	ND
TRANS-3-HEPTENE	ND	ND	ND	ND	ND	ND	ND	ND	ND
HEPTANE	3.7	3.1	3.6	4.2	3.6	3.5	2.2	2.3	2.4
CIS-3-HEPTENE	ND	ND	ND	ND	ND	ND	ND	ND	ND
UNIDENTIFIED C7	3.3	2.7	2.8	2.6	3.0	2.8	1.9	1.9	1.8
2-METHYL-2-HEXENE	0.2	0.2	0.2	ND	ND	ND	ND	ND	ND
3-METHYL-TRANS-3-HEXENE	ND	ND	ND	ND	ND	ND	ND	ND	ND
TRANS-2-HEPTENE	0.1	ND	ND	ND	ND	ND	ND	ND	ND
3-ETHYL-CIS-2-PENTENE	ND	ND	ND	ND	ND	ND	ND	ND	ND
2,4,4-TRIMETHYL-1-PENTENE	0.2	0.2	0.2	0.3	0.3	0.2	0.2	0.2	0.2
2,3-DIMETHYL-2-PENTENE	ND	ND	ND	ND	ND	ND	ND	ND	ND
CIS-2-HEPTENE	0.6	0.4	0.5	0.6	0.5	0.5	0.2	0.2	0.2
METHYLCYCLOHEXANE	5.6	4.5	5.7	5.8	6.1	5.7	3.8	3.8	4.0
CIS-1,2-DIMETHYLCYCLOPENTANE	ND	ND	ND	ND	ND	ND	ND	ND	ND
2,2-DIMETHYLHEXANE	0.3	0.2	0.4	0.4	0.4	0.4	0.2	0.2	0.2
1,1,3-TRIMETHYLCYCLOPENTANE	0.1	ND	0.2	ND	ND	ND	ND	ND	ND
2,4,4-TRIMETHYL-2-PENTENE	ND	0.1	0.4	0.2	ND	0.4	0.3	ND	ND

TABLE B-6 (CONT'D). HYDROCARBON SPECIATION DATA FOR E15 WITHOUT AFTERTREATMENT

	mg/mile			mg/mile			mg/mile		
	RFAE15-1	RFAE15-2	RFAE15-3	RFAE15-1	RFAE15-2	RFAE15-3	RFAE15-1	RFAE15-2	RFAE15-3
COMPOUND	C505	C505	C505	C867	C867	C867	H505	H505	H505
2,2,3-TRIMETHYLPENTANE	8.5	7.7	8.7	10.2	10.5	9.8	6.5	6.6	6.8
2,5-DIMETHYLHEXANE	ND	ND	ND	ND	ND	ND	ND	ND	ND
ETHYLCYCLOPENTANE	ND	ND	ND	ND	ND	ND	ND	ND	ND
2,4-DIMETHYLHEXANE	12.6	9.7	12.6	12.2	12.7	12.4	8.3	8.2	8.8
1-TRANS-2-CIS-4-TRIMETHYLCYCLOPENTANE	0.4	0.3	0.4	0.4	0.4	0.4	0.3	0.2	0.3
3,3-DIMETHYLHEXANE	ND	ND	ND	ND	ND	ND	ND	ND	ND
1-TRANS-2-CIS-3-TRIMETHYLCYCLOPENTANE	ND	ND	ND	ND	ND	ND	ND	ND	ND
2,3,4-TRIMETHYLPENTANE	29.1	24.1	29.5	31.9	33.1	31.3	20.5	21.0	22.1
2,3,3-TRIMETHYLPENTANE	19.7	17.0	19.6	19.1	17.1	20.7	13.0	12.2	14.5
TOLUENE	243.6	219.7	243.8	196.6	180.2	221.7	138.8	138.3	162.8
2,3-DIMETHYLHEXANE	ND	ND	ND	ND	ND	ND	ND	ND	ND
1,1,2-TRIMETHYLCYCLOPENTANE	ND	ND	ND	ND	ND	ND	ND	ND	ND
2-METHYLHEPTANE	1.3	1.0	1.5	1.0	1.0	1.0	0.6	0.7	1.1
3,4-DIMETHYLHEXANE, NOTE B	1.4	1.1	1.7	1.5	1.5	1.5	0.9	1.0	1.2
4-METHYLHEPTANE	ND	ND	ND	ND	ND	ND	ND	ND	ND
3-METHYLHEPTANE	0.8	0.5	1.1	0.7	0.7	0.8	0.4	0.5	0.8
1-CIS,2-TRANS,3-TRIMETHYLCYCLOPENTANE	0.7	0.3	0.7	0.4	0.4	0.7	0.3	0.3	0.5
CIS-1,3-DIMETHYLCYCLOHEXANE	ND	ND	ND	ND	ND	ND	ND	ND	ND
TRANS-1,4-DIMETHYLCYCLOHEXANE	0.2	ND	0.3	ND	ND	0.3	ND	ND	0.2
3-ETHYLHEXANE	ND	ND	ND	ND	ND	ND	ND	ND	ND
2,2,5-TRIMETHYLHEXANE	11.4	9.4	11.3	12.3	12.7	12.1	8.2	8.2	8.7
TRANS-1-METHYL-3-ETHYLCYCLOPENTANE	ND	ND	ND	ND	ND	ND	ND	ND	ND
CIS-1-METHYL-3-ETHYLCYCLOPENTANE	ND	ND	0.1	ND	ND	ND	ND	ND	ND
1,1-DIMETHYLCYCLOHEXANE	ND	ND	ND	ND	ND	ND	ND	ND	ND
TRANS-1-METHYL-2-ETHYLCYCLOPENTANE	ND	ND	ND	ND	ND	ND	ND	ND	ND
1-METHYL-1-ETHYL-CYCLOPENTANE	0.7	0.5	0.8	0.7	0.8	0.7	0.5	0.6	0.6
2,4,4-TRIMETHYLHEXANE	ND	ND	ND	ND	ND	ND	ND	ND	ND
2,2,4-TRIMETHYLHEXANE	ND	ND	ND	ND	ND	ND	ND	ND	ND
TRANS-1,2-DIMETHYLCYCLOHEXANE	0.3	0.2	0.3	0.2	0.3	0.2	0.2	0.2	0.2
1-OCTENE	ND	ND	ND	ND	ND	ND	ND	ND	ND
TRANS-4-OCTENE	ND	ND	ND	ND	ND	ND	ND	ND	ND
OCTANE	1.1	0.9	1.1	1.1	1.2	1.1	0.7	0.8	0.8
UNIDENTIFIED C8	ND	ND	ND	ND	0.2	0.2	0.1	0.1	ND
TRANS-2-OCTENE	0.1	0.1	0.1	ND	ND	ND	0.1	0.1	0.1
TRANS-1,3-DIMETHYLCYCLOHEXANE, NOTE C	0.3	0.3	0.3	0.3	0.3	0.3	0.2	0.2	0.2
CIS-2-OCTENE	0.2	0.1	ND	ND	0.2	ND	ND	ND	ND
ISOPROPYLCYCLOPENTANE	0.5	0.3	0.4	0.4	0.5	0.4	0.3	0.2	0.3
2,2-DIMETHYLHEPTANE	ND	ND	ND	ND	ND	ND	ND	ND	ND
2,3,5-TRIMETHYLHEXANE	1.8	1.6	1.7	2.0	2.0	1.9	1.4	1.3	1.3
CIS-1-METHYL-2-ETHYLCYCLOPENTANE	ND	ND	ND	ND	ND	ND	ND	ND	ND
2,4-DIMETHYLHEPTANE	0.3	0.3	0.3	0.4	0.4	0.3	0.3	0.2	0.3
4,4-DIMETHYLHEPTANE	ND	ND	ND	ND	ND	ND	ND	ND	ND
CIS-1,2-DIMETHYLCYCLOHEXANE	0.5	0.4	0.5	0.5	0.5	0.4	0.4	0.3	0.4
ETHYLCYCLOHEXANE	0.2	0.3	0.3	0.3	0.3	ND	0.2	0.2	0.2
2,6-DIMETHYLHEPTANE, NOTE D	ND	ND	ND	ND	ND	ND	ND	ND	ND
1,1,3-TRIMETHYLCYCLOHEXANE	ND	ND	ND	ND	ND	ND	ND	ND	ND
2,5-DIMETHYLHEPTANE, NOTE E	0.3	0.4	0.4	0.4	0.5	0.4	0.3	0.3	0.4
3,3-DIMETHYLHEPTANE	ND	ND	ND	ND	ND	ND	ND	ND	ND
3,5-DIMETHYLHEPTANE, NOTE E	0.3	0.4	0.4	0.4	0.5	0.4	0.3	0.3	0.4

TABLE B-6 (CONT'D). HYDROCARBON SPECIATION DATA FOR E15 WITHOUT AFTERTREATMENT

	mg/mile			mg/mile			mg/mile		
	RFAE15-1	RFAE15-2	RFAE15-3	RFAE15-1	RFAE15-2	RFAE15-3	RFAE15-1	RFAE15-2	RFAE15-3
COMPOUND	C505	C505	C505	C867	C867	C867	H505	H505	H505
ETHYLBENZENE	16.0	13.8	15.9	12.3	13.3	13.2	8.7	9.3	9.9
2,3,4-TRIMETHYLHEXANE	ND	ND	ND	ND	ND	ND	ND	ND	ND
2,3-DIMETHYLHEPTANE	ND	ND	ND	ND	ND	ND	ND	ND	ND
m-& p-XYLENE	17.1	14.9	17.3	14.1	15.2	15.0	9.8	10.9	11.5
4-METHYLOCTANE	ND	ND	ND	ND	ND	ND	ND	ND	ND
3,4-DIMETHYLHEPTANE	ND	ND	ND	ND	ND	ND	ND	ND	ND
4-ETHYLHEPTANE	ND	ND	ND	ND	ND	ND	ND	ND	ND
2-METHYLOCTANE	0.2	0.4	0.5	0.3	0.4	0.2	0.3	0.3	0.3
3-METHYLOCTANE	ND	0.2	0.2	0.2	0.2	ND	0.1	0.1	0.1
STYRENE	9.5	8.1	7.8	7.2	7.4	6.0	2.2	2.9	1.8
o-XYLENE	12.5	10.9	12.8	11.6	12.5	12.0	7.5	8.6	8.9
1-NONENE	0.9	0.8	0.9	1.0	1.0	0.9	0.6	0.7	0.7
TRANS-3-NONENE	ND	ND	ND	ND	ND	ND	ND	ND	ND
CIS-3-NONENE	ND	ND	ND	ND	ND	ND	ND	ND	ND
NONANE	1.0	0.9	1.0	1.1	1.1	1.1	0.7	0.8	0.8
TRANS-2-NONENE	ND	ND	ND	ND	ND	ND	ND	ND	ND
ISOPROPYLBENZENE (CUMENE)	0.8	0.7	0.8	0.4	0.7	0.7	0.2	0.3	0.5
2,2-DIMETHYLOCTANE	0.3	0.1	0.2	ND	0.3	0.2	0.1	0.1	0.2
2,4-DIMETHYLOCTANE	ND	ND	ND	ND	ND	ND	ND	ND	ND
n-PROPYLBENZENE	11.6	9.7	11.8	11.8	12.7	12.2	7.7	8.7	8.8
1-METHYL-3-ETHYLBENZENE	42.6	36.0	43.5	41.9	45.4	43.0	26.8	31.0	31.8
1-METHYL-4-ETHYLBENZENE	18.6	15.9	18.9	18.6	19.8	19.0	11.4	13.4	14.2
1,3,5-TRIMETHYLBENZENE	21.4	18.2	21.8	20.4	22.2	20.9	12.7	15.3	15.8
1-METHYL-2-ETHYLBENZENE	17.9	14.8	17.7	17.7	19.4	17.8	11.1	12.7	12.9
1,2,4-TRIMETHYLBENZENE	79.5	59.4	75.0	62.2	68.0	69.0	34.5	46.4	46.5
TERT-BUTYLBENZENE	ND	ND	ND	ND	ND	ND	ND	ND	ND
1-DECENE	ND	ND	ND	ND	ND	ND	ND	ND	ND
DECANE, NOTE F	7.4	1.6	0.9	0.7	0.7	0.4	0.3	0.6	0.3
ISOBUTYLBENZENE, NOTE F	7.0	1.6	0.9	0.7	0.7	0.4	0.3	0.5	0.3
1,3,-DIMETHYL-5-ETHYLBENZENE	ND	ND	ND	ND	ND	ND	ND	ND	ND
METHYLPROPYLBENZENE (sec butylbenzene)	ND	ND	ND	ND	7.3	6.9	1.8	5.0	4.9
1-METHYL-3-ISOPROPYLBENZENE	3.8	3.8	0.8	4.4	5.5	3.7	4.4	4.2	3.6
1,2,3-TRIMETHYLBENZENE	5.5	1.5	3.5	4.2	4.6	4.3	2.5	3.4	2.8
1-METHYL-4-ISOPROPYLBENZENE	ND	ND	ND	ND	ND	ND	ND	ND	ND
INDAN	ND	ND	ND	ND	ND	ND	ND	ND	ND
1-METHYL-2-ISOPROPYLBENZENE	ND	ND	ND	ND	ND	ND	ND	ND	ND
1,3-DIETHYLBENZENE	3.4	3.3	4.1	3.8	3.8	1.9	1.8	3.0	3.1
1,4-DIETHYLBENZENE	7.0	6.1	5.7	4.3	4.5	3.4	3.0	4.5	5.0
1-METHYL-3-N-PROPYLBENZENE	ND	ND	ND	ND	ND	ND	ND	ND	ND
1-METHYL-4-N-PROPYLBENZENE, NOTE G	ND	ND	ND	ND	ND	ND	ND	ND	ND
1,2 DIETHYLBENZENE	ND	ND	ND	ND	ND	ND	ND	ND	ND
1-METHYL-2-N-PROPYLBENZENE	ND	ND	ND	ND	ND	ND	ND	ND	ND
1,4-DIMETHYL-2-ETHYLBENZENE	ND	ND	ND	ND	ND	ND	ND	ND	ND
1,3-DIMETHYL-4-ETHYLBENZENE	ND	ND	ND	ND	ND	ND	ND	ND	ND
1,2-DIMETHYL-4-ETHYLBENZENE	6.3	3.2	4.0	2.7	0.8	0.5	1.3	1.3	2.4
1,3-DIMETHYL-2-ETHYLBENZENE	ND	ND	ND	ND	ND	ND	ND	ND	ND
UNDECANE	1.9	ND	ND	ND	ND	ND	ND	ND	ND
1,2-DIMETHYL-3-ETHYLBENZENE	ND	ND	ND	ND	ND	ND	ND	ND	ND
1,2,4,5-TETRAMETHYLBENZENE	0.7	ND	ND	ND	ND	ND	ND	ND	ND
2-METHYLBUTYLBENZENE (sec AMYLBENZENE)	ND	ND	ND	ND	ND	ND	ND	ND	ND

TABLE B-6 (CONT'D). HYDROCARBON SPECIATION DATA FOR E15 WITHOUT AFTERTREATMENT

	mg/mile			mg/mile			mg/mile		
	RFAE15-1	RFAE15-2	RFAE15-3	RFAE15-1	RFAE15-2	RFAE15-3	RFAE15-1	RFAE15-2	RFAE15-3
COMPOUND	C505	C505	C505	C867	C867	C867	H505	H505	H505
3,4 DIMETHYLCUMENE	ND	ND	ND	ND	ND	ND	ND	ND	ND
1,2,3,5-TETRAMETHYLBENZENE	ND	ND	ND	ND	ND	ND	ND	ND	ND
TERT-1-BUT-2-METHYLBENZENE	ND	ND	ND	ND	ND	ND	ND	ND	ND
1,2,3,4-TETRAMETHYLBENZENE	ND	ND	ND	ND	ND	ND	ND	ND	ND
N-PENT-BENZENE	ND	ND	ND	ND	ND	ND	ND	ND	ND
TERT-1-BUT-3,5-DIMETHYLBENZENE	0.4	ND	ND	ND	ND	ND	ND	ND	ND
TERT-1-BUTYL-4-ETHYLBENZENE	ND	ND	ND	ND	ND	ND	ND	ND	ND
NAPHTHALENE	ND	ND	ND	ND	ND	ND	ND	ND	ND
DODECANE	ND	ND	ND	ND	ND	ND	ND	ND	ND
1,3,5-TRIETHYLBENZENE	ND	ND	ND	ND	ND	ND	ND	ND	ND
1,2,4-TRIETHYLBENZENE	ND	ND	ND	ND	ND	ND	ND	ND	ND
HEXYLBENZENE	ND	ND	ND	ND	ND	ND	ND	ND	ND
UNIDENTIFIED C9-C12+	28.7	30.3	39.9	27.9	21.0	14.4	11.2	14.7	12.8
FORMALDEHYDE	56.3	60.1	59.0	62.2	61.4	62.2	45.6	43.8	43.5
ACETALDEHYDE	31.8	32.8	33.0	30.0	30.4	29.9	23.0	22.3	22.4
ACROLEIN	5.1	5.1	5.0	5.0	4.6	4.6	3.8	3.5	3.3
ACETONE	5.0	5.3	5.3	5.8	6.0	5.6	4.5	4.4	4.4
PROPIONALDEHYDE	3.3	4.1	4.2	3.3	4.3	3.9	2.7	3.1	2.9
CROTONALDEHYDE	2.1	2.6	2.7	2.3	2.7	2.7	2.0	2.1	2.1
METHACROLEIN	4.4	4.9	4.8	4.4	4.2	4.3	3.5	3.5	3.2
N-/ISOBUTYRALDEHYDE	1.1	1.0	0.9	1.0	1.1	1.0	0.8	0.8	0.8
METHYL ETHYL KETONE	0.5	0.6	0.6	0.6	0.7	0.7	0.5	0.5	0.6
BENZALDEHYDE	16.3	18.4	18.3	17.9	18.3	18.2	14.0	13.9	13.5
ISOVALERALDEHYDE	0.9	0.6	0.9	0.8	0.8	1.0	ND	0.7	0.5
VALERALDEHYDE	0.2	0.1	0.3	0.3	0.3	0.4	0.5	0.3	0.1
O-TOLUALDEHYDE	1.3	1.5	1.4	1.5	1.5	1.7	1.1	1.2	1.2
M/P-TOLUALDEHYDE	4.4	5.1	5.1	5.9	6.1	6.4	4.0	4.7	4.7
METHYL ISOBUTYL KETONE	ND	4.1	3.7	ND	ND	ND	ND	ND	ND
HEXANALDEHYDE	1.4	0.3	0.2	0.2	0.1	0.3	0.2	0.1	0.1
DIMETHYLBENZALDEHYDE	4.7	5.2	5.3	5.6	2.4	5.1	4.3	4.4	3.6
SUMMED SPECIATED VALUES	1653.8	1528.9	1592.5	1516.3	1499.2	1506.1	1035.4	1095.5	1125.2

A - 2,2-Dimethylpentane and methylcyclopentane co-elute. GC peak area split equally between the two compounds.
B - 3-Methyl-3-ethyl-pentane co-elutes with reported compound. Not reported separately.
C - Cis-1,4-Dimethylcyclohexane co-elutes with reported compound. Not reported separately.
D - Propylcyclopentane co-elutes with reported compound. Not reported separately.
E - 2,5-Dimethylheptane and 3,5-dimethylheptane co-elute. GC peak area split equally between the two compounds.
F - Decane and isobutylbenzene co-elute. GC peak area split equally between the two compounds.
G - n-Butylbenzene co-elutes with reported compound. Not reported separately.

TABLE B-7. COMPARISON OF COMPOSITE HYDROCARBON SPECIATION DATA WITH AFTERTREATMENT

	mg/mile			mg/mile			mg/mile		
	RFBASECAT-2	RFBASECAT-3	RFBASECAT-6	E10ADDCAT-1	E10ADDCAT-2	E10ADDCAT-3	RFAE15CAT-1	RFAE15CAT-3	RFAE15CAT-4
COMPOUND	FTP	FTP	FTP	FTP	FTP	FTP	FTP	FTP	FTP
METHANE	3.0	3.4	6.1	3.6	3.6	3.4	3.8	5.1	6.1
ETHANE	0.8	0.9	0.9	0.6	0.4	0.4	0.4	0.7	0.7
ETHYLENE	1.9	2.3	2.5	1.3	0.7	0.8	0.7	1.4	1.6
PROPANE	0.3	Trace	Trace	0.2	Trace	Trace	0.3	Trace	Trace
PROPYLENE	1.4	1.7	1.9	0.8	0.5	0.5	0.4	0.7	0.9
ACETYLENE	0.7	0.8	1.0	0.4	0.2	0.2	0.2	0.4	0.5
PROPADIENE	ND	ND	ND	ND	ND	ND	ND	ND	ND
BUTANE	0.2	0.1	0.1	Trace	0.1	0.1	0.1	0.1	Trace
TRANS-2-BUTENE	0.1	0.1	0.1	0.1	Trace	Trace	Trace	0.1	0.1
1-BUTENE	ND	ND	ND	ND	ND	ND	ND	ND	ND
2-METHYLPROPENE (ISOBUTYLENE)	0.1	0.2	0.2	0.5	0.3	0.3	0.3	0.5	0.5
2,2-DIMETHYLPROPANE (NEOPENTANE)	Trace	Trace	Trace	Trace	Trace	Trace	1.0	1.2	1.0
PROPYNE	Trace	Trace	Trace	ND	ND	ND	ND	ND	ND
1,3-BUTADIENE	0.2	0.3	0.3	0.1	Trace	Trace	Trace	0.1	0.1
2-METHYLPROPANE (ISOBUTANE)	0.1	0.2	0.2	Trace	Trace	Trace	Trace	0.1	0.1
1-BUTYNE	ND	ND	ND	ND	ND	ND	ND	ND	ND
METHANOL	ND	ND	Trace	ND	ND	ND	ND	ND	Trace
CIS-2-BUTENE	0.8	1.0	1.1	Trace	Trace	Trace	Trace	Trace	0.1
3-METHYL-1-BUTENE	Trace	Trace	Trace	ND	ND	ND	ND	ND	ND
ETHANOL	ND	ND	ND	0.2	0.2	0.3	0.4	0.6	0.9
2-METHYLBUTANE (ISOPENTANE)	1.7	1.4	3.0	1.2	1.0	1.1	Trace	Trace	0.1
2-BUTYNE	ND	ND	ND	ND	ND	ND	ND	ND	ND
1-PENTENE	ND	ND	ND	ND	ND	ND	ND	ND	ND
2-METHYL-1-BUTENE	ND	ND	ND	ND	ND	ND	ND	ND	ND
PENTANE	0.4	0.2	0.7	0.3	0.2	0.1	0.6	0.6	0.6
UNIDENTIFIED C5 OLEFINS	ND	ND	ND	ND	ND	ND	ND	ND	ND
2-METHYL-1,3-BUTADIENE	ND	ND	ND	ND	ND	ND	ND	ND	ND
TRANS-2-PENTENE	Trace	Trace	0.1	ND	ND	ND	ND	ND	ND
3,3-DIMETHYL-1-BUTENE	ND	ND	ND	ND	ND	ND	ND	ND	ND
CIS-2-PENTENE	ND	ND	ND	ND	ND	ND	ND	ND	ND
2-METHYL-2-BUTENE	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1
TERT-BUTANOL	ND	ND	ND	ND	ND	ND	ND	ND	ND
CYCLOPENTADIENE	0.1	0.1	0.1	ND	ND	ND	ND	ND	ND
2,2-DIMETHYLBUTANE	0.1	Trace	0.1	Trace	Trace	Trace	Trace	Trace	Trace
CYCLOPENTENE	ND	ND	ND	ND	ND	ND	ND	ND	ND
4-METHYL-1-PENTENE	ND	ND	ND	ND	ND	ND	ND	ND	ND
3-METHYL-1-PENTENE	ND	ND	ND	ND	ND	ND	ND	ND	ND
CYCLOPENTANE	Trace	Trace	Trace	Trace	Trace	Trace	ND	ND	ND
2,3-DIMETHYLBUTANE	0.1	0.2	0.3	0.1	0.1	0.1	0.1	0.1	0.1
MTBE	ND	ND	ND	ND	ND	ND	ND	ND	ND
4-METHYL-CIS-2-PENTENE	ND	ND	ND	ND	ND	ND	ND	ND	ND
2-METHYLPENTANE	0.2	0.5	0.5	0.2	0.1	0.1	0.2	0.2	0.2
4-METHYL-TRANS-2-PENTENE	ND	ND	ND	ND	ND	ND	ND	ND	ND
3-METHYLPENTANE	0.1	0.1	0.2	0.1	0.1	0.1	0.4	0.3	0.1
2-METHYL-1-PENTENE	ND	ND	ND	ND	ND	ND	ND	ND	ND
1-HEXENE	ND	ND	ND	ND	ND	ND	ND	ND	ND
HEXANE	0.2	0.2	0.3	0.1	0.2	0.1	0.1	0.2	0.2
UNIDENTIFIED C6	ND	ND	ND	ND	ND	ND	ND	ND	ND
TRANS-3-HEXENE	ND	ND	ND	ND	ND	ND	ND	ND	ND

TABLE B-7 (CONT'D). COMPARISON OF COMPOSITE HYDROCARBON SPECIATION DATA WITH AFTERTREATMENT

	mg/mile			mg/mile			mg/mile		
	RFBASECAT-2	RFBASECAT-3	RFBASECAT-6	E10ADDCAT-1	E10ADDCAT-2	E10ADDCAT-3	RFAE15CAT-1	RFAE15CAT-3	RFAE15CAT-4
COMPOUND	FTP	FTP	FTP	FTP	FTP	FTP	FTP	FTP	FTP
CIS-3-HEXENE	ND	ND	ND	ND	ND	ND	ND	ND	ND
DI-ISOPROPYL ETHER	ND	ND	ND	ND	ND	ND	ND	ND	ND
TRANS-2-HEXENE	ND	ND	ND	ND	ND	ND	ND	ND	ND
3-METHYL-TRANS-2-PENTENE	ND	ND	ND	ND	ND	ND	ND	ND	ND
2-METHYL-2-PENTENE	ND	ND	ND	ND	ND	ND	ND	ND	ND
3-METHYLCYCLOPENTENE	ND	ND	ND	ND	ND	ND	ND	ND	ND
CIS-2-HEXENE	ND	ND	ND	ND	ND	ND	ND	ND	ND
ETBE	ND	ND	ND	ND	ND	ND	ND	ND	ND
3-METHYL-CIS-2-PENTENE	ND	ND	ND	ND	ND	ND	ND	ND	ND
2,2-DIMETHYLPENTANE, NOTE A	0.1	0.1	0.1	Trace	Trace	Trace	Trace	0.1	0.1
METHYLCYCLOPENTANE, NOTE A	0.1	0.1	0.1	Trace	Trace	Trace	Trace	0.1	0.1
2,4-DIMETHYLPENTANE	0.1	0.1	0.3	0.1	0.1	0.1	0.1	0.2	0.2
2,2,3-TRIMETHYLBUTANE	ND	ND	ND	ND	ND	ND	ND	ND	ND
3,4-DIMETHYL-1-PENTENE	ND	ND	ND	ND	ND	ND	ND	ND	ND
1-METHYLCYCLOPENTENE	ND	ND	ND	ND	ND	ND	ND	ND	ND
BENZENE	1.2	1.4	1.2	0.9	0.5	0.5	0.6	0.9	1.0
3-METHYL-1-HEXENE	ND	ND	ND	ND	ND	ND	ND	ND	ND
3,3-DIMETHYLPENTANE	ND	ND	ND	ND	ND	ND	ND	ND	ND
CYCLOHEXANE	0.1	0.1	0.2	0.1	0.1	Trace	0.1	0.1	0.1
2-METHYLHEXANE	0.2	0.3	0.3	0.1	0.1	0.1	0.5	0.2	0.3
2,3-DIMETHYLPENTANE	ND	ND	ND	ND	ND	ND	ND	ND	ND
1,1-DIMETHYLCYCLOPENTANE	ND	ND	ND	ND	ND	ND	ND	ND	ND
TERT-AMYL METHYL ETHER	ND	ND	ND	ND	ND	ND	ND	ND	ND
CYCLOHEXENE	ND	ND	ND	ND	ND	ND	ND	ND	ND
3-METHYLHEXANE	0.1	Trace	0.1	Trace	Trace	Trace	Trace	0.1	0.1
CIS-1,3-DIMETHYLCYCLOPENTANE	ND	ND	ND	ND	ND	ND	ND	ND	ND
3-ETHYLPENTANE	Trace	Trace	Trace	ND	ND	ND	ND	ND	ND
TRANS-1,2-DIMETHYLCYCLOPENTANE	ND	ND	ND	ND	ND	ND	ND	ND	ND
TRANS-1,3-DIMETHYLCYCLOPENTANE	ND	ND	ND	ND	ND	ND	ND	ND	ND
1-HEPTENE	ND	ND	ND	ND	ND	ND	ND	ND	ND
2,2,4-TRIMETHYLPENTANE	1.7	1.9	3.2	1.2	1.2	1.0	1.9	1.6	1.5
2-METHYL-1-HEXENE	ND	ND	ND	ND	ND	ND	ND	ND	ND
TRANS-3-HEPTENE	ND	ND	ND	ND	ND	ND	ND	ND	ND
HEPTANE	0.1	Trace	0.1	0.1	Trace	Trace	0.3	0.6	0.4
CIS-3-HEPTENE	ND	ND	ND	ND	ND	ND	ND	ND	ND
UNIDENTIFIED C7	ND	ND	ND	ND	ND	ND	ND	ND	ND
2-METHYL-2-HEXENE	ND	ND	ND	ND	ND	ND	ND	ND	ND
3-METHYL-TRANS-3-HEXENE	ND	ND	ND	ND	ND	ND	ND	ND	ND
TRANS-2-HEPTENE	ND	ND	ND	ND	ND	ND	ND	ND	ND
3-ETHYL-CIS-2-PENTENE	ND	ND	ND	ND	ND	ND	ND	ND	ND
2,4,4-TRIMETHYL-1-PENTENE	ND	ND	ND	ND	ND	ND	ND	ND	ND
2,3-DIMETHYL-2-PENTENE	ND	ND	ND	ND	ND	ND	ND	ND	ND
CIS-2-HEPTENE	ND	ND	ND	ND	ND	ND	ND	ND	ND
METHYLCYCLOHEXANE	0.1	0.1	0.2	0.1	0.1	0.1	0.1	0.1	0.1
CIS-1,2-DIMETHYLCYCLOPENTANE	ND	ND	ND	ND	ND	ND	ND	ND	ND
2,2-DIMETHYLHEXANE	ND	ND	ND	ND	ND	ND	ND	ND	ND
1,1,3-TRIMETHYLCYCLOPENTANE	ND	ND	ND	ND	ND	ND	ND	ND	ND
2,4,4-TRIMETHYL-2-PENTENE	ND	ND	ND	ND	ND	ND	ND	ND	ND

TABLE B-7 (CONT'D). COMPARISON OF COMPOSITE HYDROCARBON SPECIATION DATA WITH AFTERTREATMENT

	mg/mile			mg/mile			mg/mile		
	RFBASECAT-2	RFBASECAT-3	RFBASECAT-6	E10ADDCAT-1	E10ADDCAT-2	E10ADDCAT-3	RFAE15CAT-1	RFAE15CAT-3	RFAE15CAT-4
COMPOUND	FTP	FTP	FTP	FTP	FTP	FTP	FTP	FTP	FTP
2,2,3-TRIMETHYLPENTANE	ND	ND	ND	ND	ND	ND	ND	ND	ND
2,5-DIMETHYLHEXANE	ND	ND	ND	ND	ND	ND	ND	ND	ND
ETHYLCYCLOPENTANE	ND	ND	ND	ND	ND	ND	ND	ND	ND
2,4-DIMETHYLHEXANE	0.4	0.4	0.6	0.3	0.2	0.2	0.1	0.3	0.3
1-TRANS-2-CIS-4-TRIMETHYLCYCLOPENTANE	ND	ND	ND	ND	ND	ND	ND	ND	ND
3,3-DIMETHYLHEXANE	ND	ND	ND	ND	ND	ND	ND	ND	ND
1-TRANS-2-CIS-3-TRIMETHYLCYCLOPENTANE	ND	ND	ND	ND	ND	ND	ND	ND	ND
2,3,4-TRIMETHYLPENTANE	0.5	0.5	0.8	0.4	0.3	0.3	0.4	0.6	0.6
2,3,3-TRIMETHYLPENTANE	0.4	0.4	0.6	0.4	0.3	0.3	0.4	0.5	0.4
TOLUENE	4.1	4.1	4.9	2.3	1.4	1.4	1.8	2.7	2.7
2,3-DIMETHYLHEXANE	ND	ND	ND	ND	ND	ND	ND	ND	ND
1,1,2-TRIMETHYLCYCLOPENTANE	ND	ND	ND	ND	ND	ND	ND	ND	ND
2-METHYLHEPTANE	0.2	0.2	0.3	ND	ND	ND	ND	ND	ND
3,4-DIMETHYLHEXANE, NOTE B	ND	ND	ND	ND	ND	ND	ND	ND	ND
4-METHYLHEPTANE	ND	ND	ND	ND	ND	ND	ND	ND	ND
3-METHYLHEPTANE	ND	ND	ND	ND	ND	ND	ND	ND	ND
1-CIS,2-TRANS,3-TRIMETHYLCYCLOPENTANE	Trace	Trace	Trace	ND	ND	ND	ND	ND	ND
CIS-1,3-DIMETHYLCYCLOHEXANE	ND	ND	ND	ND	ND	ND	ND	ND	ND
TRANS-1,4-DIMETHYLCYCLOHEXANE	ND	ND	ND	ND	ND	ND	ND	ND	ND
3-ETHYLHEXANE	ND	ND	ND	ND	ND	ND	ND	ND	ND
2,2,5-TRIMETHYLHEXANE	0.2	0.2	0.3	0.1	0.1	Trace	0.2	0.2	0.2
TRANS-1-METHYL-3-ETHYLCYCLOPENTANE	ND	ND	ND	ND	ND	ND	ND	ND	ND
CIS-1-METHYL-3-ETHYLCYCLOPENTANE	ND	ND	ND	ND	ND	ND	ND	ND	ND
1,1-DIMETHYLCYCLOHEXANE	ND	ND	ND	ND	ND	ND	ND	ND	ND
TRANS-1-METHYL-2-ETHYLCYCLOPENTANE	ND	ND	ND	ND	ND	ND	ND	ND	ND
1-METHYL-1-ETHYL-CYCLOPENTANE	ND	ND	ND	ND	ND	ND	ND	ND	ND
2,4,4-TRIMETHYLHEXANE	ND	ND	ND	ND	ND	ND	ND	ND	ND
2,2,4-TRIMETHYLHEXANE	ND	ND	ND	ND	ND	ND	ND	ND	ND
TRANS-1,2-DIMETHYLCYCLOHEXANE	ND	ND	ND	ND	ND	ND	ND	ND	ND
1-OCTENE	ND	ND	ND	ND	ND	ND	ND	ND	ND
TRANS-4-OCTENE	ND	ND	ND	ND	ND	ND	ND	ND	ND
OCTANE	ND	ND	ND	ND	ND	ND	ND	ND	ND
UNIDENTIFIED C8	ND	ND	ND	ND	ND	ND	ND	ND	ND
TRANS-2-OCTENE	ND	ND	ND	ND	ND	ND	ND	ND	ND
TRANS-1,3-DIMETHYLCYCLOHEXANE, NOTE C	ND	ND	ND	ND	ND	ND	ND	ND	ND
CIS-2-OCTENE	ND	ND	ND	ND	ND	ND	ND	ND	ND
ISOPROPYLCYCLOPENTANE	ND	ND	ND	ND	ND	ND	ND	ND	ND
2,2-DIMETHYLHEPTANE	ND	ND	ND	ND	ND	ND	ND	ND	ND
2,3,5-TRIMETHYLHEXANE	ND	ND	ND	ND	ND	ND	ND	ND	ND
CIS-1-METHYL-2-ETHYLCYCLOPENTANE	ND	ND	ND	ND	ND	ND	ND	ND	ND
2,4-DIMETHYLHEPTANE	ND	ND	ND	ND	ND	ND	ND	ND	ND
4,4-DIMETHYLHEPTANE	ND	ND	ND	ND	ND	ND	ND	ND	ND
CIS-1,2-DIMETHYLCYCLOHEXANE	ND	ND	ND	ND	ND	ND	ND	ND	ND
ETHYLCYCLOHEXANE	ND	ND	ND	ND	ND	ND	ND	ND	ND
2,6-DIMETHYLHEPTANE, NOTE D	ND	ND	ND	ND	ND	ND	ND	ND	ND
1,1,3-TRIMETHYLCYCLOHEXANE	ND	ND	ND	ND	ND	ND	ND	ND	ND
2,5-DIMETHYLHEPTANE, NOTE E	ND	ND	ND	ND	ND	ND	ND	ND	ND
3,3-DIMETHYLHEPTANE	ND	ND	ND	ND	ND	ND	ND	ND	ND
3,5-DIMETHYLHEPTANE, NOTE E	ND	ND	ND	ND	ND	ND	ND	ND	ND

TABLE B-7 (CONT'D). COMPARISON OF COMPOSITE HYDROCARBON SPECIATION DATA WITH AFTERTREATMENT

	mg/mile			mg/mile			mg/mile		
	RFBASECAT-2	RFBASECAT-3	RFBASECAT-6	E10ADDCAT-1	E10ADDCAT-2	E10ADDCAT-3	RFAE15CAT-1	RFAE15CAT-3	RFAE15CAT-4
COMPOUND	FTP	FTP	FTP	FTP	FTP	FTP	FTP	FTP	FTP
ETHYLBENZENE	0.3	0.2	0.1	ND	ND	ND	ND	ND	ND
2,3,4-TRIMETHYLHEXANE	ND	ND	ND	ND	ND	ND	ND	ND	ND
2,3-DIMETHYLHEPTANE	ND	ND	ND	ND	ND	ND	ND	ND	ND
m-& p-XYLENE	ND	ND	ND	ND	ND	ND	ND	ND	ND
4-METHYLOCTANE	ND	ND	ND	ND	ND	ND	ND	ND	ND
3,4-DIMETHYLHEPTANE	ND	ND	ND	ND	ND	ND	ND	ND	ND
4-ETHYLHEPTANE	ND	ND	ND	ND	ND	ND	ND	ND	ND
2-METHYLOCTANE	ND	ND	ND	ND	ND	ND	ND	ND	ND
3-METHYLOCTANE	ND	ND	ND	ND	ND	ND	ND	ND	ND
STYRENE	ND	ND	ND	ND	ND	ND	ND	ND	ND
o-XYLENE	ND	ND	ND	ND	ND	ND	ND	ND	ND
1-NONENE	ND	ND	ND	ND	ND	ND	ND	ND	ND
TRANS-3-NONENE	ND	ND	ND	ND	ND	ND	ND	ND	ND
CIS-3-NONENE	ND	ND	ND	ND	ND	ND	ND	ND	ND
NONANE	ND	ND	ND	ND	ND	ND	ND	ND	ND
TRANS-2-NONENE	ND	ND	ND	ND	ND	ND	ND	ND	ND
ISOPROPYLBENZENE (CUMENE)	ND	ND	ND	ND	ND	ND	ND	ND	ND
2,2-DIMETHYLOCTANE	ND	ND	ND	ND	ND	ND	ND	ND	ND
2,4-DIMETHYLOCTANE	ND	ND	ND	ND	ND	ND	ND	ND	ND
n-PROPYLBENZENE	ND	ND	ND	ND	ND	ND	ND	ND	ND
1-METHYL-3-ETHYLBENZENE	ND	ND	ND	ND	ND	ND	ND	ND	ND
1-METHYL-4-ETHYLBENZENE	ND	ND	ND	ND	ND	ND	ND	ND	ND
1,3,5-TRIMETHYLBENZENE	ND	ND	ND	ND	ND	ND	ND	ND	ND
1-METHYL-2-ETHYLBENZENE	ND	ND	ND	ND	ND	ND	ND	ND	ND
1,2,4-TRIMETHYLBENZENE	0.1	0.1	0.1	ND	ND	ND	ND	ND	ND
TERT-BUTYLBENZENE	ND	ND	ND	ND	ND	ND	ND	ND	ND
1-DECENE	ND	ND	ND	ND	ND	ND	ND	ND	ND
DECANE, NOTE F	ND	ND	ND	ND	ND	ND	ND	ND	ND
ISOBUTYLBENZENE, NOTE F	ND	ND	ND	ND	ND	ND	ND	ND	ND
1,3,-DIMETHYL-5-ETHYLBENZENE	ND	ND	ND	ND	ND	ND	ND	ND	ND
METHYLPROPYLBENZENE (sec butylbenzene)	ND	ND	ND	ND	ND	ND	ND	ND	ND
1-METHYL-3-ISOPROPYLBENZENE	ND	ND	ND	ND	ND	ND	ND	ND	ND
1,2,3-TRIMETHYLBENZENE	ND	ND	ND	ND	ND	ND	ND	ND	ND
1-METHYL-4-ISOPROPYLBENZENE	ND	ND	ND	ND	ND	ND	ND	ND	ND
INDAN	ND	ND	ND	ND	ND	ND	ND	ND	ND
1-METHYL-2-ISOPROPYLBENZENE	ND	ND	ND	ND	ND	ND	ND	ND	ND
1,3-DIETHYLBENZENE	ND	ND	ND	ND	ND	ND	ND	ND	ND
1,4-DIETHYLBENZENE	ND	ND	ND	ND	ND	ND	ND	ND	ND
1-METHYL-3-N-PROPYLBENZENE	ND	ND	ND	ND	ND	ND	ND	ND	ND
1-METHYL-4-N-PROPYLBENZENE, NOTE G	ND	ND	ND	ND	ND	ND	ND	ND	ND
1,2 DIETHYLBENZENE	ND	ND	ND	ND	ND	ND	ND	ND	ND
1-METHYL-2-N-PROPYLBENZENE	ND	ND	ND	ND	ND	ND	ND	ND	ND
1,4-DIMETHYL-2-ETHYLBENZENE	ND	ND	ND	ND	ND	ND	ND	ND	ND
1,3-DIMETHYL-4-ETHYLBENZENE	ND	ND	ND	ND	ND	ND	ND	ND	ND
1,2-DIMETHYL-4-ETHYLBENZENE	ND	ND	ND	ND	ND	ND	ND	ND	ND
1,3-DIMETHYL-2-ETHYLBENZENE	ND	ND	ND	ND	ND	ND	ND	ND	ND
UNDECANE	ND	ND	ND	ND	ND	ND	ND	ND	ND
1,2-DIMETHYL-3-ETHYLBENZENE	ND	ND	ND	ND	ND	ND	ND	ND	ND
1,2,4,5-TETRAMETHYLBENZENE	ND	ND	ND	ND	ND	ND	ND	ND	ND
2-METHYLBUTYLBENZENE (sec AMYLBENZENE)	ND	ND	ND	ND	ND	ND	ND	ND	ND

TABLE B-7 (CONT'D). COMPARISON OF COMPOSITE HYDROCARBON SPECIATION DATA WITH AFTERTREATMENT

	mg/mile			mg/mile			mg/mile		
	RFBASECAT-2	RFBASECAT-3	RFBASECAT-6	E10ADDCAT-1	E10ADDCAT-2	E10ADDCAT-3	RFAE15CAT-1	RFAE15CAT-3	RFAE15CAT-4
COMPOUND	FTP	FTP	FTP	FTP	FTP	FTP	FTP	FTP	FTP
3,4 DIMETHYLCUMENE	ND	ND	ND	ND	ND	ND	ND	ND	ND
1,2,3,5-TETRAMETHYLBENZENE	ND	ND	ND	ND	ND	ND	ND	ND	ND
TERT-1-BUT-2-METHYLBENZENE	ND	ND	ND	ND	ND	ND	ND	ND	ND
1,2,3,4-TETRAMETHYLBENZENE	ND	ND	ND	ND	ND	ND	ND	ND	ND
N-PENT-BENZENE	ND	ND	ND	ND	ND	ND	ND	ND	ND
TERT-1-BUT-3,5-DIMETHYLBENZENE	ND	ND	ND	ND	ND	ND	ND	ND	ND
TERT-1-BUTYL-4-ETHYLBENZENE	ND	ND	ND	ND	ND	ND	ND	ND	ND
NAPHTHALENE	ND	ND	ND	ND	ND	ND	ND	ND	ND
DODECANE	ND	ND	ND	ND	ND	ND	ND	ND	ND
1,3,5-TRIETHYLBENZENE	ND	ND	ND	ND	ND	ND	ND	ND	ND
1,2,4-TRIETHYLBENZENE	ND	ND	ND	ND	ND	ND	ND	ND	ND
HEXYLBENZENE	ND	ND	ND	ND	ND	ND	ND	ND	ND
UNIDENTIFIED C9-C12+	Trace	ND	ND	ND	ND	ND	ND	ND	ND
FORMALDEHYDE	0.3	0.3	0.3	0.2	0.2	0.1	0.1	0.3	0.3
ACETALDEHYDE	0.1	0.1	0.1	0.3	0.2	0.2	0.3	0.4	0.5
ACROLEIN	Trace	Trace	Trace	Trace	Trace	Trace	Trace	Trace	Trace
ACETONE	0.1	ND	0.1	0.1	0.1	0.1	0.1	Trace	Trace
PROPIONALDEHYDE	Trace	Trace	Trace	Trace	Trace	Trace	Trace	Trace	Trace
CROTONALDEHYDE	ND	ND	Trace	ND	ND	ND	Trace	ND	ND
METHACROLEIN	Trace	Trace	Trace	Trace	0.1	Trace	Trace	ND	Trace
N-/ISOBUTYRALDEHYDE	Trace	ND	ND	ND	ND	ND	ND	ND	ND
METHYL ETHYL KETONE	ND	ND	ND	ND	ND	ND	ND	ND	ND
BENZALDEHYDE	0.1	0.1	0.1	0.1	Trace	0.1	0.1	0.1	0.1
ISOVALERALDEHYDE	Trace	ND	Trace	ND	ND	ND	ND	ND	ND
VALERALDEHYDE	ND	ND	Trace	ND	Trace	ND	ND	ND	ND
O-TOLUALDEHYDE	ND	ND	Trace	ND	ND	ND	ND	ND	ND
M/P-TOLUALDEHYDE	Trace	Trace	Trace	Trace	ND	ND	ND	ND	ND
METHYL ISOBUTYL KETONE	ND	ND	ND	ND	ND	ND	ND	ND	ND
HEXANALDEHYDE	ND	ND	ND	ND	ND	ND	ND	ND	ND
DIMETHYLBENZALDEHYDE	ND	ND	ND	ND	ND	ND	ND	ND	ND
SUMMED SPECIATED VALUES	23.0	24.2	34.0	16.7	12.6	12.0	16.1	21.1	22.6

A - 2,2-Dimethylpentane and methylcyclopentane co-elute. GC peak area split equally between the two compounds.

B - 3-Methyl-3-ethyl-pentane co-elutes with reported compound. Not reported separately.

C - Cis-1,4-Dimethylcyclohexane co-elutes with reported compound. Not reported separately.

D - Propylcyclopentane co-elutes with reported compound. Not reported separately.

E - 2,5-Dimethylheptane and 3,5-dimethylheptane co-elute. GC peak area split equally between the two compounds.

F - Decane and isobutylbenzene co-elute. GC peak area split equally between the two compounds.

G - n-Butylbenzene co-elutes with reported compound. Not reported separately.

TABLE B-8. COMPARISON OF COMPOSITE HYDROCARBON SPECIATION DATA WITHOUT AFTERTREATMENT

	mg/mile			mg/mile			mg/mile		
	RFABASE-1	RFABASE-2	RFABASE-4	E10ADD-1	E10ADD-2	E10ADD-3	RFAE15-1	RFAE15-2	RFAE15-3
COMPOUND	FTP	FTP	FTP	FTP	FTP	FTP	FTP	FTP	FTP
METHANE	47.9	47.1	48.9	48.9	32.2	48.7	41.2	41.7	40.4
ETHANE	15.9	15.6	16.0	14.4	14.3	13.9	13.1	12.8	12.3
ETHYLENE	98.1	95.2	96.4	91.3	90.7	88.3	90.3	89.4	84.4
PROPANE	1.5	1.3	1.5	1.4	1.6	1.4	1.2	1.0	1.0
PROPYLENE	85.4	83.3	83.9	73.1	71.6	69.9	69.1	68.7	64.8
ACETYLENE	64.3	62.9	65.8	62.2	63.7	62.0	53.9	55.2	52.3
PROPADIENE	ND	ND	ND	ND	ND	ND	ND	ND	ND
BUTANE	9.4	9.6	9.8	3.5	4.6	3.1	3.5	2.7	2.6
TRANS-2-BUTENE	7.7	7.6	7.7	6.7	6.5	6.4	6.3	6.2	5.8
1-BUTENE	8.9	8.6	8.5	7.4	7.1	6.9	7.2	7.2	6.7
2-METHYLPROPENE (ISOBUTYLENE)	57.3	56.9	57.6	50.4	49.3	48.3	47.5	46.9	44.2
2,2-DIMETHYLPROPANE (NEOPENTANE)	88.7	88.2	92.1	78.7	79.8	76.9	66.8	65.6	62.7
PROPYNE	1.1	1.1	1.1	ND	Trace	Trace	Trace	ND	ND
1,3-BUTADIENE	10.2	12.0	11.9	10.2	9.3	9.4	10.5	10.0	8.4
2-METHYLPROPANE (ISOBUTANE)	6.7	6.4	5.6	6.0	4.6	5.8	5.5	5.6	5.5
1-BUTYNE	ND	ND	ND	ND	ND	ND	ND	ND	ND
METHANOL	5.7	7.8	6.5	8.0	8.3	8.0	10.7	11.0	10.2
CIS-2-BUTENE	5.4	5.4	5.4	4.7	4.6	4.5	4.5	4.4	4.2
3-METHYL-1-BUTENE	2.6	1.9	1.6	ND	ND	ND	ND	ND	ND
ETHANOL	0.7	0.6	0.5	65.5	62.9	63.6	73.8	73.3	71.8
2-METHYLBUTANE (ISOPENTANE)	8.4	8.4	8.3	7.4	7.2	7.1	7.2	7.2	6.8
2-BUTYNE	1.4	1.9	1.7	1.4	1.3	1.5	0.8	0.6	0.5
1-PENTENE	0.2	0.6	0.4	3.2	2.6	2.6	1.5	1.8	1.5
2-METHYL-1-BUTENE	5.7	7.1	6.7	6.5	5.9	6.3	4.9	5.1	5.1
PENTANE	17.2	20.5	20.7	20.4	19.5	20.3	15.4	16.0	16.4
UNIDENTIFIED C5 OLEFINS	ND	ND	ND	ND	ND	ND	ND	ND	ND
2-METHYL-1,3-BUTADIENE	2.5	4.8	4.3	4.6	4.0	3.6	4.2	4.1	3.3
TRANS-2-PENTENE	1.2	1.5	1.4	1.7	1.6	1.6	1.2	1.4	1.3
3,3-DIMETHYL-1-BUTENE	0.7	0.8	0.8	1.0	0.9	0.9	0.7	0.8	0.7
CIS-2-PENTENE	0.6	0.8	0.8	1.0	0.9	0.9	0.7	0.7	0.7
2-METHYL-2-BUTENE	5.1	6.3	6.5	5.8	5.8	5.8	7.0	4.8	4.2
TERT-BUTANOL	ND	ND	ND	ND	ND	ND	ND	ND	ND
CYCLOPENTADIENE	0.1	0.2	0.3	0.4	0.6	0.2	0.5	0.3	0.1
2,2-DIMETHYLBUTANE	1.6	2.1	2.1	2.1	2.1	1.9	1.8	1.8	1.7
CYCLOPENTENE	0.7	0.8	0.8	0.8	0.7	0.7	0.6	0.6	0.7
4-METHYL-1-PENTENE	ND	ND	ND	ND	ND	ND	ND	ND	ND
3-METHYL-1-PENTENE	1.5	2.2	2.2	2.2	2.0	2.1	2.5	2.6	2.7
CYCLOPENTANE	1.7	2.0	1.9	1.8	1.8	1.8	1.7	1.7	1.7
2,3-DIMETHYLBUTANE	5.7	7.9	7.8	7.7	7.4	7.5	6.3	6.3	6.6
MTBE	ND	ND	ND	ND	ND	ND	ND	ND	ND
4-METHYL-CIS-2-PENTENE	ND	ND	ND	ND	ND	ND	ND	ND	ND
2-METHYLPENTANE	8.6	10.2	8.5	10.2	9.6	9.8	8.1	8.1	8.5
4-METHYL-TRANS-2-PENTENE	0.6	0.6	0.5	0.6	0.6	0.6	0.6	0.6	0.6
3-METHYLPENTANE	5.2	6.1	5.7	6.0	5.3	5.6	4.7	5.0	5.0
2-METHYL-1-PENTENE	0.8	0.5	0.7	0.7	0.5	0.6	0.5	0.6	0.4
1-HEXENE	0.6	0.4	0.7	0.7	0.5	0.6	0.5	0.6	0.4
HEXANE	7.5	8.9	8.9	8.8	8.4	8.6	7.2	7.3	7.5
UNIDENTIFIED C6	3.8	2.6	3.6	3.7	3.3	2.8	2.6	2.9	3.2
TRANS-3-HEXENE	ND	ND	ND	ND	ND	ND	ND	ND	ND

TABLE B-8 (CONT'D). COMPARISON OF COMPOSITE HYDROCARBON SPECIATION DATA WITHOUT AFTERTREATMENT

	mg/mile			mg/mile			mg/mile		
	RFABASE-1	RFABASE-2	RFABASE-4	E10ADD-1	E10ADD-2	E10ADD-3	RFAE15-1	RFAE15-2	RFAE15-3
COMPOUND	FTP	FTP	FTP	FTP	FTP	FTP	FTP	FTP	FTP
CIS-3-HEXENE	Trace	Trace	0.1	Trace	Trace	Trace	Trace	0.1	Trace
DI-ISOPROPYL ETHER	ND	ND	ND	ND	ND	ND	ND	ND	ND
TRANS-2-HEXENE	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.2
3-METHYL-TRANS-2-PENTENE	0.6	0.6	0.7	0.6	0.6	0.6	0.6	0.6	0.6
2-METHYL-2-PENTENE	0.7	0.8	0.9	0.7	0.7	0.7	0.8	0.8	0.8
3-METHYLCYCLOPENTENE	ND	ND	ND	ND	ND	ND	ND	ND	ND
CIS-2-HEXENE	0.1	0.3	0.2	0.3	0.2	0.1	0.2	Trace	0.1
ETBE	ND	ND	ND	ND	ND	ND	ND	ND	ND
3-METHYL-CIS-2-PENTENE	0.3	0.4	0.4	0.3	0.3	0.3	0.4	0.3	0.3
2,2-DIMETHYLPENTANE, NOTE A	2.2	2.7	2.7	2.6	2.6	2.6	2.2	2.2	2.3
METHYLCYCLOPENTANE, NOTE A	2.2	2.6	2.7	2.5	2.5	2.6	2.2	2.2	2.3
2,4-DIMETHYLPENTANE	3.2	7.0	7.2	6.9	6.8	6.9	6.1	5.9	6.0
2,2,3-TRIMETHYLBUTANE	3.6	0.6	0.8	0.6	0.6	0.7	0.8	0.8	0.7
3,4-DIMETHYL-1-PENTENE	0.3	0.4	0.6	0.5	0.3	0.4	0.4	0.5	0.5
1-METHYLCYCLOPENTENE	0.2	4.1	0.3	0.2	0.3	0.2	0.2	0.2	0.3
BENZENE	33.0	45.7	44.0	41.0	45.3	40.5	35.3	31.2	39.7
3-METHYL-1-HEXENE	ND	ND	ND	ND	ND	ND	ND	ND	ND
3,3-DIMETHYLPENTANE	0.3	0.3	0.3	0.3	0.3	0.3	1.7	1.9	1.8
CYCLOHEXANE	3.1	3.6	4.1	3.9	4.4	3.6	3.2	2.8	3.4
2-METHYLHEXANE	7.1	8.1	8.8	8.6	8.5	8.4	7.3	7.4	7.6
2,3-DIMETHYLPENTANE	0.4	0.6	0.6	0.6	0.6	0.6	0.6	0.6	0.6
1,1-DIMETHYLCYCLOPENTANE	ND	ND	ND	ND	ND	ND	ND	ND	ND
TERT-AMYL METHYL ETHER	ND	ND	ND	ND	ND	ND	ND	ND	ND
CYCLOHEXENE	0.8	0.9	0.9	0.9	1.0	0.9	0.9	0.9	0.9
3-METHYLHEXANE	2.1	2.6	2.7	2.7	2.7	2.7	2.3	2.3	2.4
CIS-1,3-DIMETHYLCYCLOPENTANE	0.4	0.4	0.5	0.5	0.5	0.5	0.5	0.4	0.4
3-ETHYLPENTANE	0.5	0.6	0.6	0.7	0.7	0.7	0.6	0.6	0.6
TRANS-1,2-DIMETHYLCYCLOPENTANE	ND	ND	ND	ND	ND	ND	ND	ND	ND
TRANS-1,3-DIMETHYLCYCLOPENTANE	0.5	0.7	0.7	0.7	0.7	0.7	0.6	0.6	0.6
1-HEPTENE	ND	ND	ND	ND	ND	ND	ND	ND	ND
2,2,4-TRIMETHYLPENTANE	80.4	96.8	97.9	96.4	94.7	95.5	83.3	82.5	84.4
2-METHYL-1-HEXENE	ND	Trace	ND	ND	Trace	Trace	ND	ND	Trace
TRANS-3-HEPTENE	ND	ND	ND	ND	ND	ND	ND	ND	ND
HEPTANE	3.3	3.7	3.8	4.0	7.1	5.3	3.5	3.1	3.3
CIS-3-HEPTENE	ND	ND	ND	ND	ND	ND	ND	ND	ND
UNIDENTIFIED C7	1.4	1.6	1.9	1.9	1.8	1.5	2.6	2.6	2.5
2-METHYL-2-HEXENE	ND	0.1	Trace	ND	Trace	ND	Trace	Trace	Trace
3-METHYL-TRANS-3-HEXENE	ND	ND	ND	ND	ND	ND	ND	ND	ND
TRANS-2-HEPTENE	ND	Trace	Trace	0.1	Trace	Trace	Trace	ND	ND
3-ETHYL-CIS-2-PENTENE	ND	ND	ND	ND	ND	ND	ND	ND	ND
2,4,4-TRIMETHYL-1-PENTENE	0.2	0.3	0.3	0.3	0.3	0.3	0.2	0.2	0.2
2,3-DIMETHYL-2-PENTENE	ND	ND	ND	ND	ND	ND	ND	ND	ND
CIS-2-HEPTENE	0.4	0.5	0.5	0.5	0.5	0.5	0.5	0.4	0.4
METHYLCYCLOHEXANE	5.1	6.2	6.4	6.2	6.1	6.0	5.2	5.1	5.2
CIS-1,2-DIMETHYLCYCLOPENTANE	ND	ND	ND	ND	ND	ND	ND	ND	ND
2,2-DIMETHYLHEXANE	0.4	0.4	0.4	0.4	0.4	0.4	0.3	0.3	0.3
1,1,3-TRIMETHYLCYCLOPENTANE	ND	ND	Trace	ND	ND	ND	Trace	ND	Trace
2,4,4-TRIMETHYL-2-PENTENE	0.4	0.4	0.4	0.3	0.2	0.2	0.2	Trace	0.3

TABLE B-8 (CONT'D). COMPARISON OF COMPOSITE HYDROCARBON SPECIATION DATA WITHOUT AFTERTREATMENT

	mg/mile			mg/mile			mg/mile		
	RFABASE-1	RFABASE-2	RFABASE-4	E10ADD-1	E10ADD-2	E10ADD-3	RFAE15-1	RFAE15-2	RFAE15-3
COMPOUND	FTP	FTP	FTP	FTP	FTP	FTP	FTP	FTP	FTP
2,2,3-TRIMETHYLPENTANE	9.8	11.3	11.4	11.5	11.2	11.6	8.9	8.9	8.8
2,5-DIMETHYLHEXANE	ND	ND	ND	ND	ND	ND	ND	ND	ND
ETHYLCYCLOPENTANE	ND	ND	ND	ND	ND	ND	ND	ND	ND
2,4-DIMETHYLHEXANE	9.3	11.8	12.2	11.6	11.3	11.4	11.2	10.9	11.4
1-TRANS-2-CIS-4-TRIMETHYLCYCLOPENTANE	0.2	0.4	0.4	0.4	0.4	0.4	0.3	0.3	0.3
3,3-DIMETHYLHEXANE	ND	ND	ND	ND	ND	ND	ND	ND	ND
1-TRANS-2-CIS-3-TRIMETHYLCYCLOPENTANE	0.1	Trace	0.1	ND	ND	ND	ND	ND	ND
2,3,4-TRIMETHYLPENTANE	26.7	32.1	33.0	32.2	31.5	31.9	28.2	27.9	28.4
2,3,3-TRIMETHYLPENTANE	16.3	6.9	21.2	20.4	22.6	19.4	17.6	15.8	18.8
TOLUENE	213.3	210.1	246.8	224.4	235.8	218.2	190.5	176.9	210.1
2,3-DIMETHYLHEXANE	11.6	13.7	15.8	ND	ND	13.4	ND	ND	ND
1,1,2-TRIMETHYLCYCLOPENTANE	ND	ND	ND	ND	ND	ND	ND	ND	ND
2-METHYLHEPTANE	0.9	1.1	1.1	1.1	1.1	1.2	1.0	0.9	1.2
3,4-DIMETHYLHEXANE, NOTE B	1.3	1.6	1.7	1.7	1.6	1.7	1.3	1.2	1.5
4-METHYLHEPTANE	ND	ND	ND	ND	ND	ND	ND	ND	ND
3-METHYLHEPTANE	0.7	0.8	0.9	0.9	0.9	1.0	0.7	0.6	0.9
1-CIS,2-TRANS,3-TRIMETHYLCYCLOPENTANE	0.6	0.7	0.8	0.7	0.8	0.8	0.4	0.4	0.6
CIS-1,3-DIMETHYLCYCLOHEXANE	ND	ND	ND	ND	ND	ND	ND	ND	ND
TRANS-1,4-DIMETHYLCYCLOHEXANE	0.3	0.3	0.3	0.3	0.3	0.3	Trace	ND	0.3
3-ETHYLHEXANE	ND	ND	ND	ND	ND	ND	ND	ND	ND
2,2,5-TRIMETHYLHEXANE	10.6	12.5	13.0	12.7	12.5	12.9	11.0	10.8	11.0
TRANS-1-METHYL-3-ETHYLCYCLOPENTANE	ND	ND	ND	ND	ND	ND	ND	ND	ND
CIS-1-METHYL-3-ETHYLCYCLOPENTANE	ND	ND	ND	ND	ND	ND	ND	ND	Trace
1,1-DIMETHYLCYCLOHEXANE	ND	ND	ND	ND	ND	ND	ND	ND	ND
TRANS-1-METHYL-2-ETHYLCYCLOPENTANE	ND	ND	ND	ND	ND	ND	ND	ND	ND
1-METHYL-1-ETHYL-CYCLOPENTANE	0.8	0.9	1.1	0.8	0.8	0.8	0.7	0.7	0.7
2,4,4-TRIMETHYLHEXANE	ND	ND	ND	ND	ND	ND	ND	ND	ND
2,2,4-TRIMETHYLHEXANE	ND	ND	ND	ND	ND	ND	ND	ND	ND
TRANS-1,2-DIMETHYLCYCLOHEXANE	0.3	0.3	0.3	0.3	0.3	0.3	0.2	0.2	0.2
1-OCTENE	ND	ND	ND	ND	ND	ND	ND	ND	ND
TRANS-4-OCTENE	ND	ND	ND	ND	ND	ND	ND	ND	ND
OCTANE	1.0	1.2	1.3	1.2	1.2	1.2	1.0	1.0	1.0
UNIDENTIFIED C8	0.1	0.1	0.3	ND	0.3	ND	Trace	0.1	0.1
TRANS-2-OCTENE	0.2	0.1	0.2	0.2	0.2	0.2	0.1	0.1	0.1
TRANS-1,3-DIMETHYLCYCLOHEXANE, NOTE C	0.3	0.3	0.3	0.3	0.3	0.3	0.3	0.3	0.3
CIS-2-OCTENE	Trace	Trace	0.2	ND	ND	ND	Trace	0.1	ND
ISOPROPYLCYCLOPENTANE	0.4	0.4	0.4	0.4	0.4	0.4	0.4	0.4	0.3
2,2-DIMETHYLHEPTANE	ND	ND	ND	ND	ND	ND	ND	ND	ND
2,3,5-TRIMETHYLHEXANE	1.6	2.0	2.0	2.0	1.9	2.0	1.8	1.7	1.7
CIS-1-METHYL-2-ETHYLCYCLOPENTANE	ND	ND	ND	ND	Trace	ND	ND	ND	ND
2,4-DIMETHYLHEPTANE	0.3	0.4	0.3	0.3	0.3	0.3	0.3	0.3	0.3
4,4-DIMETHYLHEPTANE	ND	ND	ND	ND	ND	ND	ND	ND	ND
CIS-1,2-DIMETHYLCYCLOHEXANE	0.6	0.6	0.4	0.7	0.3	0.6	0.5	0.5	0.4
ETHYLCYCLOHEXANE	Trace	0.1	Trace	0.1	0.1	0.1	0.3	0.3	0.1
2,6-DIMETHYLHEPTANE, NOTE D	ND	ND	ND	ND	ND	ND	ND	ND	ND
1,1,3-TRIMETHYLCYCLOHEXANE	ND	ND	ND	ND	ND	ND	ND	ND	ND
2,5-DIMETHYLHEPTANE, NOTE E	0.4	0.6	0.4	0.5	0.4	0.5	0.4	0.4	0.4
3,3-DIMETHYLHEPTANE	ND	ND	ND	ND	ND	ND	ND	ND	ND
3,5-DIMETHYLHEPTANE, NOTE E	0.4	0.6	0.4	0.5	0.4	0.5	0.4	0.4	0.4

TABLE B-8 (CONT'D). COMPARISON OF COMPOSITE HYDROCARBON SPECIATION DATA WITHOUT AFTERTREATMENT

	mg/mile			mg/mile			mg/mile		
	RFABASE-1	RFABASE-2	RFABASE-4	E10ADD-1	E10ADD-2	E10ADD-3	RFAE15-1	RFAE15-2	RFAE15-3
COMPOUND	FTP	FTP	FTP	FTP	FTP	FTP	FTP	FTP	FTP
ETHYLBENZENE	12.0	15.2	15.1	14.1	13.1	13.9	12.1	12.3	12.9
2,3,4-TRIMETHYLHEXANE	ND	ND	ND	ND	ND	ND	ND	ND	ND
2,3-DIMETHYLHEPTANE	ND	ND	ND	ND	ND	ND	ND	ND	ND
m-& p-XYLENE	13.0	16.8	17.6	16.5	15.8	16.7	13.6	14.0	14.5
4-METHYLOCTANE	ND	ND	ND	ND	ND	ND	ND	ND	ND
3,4-DIMETHYLHEPTANE	ND	ND	ND	ND	ND	ND	ND	ND	ND
4-ETHYLHEPTANE	ND	ND	ND	ND	ND	ND	ND	ND	ND
2-METHYLOCTANE	0.2	0.4	0.5	0.4	0.5	0.5	0.2	0.4	0.3
3-METHYLOCTANE	0.1	0.2	0.2	0.2	0.2	0.2	0.1	0.2	0.1
STYRENE	4.0	4.8	6.7	6.8	6.1	4.9	6.3	6.3	5.2
o-XYLENE	10.0	12.5	13.6	12.5	11.9	12.6	10.7	11.1	11.3
1-NONENE	0.8	0.9	1.0	0.9	1.0	0.9	0.8	0.9	0.9
TRANS-3-NONENE	ND	ND	ND	ND	ND	ND	ND	ND	ND
CIS-3-NONENE	ND	ND	ND	ND	ND	ND	ND	ND	ND
NONANE	0.8	1.1	1.2	1.1	1.1	1.1	1.0	1.0	1.0
TRANS-2-NONENE	ND	ND	ND	ND	ND	ND	ND	ND	ND
ISOPROPYLBENZENE (CUMENE)	2.1	2.6	2.8	0.8	2.5	2.6	0.4	0.6	0.7
2,2-DIMETHYLOCTANE	0.7	0.8	0.9	0.2	0.8	0.8	0.1	0.2	0.2
2,4-DIMETHYLOCTANE	0.1	ND	ND	ND	ND	ND	ND	ND	ND
n-PROPYLBENZENE	9.6	12.3	13.3	12.1	11.5	12.1	10.7	10.9	11.2
1-METHYL-3-ETHYLBENZENE	34.0	43.6	47.5	44.8	41.2	44.1	37.9	39.5	40.0
1-METHYL-4-ETHYLBENZENE	14.8	18.9	20.8	18.9	17.9	18.9	16.6	17.2	17.6
1,3,5-TRIMETHYLBENZENE	16.5	21.4	23.6	22.2	20.4	21.8	18.5	19.5	19.7
1-METHYL-2-ETHYLBENZENE	15.8	18.9	19.5	19.6	17.1	19.0	15.9	16.6	16.4
1,2,4-TRIMETHYLBENZENE	38.8	64.2	72.3	66.2	60.9	63.0	58.2	60.3	64.1
TERT-BUTYLBENZENE	ND	ND	ND	ND	ND	ND	ND	ND	ND
1-DECENE	ND	ND	ND	ND	ND	ND	ND	ND	ND
DECANE, NOTE F	0.1	0.6	1.0	0.9	0.5	0.6	2.0	0.9	0.5
ISOBUTYLBENZENE, NOTE F	0.1	0.6	0.9	0.9	0.5	0.6	1.9	0.8	0.5
1,3,-DIMETHYL-5-ETHYLBENZENE	ND	ND	ND	ND	ND	ND	ND	ND	ND
METHYLPROPYLBENZENE (sec butylbenzene)	0.1	0.1	1.0	1.3	0.9	1.7	0.5	5.2	4.9
1-METHYL-3-ISOPROPYLBENZENE	6.6	5.4	4.7	8.9	7.2	6.9	4.3	4.8	3.1
1,2,3-TRIMETHYLBENZENE	4.4	4.1	3.6	4.5	4.6	4.4	4.0	3.7	3.7
1-METHYL-4-ISOPROPYLBENZENE	ND	ND	ND	ND	ND	ND	ND	ND	ND
INDAN	ND	ND	ND	ND	ND	ND	ND	ND	ND
1-METHYL-2-ISOPROPYLBENZENE	2.3	2.8	2.8	0.2	ND	ND	ND	ND	ND
1,3-DIETHYLBENZENE	ND	1.0	0.8	3.8	2.6	3.1	3.2	3.5	2.7
1,4-DIETHYLBENZENE	2.2	3.3	3.8	5.1	3.0	2.7	4.5	4.8	4.3
1-METHYL-3-N-PROPYLBENZENE	ND	ND	ND	ND	ND	ND	ND	ND	ND
1-METHYL-4-N-PROPYLBENZENE, NOTE G	ND	ND	ND	ND	ND	ND	ND	ND	ND
1,2 DIETHYLBENZENE	0.3	0.1	1.5	ND	ND	ND	ND	ND	ND
1-METHYL-2-N-PROPYLBENZENE	3.5	4.0	3.2	ND	ND	ND	ND	ND	ND
1,4-DIMETHYL-2-ETHYLBENZENE	1.3	2.1	2.0	ND	ND	ND	ND	ND	ND
1,3-DIMETHYL-4-ETHYLBENZENE	ND	ND	ND	ND	ND	ND	ND	ND	ND
1,2-DIMETHYL-4-ETHYLBENZENE	0.6	2.9	1.8	1.4	1.6	0.9	3.1	1.4	1.8
1,3-DIMETHYL-2-ETHYLBENZENE	0.3	Trace	0.2	ND	ND	ND	ND	ND	ND
UNDECANE	0.3	Trace	0.2	ND	ND	ND	0.4	ND	ND
1,2-DIMETHYL-3-ETHYLBENZENE	ND	ND	ND	ND	ND	ND	ND	ND	ND
1,2,4,5-TETRAMETHYLBENZENE	ND	ND	ND	ND	ND	ND	0.1	ND	ND
2-METHYLBUTYLBENZENE (sec AMYLBENZENE)	ND	ND	ND	ND	ND	ND	ND	ND	ND

TABLE B-8 (CONT'D). COMPARISON OF COMPOSITE HYDROCARBON SPECIATION DATA WITHOUT AFTERTREATMENT

	mg/mile			mg/mile			mg/mile		
	RFABASE-1	RFABASE-2	RFABASE-4	E10ADD-1	E10ADD-2	E10ADD-3	RFAE15-1	RFAE15-2	RFAE15-3
COMPOUND	FTP	FTP	FTP	FTP	FTP	FTP	FTP	FTP	FTP
3,4 DIMETHYLCUMENE	ND	ND	ND	ND	ND	ND	ND	ND	ND
1,2,3,5-TETRAMETHYLBENZENE	ND	ND	ND	ND	ND	ND	ND	ND	ND
TERT-1-BUT-2-METHYLBENZENE	ND	ND	ND	ND	ND	ND	ND	ND	ND
1,2,3,4-TETRAMETHYLBENZENE	ND	ND	ND	ND	ND	ND	ND	ND	ND
N-PENT-BENZENE	ND	ND	ND	ND	ND	ND	ND	ND	ND
TERT-1-BUT-3,5-DIMETHYLBENZENE	ND	ND	ND	ND	ND	ND	0.1	ND	ND
TERT-1-BUTYL-4-ETHYLBENZENE	ND	ND	ND	ND	ND	ND	ND	ND	ND
NAPHTHALENE	ND	ND	ND	ND	ND	ND	ND	ND	ND
DODECANE	ND	ND	ND	ND	ND	ND	ND	ND	ND
1,3,5-TRIETHYLBENZENE	ND	ND	ND	ND	ND	ND	ND	ND	ND
1,2,4-TRIETHYLBENZENE	ND	ND	ND	ND	ND	ND	ND	ND	ND
HEXYLBENZENE	ND	ND	ND	ND	ND	ND	ND	ND	ND
UNIDENTIFIED C9-C12+	16.9	20.3	25.9	21.2	24.2	20.4	23.5	21.2	19.3
FORMALDEHYDE	52.3	50.0	53.7	45.3	41.1	46.0	56.4	56.3	56.4
ACETALDEHYDE	10.1	9.6	10.3	19.3	17.6	20.0	28.4	28.7	28.5
ACROLEIN	4.2	3.7	3.8	2.3	2.9	2.9	4.7	4.4	4.3
ACETONE	6.2	6.2	6.3	5.9	6.5	6.0	5.3	5.4	5.2
PROPIONALDEHYDE	4.1	4.7	4.3	3.8	3.1	2.6	3.1	4.0	3.7
CROTONALDEHYDE	2.9	3.1	3.0	0.9	0.6	0.9	2.2	2.5	2.6
METHACROLEIN	3.5	3.0	3.4	2.9	2.0	3.0	4.1	4.2	4.1
N-/ISOBUTYRALDEHYDE	0.9	1.0	1.0	0.9	0.8	0.9	1.0	1.0	0.9
METHYL ETHYL KETONE	0.5	0.5	0.7	0.7	0.6	0.8	0.6	0.7	0.6
BENZALDEHYDE	17.4	16.2	17.3	13.0	12.2	13.8	16.5	17.1	16.9
ISOVALERALDEHYDE	0.7	0.7	0.7	0.1	ND	ND	0.6	0.7	0.8
VALERALDEHYDE	0.2	0.3	0.2	0.3	ND	ND	0.3	0.3	0.3
O-TOLUALDEHYDE	1.4	2.9	1.6	0.6	1.7	1.9	1.3	1.4	1.5
M/P-TOLUALDEHYDE	5.8	3.5	5.3	7.5	4.2	4.6	5.1	5.5	5.6
METHYL ISOBUTYL KETONE	ND	ND	ND	ND	ND	ND	ND	0.9	0.8
HEXANALDEHYDE	0.2	1.0	0.2	Trace	Trace	Trace	0.4	0.2	0.2
DIMETHYLBENZALDEHYDE	4.4	4.9	5.4	4.5	3.5	4.0	5.1	3.5	4.7
SUMMED SPECIATED VALUES	1387.9	1506.0	1601.7	1535.3	1494.9	1507.5	1412.5	1394.4	1419.2

A - 2,2-Dimethylpentane and methylcyclopentane co-elute. GC peak area split equally between the two compounds.

B - 3-Methyl-3-ethyl-pentane co-elutes with reported compound. Not reported separately.

C - Cis-1,4-Dimethylcyclohexane co-elutes with reported compound. Not reported separately.

D - Propylcyclopentane co-elutes with reported compound. Not reported separately.

E - 2,5-Dimethylheptane and 3,5-dimethylheptane co-elute. GC peak area split equally between the two compounds.

F - Decane and isobutylbenzene co-elute. GC peak area split equally between the two compounds.

G - n-Butylbenzene co-elutes with reported compound. Not reported separately.

EVAPORATIVE EMISSIONS CHARACTERIZATION OF E0, E10, AND E15 IN SUPPORT OF THE FUEL AND FUEL ADDITIVE REGISTRATION OF E15

REVISED FINAL REPORT

SwRI® Project No. 03.15812

Prepared for:

Renewable Fuels Association and Growth Energy

Prepared by:

E. Robert Fanick
Manager

February 2011

SAN ANTONIO, TEXAS
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Approved by:

Jeff J. White, Director

EMISSIONS RESEARCH AND DEVELOPMENT DEPARTMENT
ENGINE, EMISSIONS, AND VEHICLE RESEARCH DIVISION

IDENTIFICATION OF TEST SUBSTANCE

Ethanol

MANUFACTURER

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FOREWORD

This project was performed for the Renewable Fuels Association and Growth Energy under SwRI Project 03-15812. Ms. Kristy Moore was the program director for the Renewable Fuels Association (RFA), and Ms. Erin Heupel was the program director on behalf of Growth Energy. The Principal Investigator and the Project Leader was Mr. E. Robert Fanick, Manager of the Emissions Chemistry Section in the Emissions Research and Development Department. SwRI technical personnel involved in testing included: Ms. Svitlana Kroll and Mr. Joe Tsai. Data reduction was performed by Ms. Amanda L. Korzekwa. Southwest Research Institute is located at:

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TABLE OF CONTENTS

Page

LIST OF FIGURES

LIST OF TABLES

TEST SUBSTANCE INFORMATION

The fuel additive of interest for this project was ethanol, chemical formula C_2H_5OH, that would be used in a fuel blend commonly referred to as E15, a blend of 15 percent ethanol and 85 percent unleaded gasoline. Ethanol is also commonly referred to as fuel ethanol or denatured fuel ethanol; all meaning ethanol suitable for use in spark-ignition engine systems. Additional fuels tested in this evaluation were an unleaded gasoline certification fuel with no ethanol added (baseline fuel or E0) and a gasoline certification fuel blended with 10 percent ethanol (E10).

EXECUTIVE SUMMARY

The head space composition of three fuels (E0 - unleaded gasoline, E10 - ethanol blended at a concentration of 10 percent in unleaded gasoline, and E15 - ethanol blended at a concentration of 15 percent in unleaded gasoline) was evaluated for the Renewable Fuels Association and Growth Energy to address a portion of the Environmental Protection Agency (EPA) requirements for registration of designated fuels and fuel additives (F/FA) as stipulated by sections 211(b) and 211(e) of the Clean Air Act (CAA). Under the Tier I requirements of this protocol, manufacturers of F/FAs are required to supply EPA with:

- The identity and concentration of emission products from the F/FA
- Any available information regarding the health and welfare effects of the whole and speciated emissions
- A characterization of the emission products which are generated by evaporation and by combustion of the F/FA.

This report covers the evaporative emission portion of the testing. An Evaporative Emission Generator (EEG) was prepared to determine the head space composition. The system was used to test three fuels prepare by Haltermann Products:

- Base fuel (E0), EPA Tier II EEE with additives – Haltermann Product Code HF0872 (SwRI Code EM-7578-F)
- E10, 90 percent unleaded gasoline and 10 percent ethanol – Haltermann Product Code HF0869 (SwRI Code Fuel EM-7579-F)
- E15, 85 percent unleaded gasoline and 15 percent ethanol – Haltermann Product Code HF0870 (SwRI Code Fuel EM-7580-F)

The base fuel was a certification gasoline that met the specifications of 40 CFR 86.113-04 with the addition of the required additives for a 211(b) base fuel. This same fuel was then used to blend the E10 and E15 test fuels. These same three fuels were also tested for exhaust emissions in a separate program for E15, and those results will be reported separately.

Speciation of the C_1 to C_{12} hydrocarbons, alcohols, and ethers was performed on the head space from three different fuels. When the base fuel was compared to each fuel, no additional compounds were found in the fuels with additive that were not found in the base fuel with the exception of ethanol. All detected compounds were generally present at the same order of magnitude with the base fuel as with the fuels plus ethanol. In addition, two additional compounds (an unidentified C_6 and 2,2,3-trimethylpentane) were found in the base fuel. These two compounds were not detected in either of the two fuels with ethanol additive. In general, the head space composition of fuels with additive was similar to the base fuel. The addition of ethanol to the fuel did not significantly change the head space composition. Detection limits for the procedure were on the order of 0.05 mg/test. While some C_1 to C_{12} hydrocarbons were below the detection limit, one cannot necessarily conclude that these compounds were present or not present; however, if present, the compounds were below the limits of detection.

1.0 INTRODUCTION

This work was performed for the Renewable Fuels Association (RFA) and Growth Energy to address a portion of the Environmental Protection Agency (EPA) requirements for registration of designated fuels and fuel additives (F/FA) as stipulated by sections 211(b) and 211(e) of the Clean Air Act (CAA). In general, standard mandatory requirements for F/FA registrations are contained in a three tiered structure. The first two tiers generally apply to most F/FA manufacturers, but there are special provisions for certain types of additives and for small businesses. Each manufacturer is required to submit basic registration data for each product being registered. Small businesses with less than $50 million of annual sales are excused from the first two tiers of requirements for F/FA which are considered baseline or non-baseline, and small businesses with less than $10 million annual sales are excused from Tier 2 requirements for "atypical" F/FA. Definitions of baseline, non-baseline, and "atypical" F/FA are discussed in detail below. Other special provisions include experimental F/FA, relabeled products, and products exclusively for off-road use.

Each F/FA is sorted into one of two broad "fuel families": conventional or alternative. The conventional fuel families are gasoline and diesel, and the alternative fuel families include methanol, ethanol, methane, and propane. Each fuel family is then subdivided into three "F/FA categories": baseline, non-baseline, and "atypical." The baseline category consists of fuels and associated fuel additives which resemble the respective baseline fuel for a particular fuel family in terms of elemental composition (no elements other than carbon, hydrogen, oxygen, nitrogen, and sulfur), and which conforms with certain quantitative limits for particular constituents. "Atypical" is defined as those fuels which contain metals; elements other than carbon, hydrogen, nitrogen, sulfur, and oxygen or do not meet the requirements under ASTM D4814 "Standard Specifications for Automotive Spark-Ignition Engine Fuel." The non-baseline category is an intermediate category between baseline and atypical. In the gasoline fuel family, the distinction between baseline and non-baseline is based primarily on the presence of significant concentrations of oxygen-containing compounds (greater than 1.0% oxygen by weight). Ethanol in E15 is considered a non-baseline fuel additive.

A method of determining the evaporative emissions from gasoline was developed by Southwest Research Institute® SwRI® and approved by EPA uses an Evaporative Emission Generator (EEG) to determine the head space composition of the fuels rather than the method that employs an evaporative emissions enclosure that requires an entire vehicle. The fuels were introduced to the EEG, and hydrocarbon speciation was performed on the head space composition to determine volatile-phase hydrocarbons, alcohols, and ethers. This report includes the results for the measurements that were conducted on the head space composition as part of the requirements for the registration of an additive or fuel as stipulated by sections 211(b) and 211(e) of the CAA.

2.0 HEAD SPACE COMPOSITION TESTING

2.1 Objective

The objective of this program was to provide RFA and Growth Energy with measurement of the head space composition for three different fuels. An EEG was prepared according to the Tier 1 requirements as identified in Title 40 CFR Part 79, Subpart F, Section 79.57. The emission measurements for this program included the speciation of volatile hydrocarbon compounds from C_1 to C_{12}, alcohols, and ethers. Alcohols and ethers containing six carbon atoms or less were also measured for each fuel.

2.2 Scope of Work

The scope of work for this effort involved three fuels provided by RFA and Growth Energy. One fuel (EM-7578-F) was the base fuel with no additive, the second fuel (EM-7579-F) contained 10 percent denatured fuel ethanol, and the third fuel (EM-7580-F) contained 15 percent denatured fuel ethanol. Each fuel was tested to determine the head space composition. The results of these tests and a description of the test methods are reported below.

2.3 Fuel Analyses

The three fuels used for evaporative emission testing were obtained in single batches from Haltermann Products. The baseline fuel was designated with SwRI Code EM-7578-F. Traditionally, test fuel for the fuel and fuel additive Tier I test program was required to meet the 40 CFR Part 79 specifications. However per EPA request for this test program, the test fuel was requested to meet 40 CFR Part 86.113-04 specifications. The 40 CFR Part 86 specifications were required in this project by EPA because the gasoline fuel specifications changed in 2004. As a result, the fuel specifications that were established in 40 CFR Part 79 were no longer applicable for a base fuel in this project. Additionally, this base fuel also included additives for deposit control, corrosion inhibitor, demulsifier, and anti-oxidant as required in 40 CFR Part 79 Subpart F 79.55. Approval of the base fuel specifications were obtained from Mr. Jim Caldwell on July 20, 2010.[1]

The base fuel for this program had 31 ppm sulfur as opposed to the 339 ± 25 ppm sulfur as required in 40 CFR Part 79 Subpart F 79.55. In addition, the base fuel met all of the requirements under 40 CFR Part 79 except for RVP, API gravity, octane, aromatics, olefins, saturates, and 90 percent distillation. When compared to 40 CFR Part 86 specifications for a reference fuel, all requirements were met for the base fuel. The other two fuels with ethanol (EM-7579-F and EM-7580-F) met most of the requirements except for some distillation and RVP properties. Table 1 presents the test fuel properties and the 40 CFR Part 86 and Part 79 fuel specifications for comparison.

TABLE 1. 211(b) GASOLINE FUEL SPECIFICATION AND ANALYSIS

Component	40 CFR 79.55 Specifications	40 CFR 86.113-04 Specifications	EM-7578-F	EM-7579-F	EM-7580-F
Fuel Properties					
Sulfur, ppm	339±25	15-80	31	25	25
RVP, psi	8.7±0.3	8.7-9.2	9.2	9.6	9.5
API Gravity	57.4±0.3	NS[a]	59.0	57.4	56.9
Octane, (R+M)/2	87.3±0.5	NS	92.8	95.5	95.7
Research Octane, min.	NS	93	97.0	100.8	>101
Sensitivity, min.	NS	7.5	8.5	10.8	>10.6
Lead, g/gal., max.	NS	0.050	<0.001	<0.001	<0.001
Phosphorus, g/gal., max.	NS	0.005	<0.0004	<0.0001	<0.0001
Aromatics, vol %	32.0±2.7	35 max.	28	24.3	23.2
Olefins, vol %	9.2±2.5	10 max.	2	0.6	1.1
Saturates, vol %	58.8±2.0	Remainder	70	65.3	60.9
Distillation Parameters					
IBP, °F (ºC)	NS	75-95 (24-35)	85 (29.4)	95 (35.0)	96 (35.7)
10%, °F (ºC)	128±5	120-135 (49-57)	125 (51.7)	124 (51.0)	127 (52.9)
50%, °F (ºC)	218±5	200-230 (93-110)	223 (106.1)	209 (98.3)	165 (73.8)
90%, °F (ºC)	330±5	300-325 (149-163)	319 (159.4)	316 (157.9)	317 (158.1)
EP, °F (ºC), max.	NS	415 (213)	393 (200.6)	392 (199.7)	386 (196.5)
Required and Permissible Additive Types					
Deposit Control, ptb[b]	Required	NR[c]	44.04	UR[d]	UR
Corrosion Inhibitor, ptb	Required	NR	4.5	UR	UR
Demulsifier, ptb	Required	NR	1.0	UR	UR
Antioxidant, ptb	Required	NR	2.0	UR	UR
Metal Deactivator, ptb	Required	NR	2.0	UR	UR
Anti-Static	Permissible	NR	NI[e]	UR	UR

[a]NS – No standard for this property
[b]ptb – pounds per thousand barrels
[c]NR – No requirement for additives with EPA reference fuel, but included in base fuel as required by Mr. Jim Caldwell
[d]UR – Unreported
[e]NI– Not included in the fuel

3.0 DESCRIPTION OF ANALYTICAL METHODS

3.1 Evaporative Emission Generator (EEG)

An EEG is a fuel tank or vessel which is heated to cause the volatile portion of the fuel or fuel additive to evaporate at the desired rate. The SwRI EEG vessel was designed according to the requirements of the Code of Federal Regulation (CFR) Title 40 – Protection of Environment, Part 79 – Registration of Fuels and Fuel Additives, Subpart F – Testing Requirements for Registration, Section 79.57 – Emission Generation.[2] For these experiments, the EEG was a stainless steel cylinder with a flange on the top for the introduction of the fuel sample. A bleed valve, closed-tip thermocouple which extends to the liquid volume, pressure gauge, and septum-type sampling port were mounted on the top flange, and this top flange is bolted onto the main portion of the vessel with six bolts. A Teflon® ring was used to provide a seal between the bottom and top of the vessel. The assembled vessel was then wrapped with a custom-made thermal blanket which included two parts: one for covering a cylindrical vessel bottom and the other for covering the top. Both parts of the thermal blanket were connected to a temperature controller to maintain the desired temperature. Figure 1 shows a schematic of the EEG vessel, and Table 2 documents the design of the SwRI EEG.

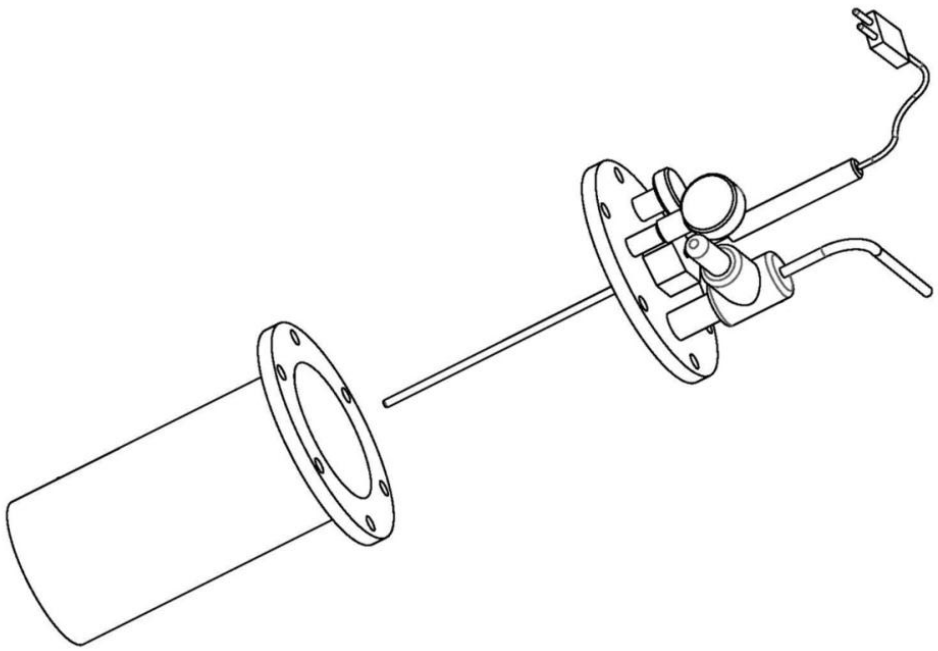

FIGURE 1. EVAPORATIVE EMISSION GENERATOR (EEG) VESSEL

To start the test, the vessel was filled to 40 ± 5 percent of the internal volume with the fuel or additive/fuel mixture being tested, and the remainder of the volume was ambient air trapped in the vessel after sealing the top flange. Internal volume of the vessel was 375 ml. For each test, 150 ml of fuel or additive/fuel mixture was added using a Class A graduated cylinder, and the vessel was sealed. The temperature of the fuel in the vessel was then heated to

TABLE 2. COMPARISON BETWEEN THE CFR AND THE SWRI EEG DESIGN AND METHOD

40 CFR Reference and Description	EEG Design and Method
§79.57(f)(2) – EEG description	Vessel rather than fuel tank heated at desired rate; EEG provides flexibility in testing multiple fuels.
§79.57(f)(2)(i) – Compatible materials	Stainless-steel sample vessel compatible with fuels tested.
§79.57(f)(2)(ii) – 40% Fuel fill	Vessel filled to 40 ± 5 percent of its interior volume.
§79.57(f)(2)(iii) – Vapor equilibrium	Vessel slightly agitated during heating to insure that the vapor space is in equilibrium and does not vary by more than 10 percent from the equilibrium concentration.
§79.57(f)(2)(iv) – Size of evaporation chamber	350 ml interior volume sufficient to reduce changes to vapor space during sample extraction.
§79.57(f)(2)(v) – Constant temperature	Place entire vessel in 130 ± 5ºF thermal blanket, and maintain until sample removal.
§79.57(f)(2)(vi) – Constant pressure	Monitor pressure in the vapor space to stay within 10 percent of ambient atmospheric pressure.
§79.57(f)(2)(vii) – Change in concentration of composition	Vessel and controls compatible with fuels tested.
§79.57(f)(2)(viii) – Verification testing	Verification performed analogous to combustion emissions.
§79.57(f)(4) – Sample collection	Sample of head space collected, introduced into Tedlar bag, and diluted prior to analysis.
§79.57(f)(5) – Request for approval of EEG	Approval received from Mr. Jim Caldwell, EPA, Transportation and Regional Programs Division on 8/11/2009[3].

130 ± 5ºF, this temperature was maintained for two hours, and the vessel was slightly agitated during the equilibration period. Samples were then taken after two hours and at one hour intervals for a total of three samples. During heating and the two hour sampling period, the pressure inside the vessel was vented to maintain the pressure within 10 percent of the ambient atmospheric pressure. The concentration of the evaporated fuel additive/fuel mixture in the vapor space of the EEG was not allowed to vary by more than 10 percent from the equilibrium concentration during the initial sample. Tests were repeated if this requirement was not achieved.

For the characterization of evaporative emissions, two separate samples were removed from the vapor space of the EEG through the sampling port. The first sample was a 100 µl sample withdrawn with a syringe, injected into a Tedlar® bag filled with 0.5 ft³ of nitrogen, and

gently mixed to obtain a homogeneous mixture. The sample was immediately analyzed for hydrocarbon speciation as specified in §79.52(b) using procedures similar to the CRC Auto/Oil Phase II methods.[4] A second 500 µl sample of the vapors was withdrawn with a syringe from the vapor space and introduced into a 50 ml volumetric flask filled with the distilled water. Contents of the flask were vigorously shaken, and a sample was analyzed for alcohols.

3.2 Speciation of Volatile Hydrocarbon Compounds

Volatile hydrocarbon compounds were determined by hydrocarbon speciation. Analytical procedures for conducting the hydrocarbon speciation (C_1-C_{12} hydrocarbons) were similar to the CRC Auto/Oil Phase II methods. With these methods, samples are analyzed for the presence of more than 200 different species. Four gas chromatography (GC) procedures were used to identify and quantify specific compounds. One GC is used for the measurement of C_2-C_4 species, a second was used for C_5-C_{12} species including three ethers (methyl tertiary butyl ether – MTBE, ethyl tertiary butyl ether – ETBE, and di-isopropyl ether – DIPE), and a third GC was used to measure 1-methylcyclopentene, cyclohexane, benzene, toluene, 2,3-dimethylhexane and 2,3,3-trimethylpentane, which co-elute and cannot be accurately quantified by other methods. A fourth GC was used to determine the C_1-C_6 alcohols and ethers. A brief description of these procedures is given in the following sections.

3.2.1 C2-C4 Species

SwRI TIP 07C-013 describes the analytical procedure for determining the C_2-C_4 hydrocarbons.[5] The C_2-C_4 hydrocarbons were determined with the aid of a DB-WAX pre-column and a 10-port switching valve. This procedure allowed the separation and determination of concentrations of C_2-C_4 individual hydrocarbon species, including: ethane; ethylene; acetylene; propane; propylene; trans-2-butene; butane; 1-butene; 2-methylpropene (isobutylene); 2,2-dimethypropane (neopentane); propyne; 1,3-butadiene; 2-methylpropane; 1-butyne; and cis-2-butene. Bag samples were analyzed with a GC system which utilized an Agilent Model 7890 GC with an FID, two pneumatically operated and electrically controlled valves, and two analytical columns. The first column separated the C_2-C_4 hydrocarbons from the higher molecular weight hydrocarbons and the polar compounds. These higher molecular weight hydrocarbons (and water and alcohols) were retained on the pre-column while the C_2-C_4 hydrocarbons were passed through to the analytical column (a 50 m Alumina PLOT/KCl with 10 µm film thickness and 0.53 mm i.d.). At the same time, the C_2-C_4 hydrocarbons were separated on the analytical column, the pre-column was back-flushed with helium to prepare for the next analysis. The carrier gas for this analysis was helium. The GC was calibrated daily using a CRC Auto/Oil 23-component calibration mixture. Analysis for the C_2-C_4 hydrocarbons were typically begun within 30 minutes after sample collection was completed.

3.2.2 C5-C12 Species

SwRI TIP 07C-013 describes the analytical procedures for the C_5-C_{12} hydrocarbons. This procedure provides separation and exhaust concentrations for more than 100 C_5-C_{12} individual hydrocarbon compounds. Bag samples were analyzed using a gas chromatograph equipped with an FID. The GC system utilized an Agilent Model 7890 GC with an FID, a pneumatically operated and electrically controlled valve, and a DB-1 fused silica open tubular (FSOT) column with a 1.0 µm film thickness and a 0.32 mm i.d. The carrier gas was helium.

Gaseous samples were pumped from the bag through a sample loop and then introduced into a liquid nitrogen cooled column. The column oven was then programmed to a maximum temperature of 200°C. The analog signal from the FID was sent to a networked computer system via a buffered analog to digital converter. The GC was calibrated daily using a CRC Auto/Oil 23-component calibration mixture.

3.2.3 Benzene and Toluene

The analytical procedure for benzene and toluene is also described in SwRI TIP 07C-013. This procedure used a separate system configured similarly to the C_5-C_{12} GC method (with a 30 m DB-5 analytical column in place of the DB-1 FSOT column) to resolve individual concentrations of benzene and toluene according to the CRC Auto/Oil Phase II Protocols. Separation of benzene and toluene from co-eluting peaks was carried out by fine-tuning the column head pressure to give benzene a retention time of 22 to 23 minutes. The GC was calibrated daily using a CRC 7-component calibration mixture.

3.2.4 Alcohols and Ethers

For the alcohol and ethers analysis, the procedure was similar to the CARB SOP No. MLD 101 for the determination of alcohols in automotive sources.[6] A 1.5 µl portion of the aqueous solution was injected into a gas chromatograph equipped with a FID and an autosampler. The analytical column was a 30 m × 0.53 mm i.d. capillary column of 1 µm DB-Wax film thickness. The carrier gas was helium and was set to give optimum separation (18 ml/min.). External standards containing methanol, ethanol, isopropyl alcohol, n-propanol, n-butanol, and ethanol in deionized water were used to quantify the results. Sample chromatograms were also searched for the presence of a number of other alcohols using predetermined retention times. The search list included: tert-butanol (CAS# 75-65-0), 2-methyl-2-butanol (CAS# 75-85-4), 2-butanol (CAS# 78-92-2), 3-pentanol (CAS# 584-02-1), 3-methyl-3-pentanol (CAS# 77-74-7), 3,3-dimethyl-2-butanol (CAS# 464-07-3), 2-pentanol (CAS# 6032-29-7), 4-methyl-2-pentanol (CAS# 108-11-2), 2-methyl-1-butanol (CAS# 137-32-6), 3-methyl-1-butanol (CAS# 123-51-3), 1-pentanol (CAS# 71-41-0), 2-methyl-1-pentanol (CAS# 105-30-6), and 2-ethyl-1-butanol (CAS# 97-95-0). Table 3 lists a number of alcohols that range in solubility from miscible to slightly soluble in water.

TABLE 3. SELECTED C1 TO C6 ALCOHOLS THAT HAVE SOME SOLUBILITY IN WATER

COMPOUND	EMPIRICAL FORMULA	COMPOUND	EMPIRICAL FORMULA
Water Soluble Alcohols			
Methanol	CH_4O	3-Methyl-2-butanol	$C_5H_{12}O$
Ethanol	C_2H_6O	Neopentanol (2,2-dimethyl-1-propanol)	$C_5H_{12}O$
2-Propyn-1-ol	C_3H_4O	1-Pentanol	$C_5H_{12}O$
Allyl alcohol (2-propen-1-ol)	C_3H_6O	2-Pentanol	$C_5H_{12}O$
Isopropanol	C_3H_8O	3-Pentanol	$C_5H_{12}O$
n-Propanol	C_3H_8O	tert-Pentanol (2-methyl-2-butanol)	$C_5H_{12}O$
Crotyl alcohol (2-buten-1-ol)	C_4H_8O	Phenol	C_6H_6O
n-Butanol	$C_4H_{10}O$	3-Methyl-1-pentyn-3-ol	$C_6H_{10}O$
Ethanol (2-methyl-1-propanol)	$C_4H_{10}O$	Cyclohexanol	$C_6H_{12}O$
sec-Butanol (2-butanol)	$C_4H_{10}O$	4-Hydroxy-4-methyl-2-pentanone	$C_6H_{12}O_2$
tert-Butanol (2-methyl-2-propanol)	$C_4H_{10}O$	2,2-Dimethyl-1,3-dioxolane-4-methanol	$C_6H_{12}O_3$
Furfuryl alcohol	$C_5H_6O_2$	2,5-Tetrahydrofurandimethanol	$C_6H_{12}O_3$
2-Methyl-3-butyn-2-ol	C_5H_8O	1-Hexanol	$C_6H_{14}O$
Cyclopentanol	$C_5H_{10}O$	2-Methyl-1-pentanol	$C_6H_{14}O$
Tetrahydrofurfuryl alcohol	$C_5H_{10}O_2$	3-Methyl-3-pentanol	$C_6H_{14}O$
Isopentanol (3-methyl-1-butanol)	$C_5H_{12}O$	4-Methyl-2-pentanol	$C_6H_{14}O$
2-Methyl-1-butanol	$C_5H_{12}O$	3,3-Dimethyl-2-butanol	$C_6H_{14}O$
3-Methyl-1-butanol	$C_5H_{12}O$	2-Ethyl-1-butanol	$C_6H_{14}O$
Water Soluble Ethers			
Methyl ether	C_2H_6O	Ethyl ether	$C_4H_{10}O$
Methyl ethyl ether	C_3H_8O	Methyl propyl ether	$C_4H_{10}O$
Vinyl ether	C_4H_6O	Isopropyl ether	$C_6H_{14}O$
Cyclopropyl methyl ether	C_4H_8O	Propyl ether	$C_6H_{14}O$

4.0 QUALITY CONTROL AND QUALITY ASSURANCE

In order to demonstrate SwRI's goal to provide quality emissions data in our project efforts, the Engine, Emissions, and Vehicle Research Division (EEVRD) maintains certification to ISO 9001:2000 and accreditation to ISO/IEC 17025:2005 standards. Standard operating procedures and routine instrument calibration and calibration records are included in these standards. Based on the successful completion of third party audits, the EEVRD is able to maintain registration under ISO 9001:2000, "Quality Management System," and accreditation by ISO/IEC 17025:2005, General Requirements for the Competence of Testing and Calibration Laboratories." The SwRI Office of Automotive Engineering (OAE) Quality Policy Statement is as follows:

SwRI OAE Quality Policy Statement

> *"The Office of Automotive Engineering provides unequaled capabilities for the research, development, evaluation, and qualification of transportation systems, vehicles, engines, fuels, lubricants, and emissions-related products. Quality excellence is the foundation for the management of our business and the keystone to customer satisfaction. It is our objective to ensure that our final products are internationally recognized with unquestioned quality and are delivered to our clients in a professional, cost effective, and timely manner.*
>
> *We are committed to comply with ISO 17025, ISO 9001:2000, and all customer-required standards of excellence. Continual improvement of this policy occurs through regular review of the quality system's suitability to meet customer, employee, and supplier needs."*

Throughout this project, SwRI implemented our QA/QC plan in a manner consistent with the program objectives, including spot-checking of records, accuracy/precision charts, notebooks, calibration tags, and other quality control elements including chain of custody of samples. Listed below are a few of the key process that ensure the quality standards are implemented.

Senior Scientist/Technician Review - A system for formal data review is in place in the SwRI Emissions Research and Development Department (ER&DD). All technicians review their work prior to submitting it to the data computations laboratory for calculation of final concentrations. The Project Leader performs the final review before test results are accepted.

Interlaboratory Comparisons/Round Robins - SwRI has participated in numerous Round Robin exercises to correlate the results of our laboratory with other accepted facilities. Those Round Robin studies which are directly related to this project include: CRC Round Robin Analysis of Alcohol and Carbonyl Synthetic Exhaust Samples; and CRC Round Robin Hydrocarbon Speciation Analysis of Synthetic Exhaust Gas.

Project Records - Documents directly associated with a technical project, such as: correspondence, proposals, contracts, work orders, interim and final reports, and follow-up contacts are maintained. These records are handled in accordance with Standard Operating Procedure (SOP), document SOP-4.16 "Quality Records"[7].

Calibration Records - Data sheets, chart recordings, computer printouts, logbooks, calibration and maintenance logs, and spreadsheets associated with the calibration of measurement equipment are maintained. Calibration results from external suppliers are also included.

Chemistry Calibration and Analysis Records - Data sheets, logbooks, and spreadsheets associated with calibration and analyses performed in the chemistry areas are retained.

Data Reduction and Test Result Records - Computed results, tables, and spreadsheets generated using information obtained from emissions testing and chemical analysis are maintained. Records developed in the areas specified in SOP 4.16 are retained for a period of ten years.

Training and Competency Evaluation: Personnel are trained to applicable SOP, TIP, and Design and Analysis Procedures and Safety Requirements. Staff members that perform an individual test are certified before performing these tests without supervision. Management encourages personnel to avail themselves to attend appropriate seminars, conferences, and continuing/higher education opportunities to continually enhance their skill set.

5.0 TEST RESULTS

Speciation results for volatile hydrocarbon compounds with carbon numbers from C_1 to C_{12} plus alcohols and ethers are included in this section. Speciation was performed on samples of each fuel. More than 200 compounds were checked for their presence in the head space of the EEG. The data is reported in two ways: 1) as mass of hydrocarbons detected in the head space and 2) normalized by mass percentage of the total hydrocarbons in the head space. Data for the individual compounds are included in Tables 4 and 5. Table 4 shows the mass in milligrams of the individual hydrocarbons in the head space, and Table 5 presents the normalized results in terms of mass percentage of the total hydrocarbons.

Fuel EM-7578-F was the base fuel without additive, Fuel EM-7579-F contained 10 percent ethanol, and Fuel EM-7580-F contained 15 percent ethanol. Ethanol was the only alcohol or ether detected in any of the fuels. No ethanol was detected in the base fuel as expected; and two compounds, an unidentified C_6 and 2,2,3-trimethylpentane, were detected in the base fuel. Ethanol was detected in both with additive, and the two compounds (an unidentified C_6 and 2,2,3-trimethylpentane) were not found in either of other two fuels with additive. When the three fuels were compared, no trans-2-butene was detected in EM-7579-F; and four compounds, 2,4-dimethylhexane, 2,3,4-trimethylpentane, decane, and isobutylbenzene were not found in EM-7580-F. All other compounds, when detected, were generally present at concentrations in the same order of magnitude as the base fuel.

When normalized by the percentage of total hydrocarbons in the head space, similar results were observed. The compounds with the highest concentration in the head space were:

- Propane
- Butane
- 2-methylpropane
- 2-methylbutane
- Pentane
- Ethanol for the fuel plus additive
- 3-methylpentane

All of these detected compounds were lower in molecular weight (less than six carbons) and were the more volatile components in each fuel.

TABLE 4. HEAD SPACE HYDROCARBON SPECIATION OF THREE FUELS

	EM-7578-F			EM-7579-F			EM-7580-F		
	TEST 1	TEST 3	Average	TEST 1	TEST 2	Average	TEST 2	TEST 4	Average
COMPOUND	mg	mg	mg	mg	mg	mg	mg	mg	mg
METHANE	ND	ND	ND	ND	ND	ND	ND	ND	ND
ETHANE	2.0	2.6	2.3	1.6	1.4	1.5	1.9	1.4	1.7
ETHYLENE	ND	ND	ND	ND	ND	ND	ND	ND	ND
PROPANE	22.6	29.4	26.0	32.6	27.9	30.3	28.5	24.7	26.6
PROPYLENE	ND	ND	ND	ND	ND	ND	ND	ND	ND
ACETYLENE	ND	ND	ND	ND	ND	ND	ND	ND	ND
PROPADIENE	ND	ND	ND	ND	ND	ND	ND	ND	ND
BUTANE	108.6	128.7	118.7	81.7	71.3	76.5	66.2	73.4	69.8
TRANS-2-BUTENE	ND	0.3	0.1	ND	ND	ND	0.3	ND	0.2
1-BUTENE	ND	ND	ND	ND	ND	ND	ND	ND	ND
2-METHYLPROPENE (ISOBUTYLENE)	0.3	ND	0.1	0.5	0.4	0.4	0.3	0.3	0.3
2,2-DIMETHYLPROPANE (NEOPENTANE)	3.0	2.7	2.9	4.7	4.2	4.5	3.8	4.2	4.0
PROPYNE	3.5	2.5	3.0	5.2	4.9	5.0	4.1	4.0	4.0
1,3-BUTADIENE	ND	ND	ND	ND	ND	ND	ND	ND	ND
2-METHYLPROPANE (ISOBUTANE)	132.8	149.5	141.2	213.7	185.4	199.5	175.8	184.1	179.9
1-BUTYNE	ND	ND	ND	ND	ND	ND	ND	ND	ND
METHANOL	ND	ND	ND	ND	ND	ND	ND	ND	ND
CIS-2-BUTENE	ND	ND	ND	ND	ND	ND	ND	ND	ND
3-METHYL-1-BUTENE	ND	ND	ND	ND	ND	ND	ND	ND	ND
ETHANOL	ND	ND	ND	95.4	86.7	91.1	142.8	130.6	136.7
2-METHYLBUTANE (ISOPENTANE)	416.3	365.3	390.8	671.0	606.4	638.7	541.2	581.4	561.3
2-BUTYNE	ND	ND	ND	ND	ND	ND	ND	ND	ND
1-PENTENE	ND	ND	ND	ND	ND	ND	ND	ND	ND
2-METHYL-1-BUTENE	ND	ND	ND	ND	ND	ND	ND	ND	ND
PENTANE	51.0	50.0	50.5	80.5	75.1	77.8	69.6	74.4	72.0
UNIDENTIFIED C5 OLEFINS	ND	ND	ND	ND	ND	ND	ND	ND	ND
2-METHYL-1,3-BUTADIENE	ND	ND	ND	ND	ND	ND	ND	ND	ND
TRANS-2-PENTENE	ND	ND	ND	ND	ND	ND	ND	ND	ND
3,3-DIMETHYL-1-BUTENE	ND	ND	ND	ND	ND	ND	ND	ND	ND
CIS-2-PENTENE	ND	ND	ND	ND	ND	ND	ND	ND	ND
2-METHYL-2-BUTENE	ND	ND	ND	ND	ND	ND	ND	ND	ND
ISOBUTANOL	ND	ND	ND	ND	ND	ND	ND	ND	ND
CYCLOPENTADIENE	ND	ND	ND	ND	ND	ND	ND	ND	ND
2,2-DIMETHYLBUTANE	2.1	2.0	2.0	3.1	3.0	3.1	2.7	2.8	2.7
CYCLOPENTENE	ND	ND	ND	ND	ND	ND	ND	ND	ND
4-METHYL-1-PENTENE	ND	ND	ND	ND	ND	ND	ND	ND	ND
3-METHYL-1-PENTENE	ND	ND	ND	ND	ND	ND	ND	ND	ND
CYCLOPENTANE	1.8	1.9	1.9	3.0	2.8	2.9	2.5	2.5	2.5
2,3-DIMETHYLBUTANE	6.3	6.7	6.5	10.7	10.5	10.6	8.8	8.2	8.5
MTBE	ND	ND	ND	ND	ND	ND	ND	ND	ND
4-METHYL-CIS-2-PENTENE	ND	ND	ND	ND	ND	ND	ND	ND	ND
2-METHYLPENTANE	6.8	8.0	7.4	12.9	12.6	12.7	10.4	9.7	10.1
4-METHYL-TRANS-2-PENTENE	ND	ND	ND	ND	ND	ND	ND	ND	ND
3-METHYLPENTANE	3.1	3.9	3.5	6.2	6.0	6.1	4.9	4.5	4.7
2-METHYL-1-PENTENE	ND	ND	ND	ND	ND	ND	ND	ND	ND
1-HEXENE	ND	ND	ND	ND	ND	ND	ND	ND	ND
HEXANE	2.9	4.9	3.9	7.6	7.2	7.4	5.6	5.2	5.4
UNIDENTIFIED C6	3.2	ND	1.6	ND	ND	ND	ND	ND	ND
TRANS-3-HEXENE	ND	ND	ND	ND	ND	ND	ND	ND	ND
CIS-3-HEXENE	ND	ND	ND	ND	ND	ND	ND	ND	ND

TABLE 4. HEAD SPACE HYDROCARBON SPECIATION OF THREE FUELS (CONT'D)

	EM-7578-F			EM-7579-F			EM-7580-F		
	TEST 1	TEST 3	Average	TEST 1	TEST 2	Average	TEST 2	TEST 4	Average
COMPOUND	mg	mg	mg	mg	mg	mg	mg	mg	mg
DI-ISOPROPYL ETHER	ND	ND	ND	ND	ND	ND	ND	ND	ND
TRANS-2-HEXENE	ND	ND	ND	ND	ND	ND	ND	ND	ND
3-METHYL-TRANS-2-PENTENE	ND	ND	ND	ND	ND	ND	ND	ND	ND
2-METHYL-2-PENTENE	ND	ND	ND	ND	ND	ND	ND	ND	ND
3-METHYLCYCLOPENTENE	ND	ND	ND	ND	ND	ND	ND	ND	ND
CIS-2-HEXENE	ND	ND	ND	ND	ND	ND	ND	ND	ND
ETBE	ND	ND	ND	ND	ND	ND	ND	ND	ND
3-METHYL-CIS-2-PENTENE	ND	ND	ND	ND	ND	ND	ND	ND	ND
2,2-DIMETHYLPENTANE, NOTE A	0.9	1.1	1.0	1.3	1.3	1.3	1.0	1.0	1.0
METHYLCYCLOPENTANE, NOTE A	1.1	1.2	1.1	1.5	1.5	1.5	1.2	1.1	1.1
2,4-DIMETHYLPENTANE	1.9	1.9	1.9	2.7	1.6	2.1	1.0	1.7	1.4
2,2,3-TRIMETHYLBUTANE	ND	ND	ND	ND	ND	ND	ND	ND	ND
3,4-DIMETHYL-1-PENTENE	ND	ND	ND	ND	ND	ND	ND	ND	ND
1-METHYLCYCLOPENTENE	ND	ND	ND	ND	ND	ND	ND	ND	ND
BENZENE	ND	ND	ND	ND	ND	ND	ND	ND	ND
3-METHYL-1-HEXENE	ND	ND	ND	ND	ND	ND	ND	ND	ND
3,3-DIMETHYLPENTANE	ND	ND	ND	ND	ND	ND	ND	ND	ND
CYCLOHEXANE	1.7	1.3	1.5	2.3	2.0	2.1	1.3	ND	0.6
2-METHYLHEXANE	1.6	1.6	1.6	1.9	ND	0.9	ND	ND	ND
2,3-DIMETHYLPENTANE	ND	ND	ND	ND	ND	ND	ND	ND	ND
1,1-DIMETHYLCYCLOPENTANE	ND	ND	ND	ND	ND	ND	ND	ND	ND
TERT-AMYL METHYL ETHER	ND	ND	ND	ND	ND	ND	ND	ND	ND
CYCLOHEXENE	ND	ND	ND	ND	ND	ND	ND	ND	ND
3-METHYLHEXANE	0.4	0.4	0.4	0.5	1.1	0.8	0.6	0.3	0.4
CIS-1,3-DIMETHYLCYCLOPENTANE	ND	ND	ND	ND	ND	ND	ND	ND	ND
3-ETHYLPENTANE	ND	ND	ND	ND	ND	ND	ND	ND	ND
TRANS-1,2-DIMETHYLCYCLOPENTANE	ND	ND	ND	ND	ND	ND	ND	ND	ND
TRANS-1,3-DIMETHYLCYCLOPENTANE	ND	ND	ND	ND	ND	ND	ND	ND	ND
1-HEPTENE	ND	ND	ND	ND	ND	ND	ND	ND	ND
2,2,4-TRIMETHYLPENTANE	6.7	9.4	8.1	7.2	7.9	7.6	3.9	6.1	5.0
2-METHYL-1-HEXENE	ND	ND	ND	ND	ND	ND	ND	ND	ND
TRANS-3-HEPTENE	ND	ND	ND	ND	ND	ND	ND	ND	ND
HEPTANE	ND	ND	ND	ND	ND	ND	ND	ND	ND
CIS-3-HEPTENE	ND	ND	ND	ND	ND	ND	ND	ND	ND
UNIDENTIFIED C7	0.3	0.4	0.4	0.4	0.7	0.5	ND	2.1	1.0
2-METHYL-2-HEXENE	ND	ND	ND	ND	ND	ND	ND	ND	ND
3-METHYL-TRANS-3-HEXENE	ND	ND	ND	ND	ND	ND	ND	ND	ND
TRANS-2-HEPTENE	ND	ND	ND	ND	ND	ND	ND	ND	ND
3-ETHYL-CIS-2-PENTENE	ND	ND	ND	ND	ND	ND	ND	ND	ND
2,4,4-TRIMETHYL-1-PENTENE	ND	ND	ND	ND	ND	ND	ND	ND	ND
2,3-DIMETHYL-2-PENTENE	ND	ND	ND	ND	ND	ND	ND	ND	ND
CIS-2-HEPTENE	ND	ND	ND	ND	ND	ND	ND	ND	ND
METHYLCYCLOHEXANE	0.8	0.7	0.8	0.9	0.2	0.5	ND	0.5	0.3
CIS-1,2-DIMETHYLCYCLOPENTANE	ND	ND	ND	ND	ND	ND	ND	ND	ND
2,2-DIMETHYLHEXANE	ND	ND	ND	ND	ND	ND	ND	ND	ND
1,1,3-TRIMETHYLCYCLOPENTANE	ND	ND	ND	ND	ND	ND	ND	ND	ND
2,4,4-TRIMETHYL-2-PENTENE	ND	ND	ND	ND	ND	ND	ND	ND	ND
2,2,3-TRIMETHYLPENTANE	ND	0.2	0.1	ND	ND	ND	ND	ND	ND
2,5-DIMETHYLHEXANE	ND	ND	ND	ND	ND	ND	ND	ND	ND
ETHYLCYCLOPENTANE	ND	ND	ND	ND	ND	ND	ND	ND	ND

TABLE 4. HEAD SPACE HYDROCARBON SPECIATION OF THREE FUELS (CONT'D)

	EM-7578-F			EM-7579-F			EM-7580-F		
	TEST 1	TEST 3	Average	TEST 1	TEST 2	Average	TEST 2	TEST 4	Average
COMPOUND	mg	mg	mg	mg	mg	mg	mg	mg	mg
2,4-DIMETHYLHEXANE	0.2	0.2	0.2	0.2	ND	0.1	ND	ND	ND
1-TRANS-2-CIS-4-TRIMETHYLCYCLOPENTANE	ND	ND	ND	ND	ND	ND	ND	ND	ND
3,3-DIMETHYLHEXANE	ND	ND	ND	ND	ND	ND	ND	ND	ND
1-TRANS-2-CIS-3-TRIMETHYLCYCLOPENTANE	ND	ND	ND	ND	ND	ND	ND	ND	ND
2,3,4-TRIMETHYLPENTANE	0.3	0.8	0.6	0.3	ND	0.2	ND	ND	ND
2,3,3-TRIMETHYLPENTANE	ND	ND	ND	ND	ND	ND	ND	ND	ND
TOLUENE	ND	ND	ND	ND	ND	ND	ND	ND	ND
2,3-DIMETHYLHEXANE	ND	ND	ND	ND	ND	ND	ND	ND	ND
1,1,2-TRIMETHYLCYCLOPENTANE	ND	ND	ND	ND	ND	ND	ND	ND	ND
2-METHYLHEPTANE	ND	ND	ND	ND	ND	ND	ND	ND	ND
3,4-DIMETHYLHEXANE, NOTE B	ND	ND	ND	ND	ND	ND	ND	ND	ND
4-METHYLHEPTANE	ND	ND	ND	ND	ND	ND	ND	ND	ND
3-METHYLHEPTANE	ND	ND	ND	ND	ND	ND	ND	ND	ND
1-CIS,2-TRANS,3-TRIMETHYLCYCLOPENTANE	ND	ND	ND	ND	ND	ND	ND	ND	ND
CIS-1,3-DIMETHYLCYCLOHEXANE	ND	ND	ND	ND	ND	ND	ND	ND	ND
TRANS-1,4-DIMETHYLCYCLOHEXANE	ND	ND	ND	ND	ND	ND	ND	ND	ND
3-ETHYLHEXANE	ND	ND	ND	ND	ND	ND	ND	ND	ND
2,2,5-TRIMETHYLHEXANE	ND	ND	ND	ND	ND	ND	ND	ND	ND
TRANS-1-METHYL-3-ETHYLCYCLOPENTANE	ND	ND	ND	ND	ND	ND	ND	ND	ND
CIS-1-METHYL-3-ETHYLCYCLOPENTANE	ND	ND	ND	ND	ND	ND	ND	ND	ND
1,1-DIMETHYLCYCLOHEXANE	ND	ND	ND	ND	ND	ND	ND	ND	ND
TRANS-1-METHYL-2-ETHYLCYCLOPENTANE	ND	ND	ND	ND	ND	ND	ND	ND	ND
1-METHYL-1-ETHYL-CYCLOPENTANE	ND	ND	ND	ND	ND	ND	ND	ND	ND
2,4,4-TRIMETHYLHEXANE	ND	ND	ND	ND	ND	ND	ND	ND	ND
2,2,4-TRIMETHYLHEXANE	ND	ND	ND	ND	ND	ND	ND	ND	ND
TRANS-1,2-DIMETHYLCYCLOHEXANE	ND	ND	ND	ND	ND	ND	ND	ND	ND
1-OCTENE	ND	ND	ND	ND	ND	ND	ND	ND	ND
TRANS-4-OCTENE	ND	ND	ND	ND	ND	ND	ND	ND	ND
OCTANE	ND	ND	ND	ND	ND	ND	ND	ND	ND
UNIDENTIFIED C8	ND	0.3	0.1	ND	1.3	0.6	0.8	ND	0.4
TRANS-2-OCTENE	ND	ND	ND	ND	ND	ND	ND	ND	ND
TRANS-1,3-DIMETHYLCYCLOHEXANE, NOTE C	ND	ND	ND	ND	ND	ND	ND	ND	ND
CIS-2-OCTENE	ND	ND	ND	ND	ND	ND	ND	ND	ND
ISOPROPYLCYCLOPENTANE	ND	ND	ND	ND	ND	ND	ND	ND	ND
2,2-DIMETHYLHEPTANE	ND	ND	ND	ND	ND	ND	ND	ND	ND
2,3,5-TRIMETHYLHEXANE	ND	ND	ND	ND	ND	ND	ND	ND	ND
CIS-1-METHYL-2-ETHYLCYCLOPENTANE	ND	ND	ND	ND	ND	ND	ND	ND	ND
2,4-DIMETHYLHEPTANE	ND	ND	ND	ND	ND	ND	ND	ND	ND
4,4-DIMETHYLHEPTANE	ND	ND	ND	ND	ND	ND	ND	ND	ND
CIS-1,2-DIMETHYLCYCLOHEXANE	ND	ND	ND	ND	ND	ND	ND	ND	ND
ETHYLCYCLOHEXANE	ND	ND	ND	ND	ND	ND	ND	ND	ND
2,6-DIMETHYLHEPTANE, NOTE D	ND	ND	ND	ND	ND	ND	ND	ND	ND
1,1,3-TRIMETHYLCYCLOHEXANE	ND	ND	ND	ND	ND	ND	ND	ND	ND
2,5-DIMETHYLHEPTANE, NOTE E	ND	ND	ND	ND	ND	ND	ND	ND	ND
3,3-DIMETHYLHEPTANE	ND	ND	ND	ND	ND	ND	ND	ND	ND
3,5-DIMETHYLHEPTANE, NOTE E	ND	ND	ND	ND	ND	ND	ND	ND	ND
ETHYLBENZENE	ND	ND	ND	ND	ND	ND	ND	ND	ND
2,3,4-TRIMETHYLHEXANE	ND	ND	ND	ND	ND	ND	ND	ND	ND
2,3-DIMETHYLHEPTANE	ND	ND	ND	ND	ND	ND	ND	ND	ND
m-& p-XYLENE	ND	ND	ND	ND	ND	ND	ND	ND	ND

TABLE 4. HEAD SPACE HYDROCARBON SPECIATION OF THREE FUELS (CONT'D)

	EM-7578-F			EM-7579-F			EM-7580-F		
	TEST 1	TEST 3	Average	TEST 1	TEST 2	Average	TEST 2	TEST 4	Average
COMPOUND	mg	mg	mg	mg	mg	mg	mg	mg	mg
4-METHYLOCTANE	ND	ND	ND	ND	ND	ND	ND	ND	ND
3,4-DIMETHYLHEPTANE	ND	ND	ND	ND	ND	ND	ND	ND	ND
4-ETHYLHEPTANE	ND	ND	ND	ND	ND	ND	ND	ND	ND
2-METHYLOCTANE	ND	ND	ND	ND	ND	ND	ND	ND	ND
3-METHYLOCTANE	ND	ND	ND	ND	ND	ND	ND	ND	ND
STYRENE	ND	ND	ND	ND	ND	ND	ND	ND	ND
o-XYLENE	ND	ND	ND	ND	ND	ND	ND	ND	ND
1-NONENE	ND	ND	ND	ND	ND	ND	ND	ND	ND
TRANS-3-NONENE	ND	ND	ND	ND	ND	ND	ND	ND	ND
CIS-3-NONENE	ND	ND	ND	ND	ND	ND	ND	ND	ND
NONANE	ND	ND	ND	ND	ND	ND	ND	ND	ND
TRANS-2-NONENE	ND	ND	ND	ND	ND	ND	ND	ND	ND
ISOPROPYLBENZENE (CUMENE)	ND	ND	ND	ND	ND	ND	ND	ND	ND
2,2-DIMETHYLOCTANE	ND	ND	ND	ND	ND	ND	ND	ND	ND
2,4-DIMETHYLOCTANE	ND	ND	ND	ND	ND	ND	ND	ND	ND
n-PROPYLBENZENE	ND	ND	ND	ND	ND	ND	ND	ND	ND
1-METHYL-3-ETHYLBENZENE	ND	ND	ND	ND	ND	ND	ND	ND	ND
1-METHYL-4-ETHYLBENZENE	ND	ND	ND	ND	ND	ND	ND	ND	ND
1,3,5-TRIMETHYLBENZENE	ND	ND	ND	ND	ND	ND	ND	ND	ND
1-METHYL-2-ETHYLBENZENE	ND	ND	ND	ND	ND	ND	ND	ND	ND
1,2,4-TRIMETHYLBENZENE	ND	ND	ND	ND	ND	ND	ND	ND	ND
TERT-BUTYLBENZENE	ND	ND	ND	ND	ND	ND	ND	ND	ND
1-DECENE	ND	ND	ND	ND	ND	ND	ND	ND	ND
DECANE, NOTE F	0.4	ND	0.2	ND	0.2	0.1	ND	ND	ND
ISOBUTYLBENZENE, NOTE F	0.3	ND	0.2	ND	0.2	0.1	ND	ND	ND
1,3,-DIMETHYL-5-ETHYLBENZENE	ND	ND	ND	ND	ND	ND	ND	ND	ND
METHYLPROPYLBENZENE (sec butylbenzene)	ND	ND	ND	ND	ND	ND	ND	ND	ND
1-METHYL-3-ISOPROPYLBENZENE	ND	ND	ND	ND	ND	ND	ND	ND	ND
1,2,3-TRIMETHYLBENZENE	ND	ND	ND	ND	ND	ND	ND	ND	ND
1-METHYL-4-ISOPROPYLBENZENE	ND	ND	ND	ND	ND	ND	ND	ND	ND
INDAN	ND	ND	ND	ND	ND	ND	ND	ND	ND
1-METHYL-2-ISOPROPYLBENZENE	ND	ND	ND	ND	ND	ND	ND	ND	ND
1,3-DIETHYLBENZENE	ND	ND	ND	ND	ND	ND	ND	ND	ND
1,4-DIETHYLBENZENE	ND	ND	ND	ND	ND	ND	ND	ND	ND
1-METHYL-3-N-PROPYLBENZENE	ND	ND	ND	ND	ND	ND	ND	ND	ND
1-METHYL-4-N-PROPYLBENZENE, NOTE G	ND	ND	ND	ND	ND	ND	ND	ND	ND
1,2 DIETHYLBENZENE	ND	ND	ND	ND	ND	ND	ND	ND	ND
1-METHYL-2-N-PROPYLBENZENE	ND	ND	ND	ND	ND	ND	ND	ND	ND
1,4-DIMETHYL-2-ETHYLBENZENE	ND	ND	ND	ND	ND	ND	ND	ND	ND
1,3-DIMETHYL-4-ETHYLBENZENE	ND	ND	ND	ND	ND	ND	ND	ND	ND
1,2-DIMETHYL-4-ETHYLBENZENE	ND	ND	ND	ND	ND	ND	ND	ND	ND
1,3-DIMETHYL-2-ETHYLBENZENE	ND	ND	ND	ND	ND	ND	ND	ND	ND
UNDECANE	ND	ND	ND	ND	ND	ND	ND	ND	ND
1,2-DIMETHYL-3-ETHYLBENZENE	ND	ND	ND	ND	ND	ND	ND	ND	ND
1,2,4,5-TETRAMETHYLBENZENE	ND	ND	ND	ND	ND	ND	ND	ND	ND
2-METHYLBUTYLBENZENE (sec AMYLBENZENE)	ND	ND	ND	ND	ND	ND	ND	ND	ND
3,4 DIMETHYLCUMENE	ND	ND	ND	ND	ND	ND	ND	ND	ND
1,2,3,5-TETRAMETHYLBENZENE	ND	ND	ND	ND	ND	ND	ND	ND	ND
TERT-1-BUT-2-METHYLBENZENE	ND	ND	ND	ND	ND	ND	ND	ND	ND
1,2,3,4-TETRAMETHYLBENZENE	ND	ND	ND	ND	ND	ND	ND	ND	ND

TABLE 4. HEAD SPACE HYDROCARBON SPECIATION OF THREE FUELS (CONT'D)

	EM-7578-F			EM-7579-F			EM-7580-F		
	TEST 1	TEST 3	Average	TEST 1	TEST 2	Average	TEST 2	TEST 4	Average
COMPOUND	mg	mg	mg	mg	mg	mg	mg	mg	mg
N-PENT-BENZENE	ND	ND	ND	ND	ND	ND	ND	ND	ND
TERT-1-BUT-3,5-DIMETHYLBENZENE	ND	ND	ND	ND	ND	ND	ND	ND	ND
TERT-1-BUTYL-4-ETHYLBENZENE	ND	ND	ND	ND	ND	ND	ND	ND	ND
NAPHTHALENE	ND	ND	ND	ND	ND	ND	ND	ND	ND
DODECANE	ND	ND	ND	ND	ND	ND	ND	ND	ND
1,3,5-TRIETHYLBENZENE	ND	ND	ND	ND	ND	ND	ND	ND	ND
1,2,4-TRIETHYLBENZENE	ND	ND	ND	ND	ND	ND	ND	ND	ND
HEXYLBENZENE	ND	ND	ND	ND	ND	ND	ND	ND	ND
UNIDENTIFIED C9-C12+	0.7	4.4	2.6	1.8	0.1	1.0	0.7	1.5	1.1
SUMMED SPECIATED VALUES	**784**	**782**	**783**	**1251**	**1124**	**1188**	**1080**	**1126**	**1103**

A - 2,2-Dimethylpentane and methylcyclopentane co-elute. GC peak area split equally between the two compounds.
B - 3-Methyl-3-ethylpentane co-elutes with reported compound. Not reported separately.
C - Cis-1,4-Dimethylcyclohexane co-elutes with reported compound. Not reported separately.
D - Propylcyclopentane co-elutes with reported compound. Not reported separately.
E - 2,5-Dimethylheptane and 3,5-dimethylheptane co-elute. GC peak area split equally between the two compounds.
F - Decane and isobutylbenzene co-elute. GC peak area split equally between the two compounds.
G - n-Butylbenzene co-elutes with reported compound. Not reported separately.

TABLE 5. NORMALIZED HEAD SPACE HYDROCARBON SPECIATION OF THREE FUELS

	EM-7578-F			EM-7579-F			EM-7580-F		
	TEST 1	TEST 3	Average	TEST 1	TEST 2	Average	TEST 2	TEST 4	Average
COMPOUND	% of Total	% of Total	% of Total	% of Total	% of Total	% of Total	% of Total	% of Total	% of Total
METHANE	ND	ND	ND	ND	ND	ND	ND	ND	ND
ETHANE	0.25	0.33	0.29	0.13	0.13	0.13	0.18	0.13	0.15
ETHYLENE	ND	ND	ND	ND	ND	ND	ND	ND	ND
PROPANE	2.88	3.76	3.32	2.61	2.48	2.55	2.64	2.19	2.41
PROPYLENE	ND	ND	ND	ND	ND	ND	ND	ND	ND
ACETYLENE	ND	ND	ND	ND	ND	ND	ND	ND	ND
PROPADIENE	ND	ND	ND	ND	ND	ND	ND	ND	ND
BUTANE	13.86	16.45	15.15	6.53	6.35	6.44	6.13	6.52	6.33
TRANS-2-BUTENE	ND	0.03	0.02	ND	ND	ND	0.03	ND	0.01
1-BUTENE	ND	ND	ND	ND	ND	ND	ND	ND	ND
2-METHYLPROPENE (ISOBUTYLENE)	0.04	ND	0.02	0.04	0.03	0.04	0.03	0.03	0.03
2,2-DIMETHYLPROPANE (NEOPENTANE)	0.39	0.35	0.37	0.38	0.38	0.38	0.36	0.38	0.37
PROPYNE	0.45	0.31	0.38	0.41	0.44	0.42	0.38	0.35	0.36
1,3-BUTADIENE	ND	ND	ND	ND	ND	ND	ND	ND	ND
2-METHYLPROPANE (ISOBUTANE)	16.95	19.12	18.03	17.07	16.49	16.80	16.28	16.35	16.32
1-BUTYNE	ND	ND	ND	ND	ND	ND	ND	ND	ND
METHANOL	ND	ND	ND	ND	ND	ND	ND	ND	ND
CIS-2-BUTENE	ND	ND	ND	ND	ND	ND	ND	ND	ND
3-METHYL-1-BUTENE	ND	ND	ND	ND	ND	ND	ND	ND	ND
ETHANOL	ND	ND	ND	7.63	7.72	7.67	13.23	11.60	12.40
2-METHYLBUTANE (ISOPENTANE)	53.12	46.70	49.91	53.62	53.95	53.77	50.12	51.64	50.90
2-BUTYNE	ND	ND	ND	ND	ND	ND	ND	ND	ND
1-PENTENE	ND	ND	ND	ND	ND	ND	ND	ND	ND
2-METHYL-1-BUTENE	ND	ND	ND	ND	ND	ND	ND	ND	ND
PENTANE	6.51	6.39	6.45	6.43	6.68	6.55	6.44	6.61	6.53
UNIDENTIFIED C5 OLEFINS	ND	ND	ND	ND	ND	ND	ND	ND	ND
2-METHYL-1,3-BUTADIENE	ND	ND	ND	ND	ND	ND	ND	ND	ND
TRANS-2-PENTENE	ND	ND	ND	ND	ND	ND	ND	ND	ND
3,3-DIMETHYL-1-BUTENE	ND	ND	ND	ND	ND	ND	ND	ND	ND
CIS-2-PENTENE	ND	ND	ND	ND	ND	ND	ND	ND	ND
2-METHYL-2-BUTENE	ND	ND	ND	ND	ND	ND	ND	ND	ND
ISOBUTANOL	ND	ND	ND	ND	ND	ND	ND	ND	ND
CYCLOPENTADIENE	ND	ND	ND	ND	ND	ND	ND	ND	ND
2,2-DIMETHYLBUTANE	0.27	0.25	0.26	0.25	0.27	0.26	0.25	0.25	0.25
CYCLOPENTENE	ND	ND	ND	ND	ND	ND	ND	ND	ND
4-METHYL-1-PENTENE	ND	ND	ND	ND	ND	ND	ND	ND	ND
3-METHYL-1-PENTENE	ND	ND	ND	ND	ND	ND	ND	ND	ND
CYCLOPENTANE	0.23	0.24	0.24	0.24	0.25	0.24	0.23	0.22	0.23
2,3-DIMETHYLBUTANE	0.81	0.85	0.83	0.86	0.93	0.89	0.81	0.73	0.77
MTBE	ND	ND	ND	ND	ND	ND	ND	ND	ND
4-METHYL-CIS-2-PENTENE	ND	ND	ND	ND	ND	ND	ND	ND	ND
2-METHYLPENTANE	0.87	1.02	0.95	1.03	1.12	1.07	0.97	0.86	0.91
4-METHYL-TRANS-2-PENTENE	ND	ND	ND	ND	ND	ND	ND	ND	ND
3-METHYLPENTANE	0.39	0.49	0.44	0.49	0.54	0.51	0.45	0.40	0.43
2-METHYL-1-PENTENE	ND	ND	ND	ND	ND	ND	ND	ND	ND
1-HEXENE	ND	ND	ND	ND	ND	ND	ND	ND	ND
HEXANE	0.37	0.63	0.50	0.61	0.64	0.62	0.52	0.46	0.49
UNIDENTIFIED C6	0.41	ND	0.20	ND	ND	ND	ND	ND	ND
TRANS-3-HEXENE	ND	ND	ND	ND	ND	ND	ND	ND	ND
CIS-3-HEXENE	ND	ND	ND	ND	ND	ND	ND	ND	ND

TABLE 5. NORMALIZED HEAD SPACE HYDROCARBON SPECIATION OF THREE FUELS (CONT'D)

	EM-7578-F			EM-7579-F			EM-7580-F		
	TEST 1	TEST 3	Average	TEST 1	TEST 2	Average	TEST 2	TEST 4	Average
COMPOUND	% of Total	% of Total	% of Total	% of Total	% of Total	% of Total	% of Total	% of Total	% of Total
DI-ISOPROPYL ETHER	ND	ND	ND	ND	ND	ND	ND	ND	ND
TRANS-2-HEXENE	ND	ND	ND	ND	ND	ND	ND	ND	ND
3-METHYL-TRANS-2-PENTENE	ND	ND	ND	ND	ND	ND	ND	ND	ND
2-METHYL-2-PENTENE	ND	ND	ND	ND	ND	ND	ND	ND	ND
3-METHYLCYCLOPENTENE	ND	ND	ND	ND	ND	ND	ND	ND	ND
CIS-2-HEXENE	ND	ND	ND	ND	ND	ND	ND	ND	ND
ETBE	ND	ND	ND	ND	ND	ND	ND	ND	ND
3-METHYL-CIS-2-PENTENE	ND	ND	ND	ND	ND	ND	ND	ND	ND
2,2-DIMETHYLPENTANE, NOTE A	0.12	0.14	0.13	0.11	0.12	0.11	0.10	0.09	0.09
METHYLCYCLOPENTANE, NOTE A	0.13	0.16	0.14	0.12	0.14	0.13	0.11	0.10	0.10
2,4-DIMETHYLPENTANE	0.25	0.24	0.24	0.21	0.14	0.18	0.10	0.15	0.12
2,2,3-TRIMETHYLBUTANE	ND	ND	ND	ND	ND	ND	ND	ND	ND
3,4-DIMETHYL-1-PENTENE	ND	ND	ND	ND	ND	ND	ND	ND	ND
1-METHYLCYCLOPENTENE	ND	ND	ND	ND	ND	ND	ND	ND	ND
BENZENE	ND	ND	ND	ND	ND	ND	ND	ND	ND
3-METHYL-1-HEXENE	ND	ND	ND	ND	ND	ND	ND	ND	ND
3,3-DIMETHYLPENTANE	ND	ND	ND	ND	ND	ND	ND	ND	ND
CYCLOHEXANE	0.21	0.17	0.19	0.18	0.18	0.18	0.12	ND	0.06
2-METHYLHEXANE	0.20	0.21	0.20	0.15	ND	0.08	ND	ND	ND
2,3-DIMETHYLPENTANE	ND	ND	ND	ND	ND	ND	ND	ND	ND
1,1-DIMETHYLCYCLOPENTANE	ND	ND	ND	ND	ND	ND	ND	ND	ND
TERT-AMYL METHYL ETHER	ND	ND	ND	ND	ND	ND	ND	ND	ND
CYCLOHEXENE	ND	ND	ND	ND	ND	ND	ND	ND	ND
3-METHYLHEXANE	0.05	0.05	0.05	0.04	0.10	0.07	0.05	0.03	0.04
CIS-1,3-DIMETHYLCYCLOPENTANE	ND	ND	ND	ND	ND	ND	ND	ND	ND
3-ETHYLPENTANE	ND	ND	ND	ND	ND	ND	ND	ND	ND
TRANS-1,2-DIMETHYLCYCLOPENTANE	ND	ND	ND	ND	ND	ND	ND	ND	ND
TRANS-1,3-DIMETHYLCYCLOPENTANE	ND	ND	ND	ND	ND	ND	ND	ND	ND
1-HEPTENE	ND	ND	ND	ND	ND	ND	ND	ND	ND
2,2,4-TRIMETHYLPENTANE	0.86	1.21	1.03	0.58	0.70	0.64	0.36	0.54	0.45
2-METHYL-1-HEXENE	ND	ND	ND	ND	ND	ND	ND	ND	ND
TRANS-3-HEPTENE	ND	ND	ND	ND	ND	ND	ND	ND	ND
HEPTANE	ND	ND	ND	ND	ND	ND	ND	ND	ND
CIS-3-HEPTENE	ND	ND	ND	ND	ND	ND	ND	ND	ND
UNIDENTIFIED C7	0.03	0.06	0.04	0.03	0.06	0.04	ND	0.19	0.09
2-METHYL-2-HEXENE	ND	ND	ND	ND	ND	ND	ND	ND	ND
3-METHYL-TRANS-3-HEXENE	ND	ND	ND	ND	ND	ND	ND	ND	ND
TRANS-2-HEPTENE	ND	ND	ND	ND	ND	ND	ND	ND	ND
3-ETHYL-CIS-2-PENTENE	ND	ND	ND	ND	ND	ND	ND	ND	ND
2,4,4-TRIMETHYL-1-PENTENE	ND	ND	ND	ND	ND	ND	ND	ND	ND
2,3-DIMETHYL-2-PENTENE	ND	ND	ND	ND	ND	ND	ND	ND	ND
CIS-2-HEPTENE	ND	ND	ND	ND	ND	ND	ND	ND	ND
METHYLCYCLOHEXANE	0.11	0.09	0.10	0.07	0.02	0.04	ND	0.05	0.02
CIS-1,2-DIMETHYLCYCLOPENTANE	ND	ND	ND	ND	ND	ND	ND	ND	ND
2,2-DIMETHYLHEXANE	ND	ND	ND	ND	ND	ND	ND	ND	ND
1,1,3-TRIMETHYLCYCLOPENTANE	ND	ND	ND	ND	ND	ND	ND	ND	ND
2,4,4-TRIMETHYL-2-PENTENE	ND	ND	ND	ND	ND	ND	ND	ND	ND
2,2,3-TRIMETHYLPENTANE	ND	0.03	0.01	ND	ND	ND	ND	ND	ND
2,5-DIMETHYLHEXANE	ND	ND	ND	ND	ND	ND	ND	ND	ND
ETHYLCYCLOPENTANE	ND	ND	ND	ND	ND	ND	ND	ND	ND

TABLE 5. NORMALIZED HEAD SPACE HYDROCARBON SPECIATION OF THREE FUELS (CONT'D)

	EM-7578-F			EM-7579-F			EM-7580-F		
	TEST 1	TEST 3	Average	TEST 1	TEST 2	Average	TEST 2	TEST 4	Average
COMPOUND	% of Total	% of Total	% of Total	% of Total	% of Total	% of Total	% of Total	% of Total	% of Total
2,4-DIMETHYLHEXANE	0.02	0.03	0.02	0.02	ND	0.01	ND	ND	ND
1-TRANS-2-CIS-4-TRIMETHYLCYCLOPENTANE	ND	ND	ND	ND	ND	ND	ND	ND	ND
3,3-DIMETHYLHEXANE	ND	ND	ND	ND	ND	ND	ND	ND	ND
1-TRANS-2-CIS-3-TRIMETHYLCYCLOPENTANE	ND	ND	ND	ND	ND	ND	ND	ND	ND
2,3,4-TRIMETHYLPENTANE	0.04	0.11	0.07	0.03	ND	0.01	ND	ND	ND
2,3,3-TRIMETHYLPENTANE	ND	ND	ND	ND	ND	ND	ND	ND	ND
TOLUENE	ND	ND	ND	ND	ND	ND	ND	ND	ND
2,3-DIMETHYLHEXANE	ND	ND	ND	ND	ND	ND	ND	ND	ND
1,1,2-TRIMETHYLCYCLOPENTANE	ND	ND	ND	ND	ND	ND	ND	ND	ND
2-METHYLHEPTANE	ND	ND	ND	ND	ND	ND	ND	ND	ND
3,4-DIMETHYLHEXANE, NOTE B	ND	ND	ND	ND	ND	ND	ND	ND	ND
4-METHYLHEPTANE	ND	ND	ND	ND	ND	ND	ND	ND	ND
3-METHYLHEPTANE	ND	ND	ND	ND	ND	ND	ND	ND	ND
1-CIS,2-TRANS,3-TRIMETHYLCYCLOPENTANE	ND	ND	ND	ND	ND	ND	ND	ND	ND
CIS-1,3-DIMETHYLCYCLOHEXANE	ND	ND	ND	ND	ND	ND	ND	ND	ND
TRANS-1,4-DIMETHYLCYCLOHEXANE	ND	ND	ND	ND	ND	ND	ND	ND	ND
3-ETHYLHEXANE	ND	ND	ND	ND	ND	ND	ND	ND	ND
2,2,5-TRIMETHYLHEXANE	ND	ND	ND	ND	ND	ND	ND	ND	ND
TRANS-1-METHYL-3-ETHYLCYCLOPENTANE	ND	ND	ND	ND	ND	ND	ND	ND	ND
CIS-1-METHYL-3-ETHYLCYCLOPENTANE	ND	ND	ND	ND	ND	ND	ND	ND	ND
1,1-DIMETHYLCYCLOHEXANE	ND	ND	ND	ND	ND	ND	ND	ND	ND
TRANS-1-METHYL-2-ETHYLCYCLOPENTANE	ND	ND	ND	ND	ND	ND	ND	ND	ND
1-METHYL-1-ETHYL-CYCLOPENTANE	ND	ND	ND	ND	ND	ND	ND	ND	ND
2,4,4-TRIMETHYLHEXANE	ND	ND	ND	ND	ND	ND	ND	ND	ND
2,2,4-TRIMETHYLHEXANE	ND	ND	ND	ND	ND	ND	ND	ND	ND
TRANS-1,2-DIMETHYLCYCLOHEXANE	ND	ND	ND	ND	ND	ND	ND	ND	ND
1-OCTENE	ND	ND	ND	ND	ND	ND	ND	ND	ND
TRANS-4-OCTENE	ND	ND	ND	ND	ND	ND	ND	ND	ND
OCTANE	ND	ND	ND	ND	ND	ND	ND	ND	ND
UNIDENTIFIED C8	ND	0.04	0.02	ND	0.11	0.05	0.08	ND	0.04
TRANS-2-OCTENE	ND	ND	ND	ND	ND	ND	ND	ND	ND
TRANS-1,3-DIMETHYLCYCLOHEXANE, NOTE C	ND	ND	ND	ND	ND	ND	ND	ND	ND
CIS-2-OCTENE	ND	ND	ND	ND	ND	ND	ND	ND	ND
ISOPROPYLCYCLOPENTANE	ND	ND	ND	ND	ND	ND	ND	ND	ND
2,2-DIMETHYLHEPTANE	ND	ND	ND	ND	ND	ND	ND	ND	ND
2,3,5-TRIMETHYLHEXANE	ND	ND	ND	ND	ND	ND	ND	ND	ND
CIS-1-METHYL-2-ETHYLCYCLOPENTANE	ND	ND	ND	ND	ND	ND	ND	ND	ND
2,4-DIMETHYLHEPTANE	ND	ND	ND	ND	ND	ND	ND	ND	ND
4,4-DIMETHYLHEPTANE	ND	ND	ND	ND	ND	ND	ND	ND	ND
CIS-1,2-DIMETHYLCYCLOHEXANE	ND	ND	ND	ND	ND	ND	ND	ND	ND
ETHYLCYCLOHEXANE	ND	ND	ND	ND	ND	ND	ND	ND	ND
2,6-DIMETHYLHEPTANE, NOTE D	ND	ND	ND	ND	ND	ND	ND	ND	ND
1,1,3-TRIMETHYLCYCLOHEXANE	ND	ND	ND	ND	ND	ND	ND	ND	ND
2,5-DIMETHYLHEPTANE, NOTE E	ND	ND	ND	ND	ND	ND	ND	ND	ND
3,3-DIMETHYLHEPTANE	ND	ND	ND	ND	ND	ND	ND	ND	ND
3,5-DIMETHYLHEPTANE, NOTE E	ND	ND	ND	ND	ND	ND	ND	ND	ND
ETHYLBENZENE	ND	ND	ND	ND	ND	ND	ND	ND	ND
2,3,4-TRIMETHYLHEXANE	ND	ND	ND	ND	ND	ND	ND	ND	ND
2,3-DIMETHYLHEPTANE	ND	ND	ND	ND	ND	ND	ND	ND	ND
m-& p-XYLENE	ND	ND	ND	ND	ND	ND	ND	ND	ND

TABLE 5. NORMALIZED HEAD SPACE HYDROCARBON SPECIATION OF THREE FUELS (CONT'D)

	EM-7578-F			EM-7579-F			EM-7580-F		
	TEST 1	TEST 3	Average	TEST 1	TEST 2	Average	TEST 2	TEST 4	Average
COMPOUND	% of Total	% of Total	% of Total	% of Total	% of Total	% of Total	% of Total	% of Total	% of Total
4-METHYLOCTANE	ND	ND	ND	ND	ND	ND	ND	ND	ND
3,4-DIMETHYLHEPTANE	ND	ND	ND	ND	ND	ND	ND	ND	ND
4-ETHYLHEPTANE	ND	ND	ND	ND	ND	ND	ND	ND	ND
2-METHYLOCTANE	ND	ND	ND	ND	ND	ND	ND	ND	ND
3-METHYLOCTANE	ND	ND	ND	ND	ND	ND	ND	ND	ND
STYRENE	ND	ND	ND	ND	ND	ND	ND	ND	ND
o-XYLENE	ND	ND	ND	ND	ND	ND	ND	ND	ND
1-NONENE	ND	ND	ND	ND	ND	ND	ND	ND	ND
TRANS-3-NONENE	ND	ND	ND	ND	ND	ND	ND	ND	ND
CIS-3-NONENE	ND	ND	ND	ND	ND	ND	ND	ND	ND
NONANE	ND	ND	ND	ND	ND	ND	ND	ND	ND
TRANS-2-NONENE	ND	ND	ND	ND	ND	ND	ND	ND	ND
ISOPROPYLBENZENE (CUMENE)	ND	ND	ND	ND	ND	ND	ND	ND	ND
2,2-DIMETHYLOCTANE	ND	ND	ND	ND	ND	ND	ND	ND	ND
2,4-DIMETHYLOCTANE	ND	ND	ND	ND	ND	ND	ND	ND	ND
n-PROPYLBENZENE	ND	ND	ND	ND	ND	ND	ND	ND	ND
1-METHYL-3-ETHYLBENZENE	ND	ND	ND	ND	ND	ND	ND	ND	ND
1-METHYL-4-ETHYLBENZENE	ND	ND	ND	ND	ND	ND	ND	ND	ND
1,3,5-TRIMETHYLBENZENE	ND	ND	ND	ND	ND	ND	ND	ND	ND
1-METHYL-2-ETHYLBENZENE	ND	ND	ND	ND	ND	ND	ND	ND	ND
1,2,4-TRIMETHYLBENZENE	ND	ND	ND	ND	ND	ND	ND	ND	ND
TERT-BUTYLBENZENE	ND	ND	ND	ND	ND	ND	ND	ND	ND
1-DECENE	ND	ND	ND	ND	ND	ND	ND	ND	ND
DECANE, NOTE F	0.05	ND	0.02	ND	0.02	0.01	ND	ND	ND
ISOBUTYLBENZENE, NOTE F	0.04	ND	0.02	ND	0.02	0.01	ND	ND	ND
1,3,-DIMETHYL-5-ETHYLBENZENE	ND	ND	ND	ND	ND	ND	ND	ND	ND
METHYLPROPYLBENZENE (sec butylbenzene)	ND	ND	ND	ND	ND	ND	ND	ND	ND
1-METHYL-3-ISOPROPYLBENZENE	ND	ND	ND	ND	ND	ND	ND	ND	ND
1,2,3-TRIMETHYLBENZENE	ND	ND	ND	ND	ND	ND	ND	ND	ND
1-METHYL-4-ISOPROPYLBENZENE	ND	ND	ND	ND	ND	ND	ND	ND	ND
INDAN	ND	ND	ND	ND	ND	ND	ND	ND	ND
1-METHYL-2-ISOPROPYLBENZENE	ND	ND	ND	ND	ND	ND	ND	ND	ND
1,3-DIETHYLBENZENE	ND	ND	ND	ND	ND	ND	ND	ND	ND
1,4-DIETHYLBENZENE	ND	ND	ND	ND	ND	ND	ND	ND	ND
1-METHYL-3-N-PROPYLBENZENE	ND	ND	ND	ND	ND	ND	ND	ND	ND
1-METHYL-4-N-PROPYLBENZENE, NOTE G	ND	ND	ND	ND	ND	ND	ND	ND	ND
1,2 DIETHYLBENZENE	ND	ND	ND	ND	ND	ND	ND	ND	ND
1-METHYL-2-N-PROPYLBENZENE	ND	ND	ND	ND	ND	ND	ND	ND	ND
1,4-DIMETHYL-2-ETHYLBENZENE	ND	ND	ND	ND	ND	ND	ND	ND	ND
1,3-DIMETHYL-4-ETHYLBENZENE	ND	ND	ND	ND	ND	ND	ND	ND	ND
1,2-DIMETHYL-4-ETHYLBENZENE	ND	ND	ND	ND	ND	ND	ND	ND	ND
1,3-DIMETHYL-2-ETHYLBENZENE	ND	ND	ND	ND	ND	ND	ND	ND	ND
UNDECANE	ND	ND	ND	ND	ND	ND	ND	ND	ND
1,2-DIMETHYL-3-ETHYLBENZENE	ND	ND	ND	ND	ND	ND	ND	ND	ND
1,2,4,5-TETRAMETHYLBENZENE	ND	ND	ND	ND	ND	ND	ND	ND	ND
2-METHYLBUTYLBENZENE (sec AMYLBENZENE)	ND	ND	ND	ND	ND	ND	ND	ND	ND
3,4 DIMETHYLCUMENE	ND	ND	ND	ND	ND	ND	ND	ND	ND
1,2,3,5-TETRAMETHYLBENZENE	ND	ND	ND	ND	ND	ND	ND	ND	ND
TERT-1-BUT-2-METHYLBENZENE	ND	ND	ND	ND	ND	ND	ND	ND	ND
1,2,3,4-TETRAMETHYLBENZENE	ND	ND	ND	ND	ND	ND	ND	ND	ND

TABLE 5. NORMALIZED HEAD SPACE HYDROCARBON SPECIATION OF THREE FUELS (CONT'D)

	EM-7578-F			EM-7579-F			EM-7580-F		
	TEST 1	TEST 3	Average	TEST 1	TEST 2	Average	TEST 2	TEST 4	Average
COMPOUND	% of Total	% of Total	% of Total	% of Total	% of Total	% of Total	% of Total	% of Total	% of Total
N-PENT-BENZENE	ND	ND	ND	ND	ND	ND	ND	ND	ND
TERT-1-BUT-3,5-DIMETHYLBENZENE	ND	ND	ND	ND	ND	ND	ND	ND	ND
TERT-1-BUTYL-4-ETHYLBENZENE	ND	ND	ND	ND	ND	ND	ND	ND	ND
NAPHTHALENE	ND	ND	ND	ND	ND	ND	ND	ND	ND
DODECANE	ND	ND	ND	ND	ND	ND	ND	ND	ND
1,3,5-TRIETHYLBENZENE	ND	ND	ND	ND	ND	ND	ND	ND	ND
1,2,4-TRIETHYLBENZENE	ND	ND	ND	ND	ND	ND	ND	ND	ND
HEXYLBENZENE	ND	ND	ND	ND	ND	ND	ND	ND	ND
UNIDENTIFIED C9-C12+	0.09	0.56	0.33	0.15	0.01	0.08	0.06	0.14	0.10
SUMMED SPECIATED VALUES	**100**	**100**	**100**	**100**	**100**	**100**	**100**	**100**	**100**

A - 2,2-Dimethylpentane and methylcyclopentane co-elute. GC peak area split equally between the two compounds.
B - 3-Methyl-3-ethylpentane co-elutes with reported compound. Not reported separately.
C - Cis-1,4-Dimethylcyclohexane co-elutes with reported compound. Not reported separately.
D - Propylcyclopentane co-elutes with reported compound. Not reported separately.
E - 2,5-Dimethylheptane and 3,5-dimethylheptane co-elute. GC peak area split equally between the two compounds.
F - Decane and isobutylbenzene co-elute. GC peak area split equally between the two compounds.
G - n-Butylbenzene co-elutes with reported compound. Not reported separately.

6.0 SUMMARY

Head space testing was performed on three fuels for RFA and Growth Energy to provide data in regard to the EPA requirements for registration of a designated F/FA as stipulated by section 211 (b) and 211 (e) of the CAA. Speciation of the C_1 to C_{12} hydrocarbons, alcohols, and ethers was performed on each fuel. When the base fuel was compared to the fuels with ethanol:

- The fuels with additive (EM-7579-F and EM-7580-F) had all of the same compounds as the base fuel (EM-7578-F) except for the presence of ethanol which was expected.
- Two additional compounds (an unidentified C_6 and 2,2,3-trimethylpentane) were found in the base fuel; these two compounds were not found in the fuels with the additive.
- All compounds were generally at the same order of magnitude with the base fuel as the fuels with ethanol.
- No additional compounds were found in the fuels with additive that were not in the base fuel with the exception of ethanol.

In general, the head space composition for the base fuel was similar to the fuels with additive. The addition of ethanol as an additive did not affect the head space composition of the fuels. While some C_1 to C_{12} hydrocarbons were below the detection limit, one cannot necessarily conclude that these compounds were present or not present; however, if present, the compounds were below the limits of detection.

7.0 REFERENCES

1. Caldwell, J.W., e-mail to Mr. E. Robert Fanick, July 20, 2010, 12:47 p.m.

2. Code of Federal Regulation (CFR) Title 40 – Protection of Environment, Part 79 – Registration of Fuels and Fuel Additives, Subpart F – Testing Requirements for Registration

3. Caldwell, J.W., e-mail to Mr. E. Robert Fanick, August 11, 2009, 6:42 a.m.

4. Siegl, Walter O., et. al., "Improved Emissions Speciation Methodology for Phase II of the Auto/Oil Air Quality Improvement Research Program - Hydrocarbons and Oxygenates," SAE Paper No. 930142, International Congress and Exposition, Detroit, Michigan, March 1-3, 1993

5. DEER SwRI TIP 07C-013, "Hydrocarbon Speciation."

6. "Determination of Alcohols in Automotive Source Samples by Gas Chromatography," California Air Resources Board, SOP No. MLD 101, revision 2, January 2005.

7. DEER SwRI SOP-4.16, "Quality Records."

Evaporative and Exhaust Emissions Characterization of 2011 E0, E10, and E15: Comparison to Data Developed by the Section 211(b) Research Group

Prepared for:
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Table of Contents

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List of Tables

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List of Figures

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1. Executive summary

Sections 211(b)(2) and 211(e) of the Clean Air Act require that fuels and fuel additives be registered with U.S. EPA and undergo Tier 1 and Tier 2 testing, as outlined in 40 CFR 79.52-53, in order to identify and evaluate the potential adverse effects of the fuel additive emissions and to help guide EPA's future regulatory activities. Company registration data are also required. Tier 1 requires that exhaust and evaporative emissions be collected and characterized and that the scientific literature for the past 30 years be searched for information about the health and welfare effects of the fuels, fuel additives, and exhaust constituents. Tier 2 requires that the fuels and additives undergo health effects testing in rodents. Requirements for Tier 1 and 2 are specified in the regulations.

This submission addresses Tier 1 data for E15, that is, gasoline containing 15% ethanol, in support of the registration effort for E15. The ethanol in E15 is considered a non-baseline fuel additive per 40 CFR 79.56. Tier 1 evaporative and exhaust emission characterizations were recently developed for ethanol in E15 fuel blends, as well as for E0 and E10, by Southwest Research Institute (SwRI 2011a and b). The analyses herein consider these data for E0, E10, and E15 in light of data developed over the last 15 or so years by the Section 211(b) Research Group for baseline gasoline (BG) and baseline gasoline with 10% ethanol (BG+E).[1] Note that the baseline fuels used in 1997 and 2011 were formulated to specifications in force at the time.

This report supports the argument that no additional Tier 1 literature search and no Tier 2 health effects testing are merited for E15. First, evaporative and combustion emissions of E15 are highly similar to E10 emissions: no new species were consistently identified in E15 compared to E10, an approved fuel. Second, the existing database demonstrates that the evaporative and exhaust emissions for E15 are reasonably comparable (*per* 40 CFR 79.52(b)) to those already addressed by the Section 211(b) Research Group in its submissions to U.S. EPA in support of the registration of conventional gasoline containing 10% ethanol (BG+E). The data therefore support the extension of the Alternative Tier 2 test program to E15.

The following abbreviations are used in this report:

E0 baseline gasoline used in 2011 studies by RFA and Growth Energy; specified by 40 CFR 86.113-04; blended by Haltermann Products.
E10 baseline gasoline (E0) containing 10% by volume ethanol; blended by Haltermann Products.
E15 baseline gasoline (E0) containing 15% by volume ethanol; blended by Haltermann Products.
BG baseline gasoline used by the Section 211(b) Research Group for Tier 1 and Tier 2 studies; specified by 40 CFR 79.55.
BG+E baseline gasoline (BG) containing 10% ethanol, used by the Section 211(b) Research Group.

[1] The Section 211(b) Research Group is the owner of the 1997 data regarding BG and BG+E, the 1997 Tier 1 literature search, and the associated Alternative Tier 2 health effects data.

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GEVC vapor condensate of BG+E used in Alternative Tier 2 health effects studies by the Section 211(b) Research Group.

Comparisons among the data sets show the following, with respect to evaporative emissions:

- Evaporative emissions profiles of 2011 E0, E10, and E15, determined by a headspace method, are highly similar. Addition of ethanol causes only one new species, ethanol itself, to appear in vapors of E10 and/or E15.
- E15 headspace does not contain any new compounds, compared to E10 headspace, with the possible exception of trans-2-butene. This compound was inconsistently detected in E15 and E0 evaporative emissions but consistently found in BG and BG+E evaporative emissions.
- Compared to the 1997 reference fuel, BG, evaporative emissions of E0 contain far fewer components. Four minor components of E0 headspace, propane, propyne, ethane, and 2-methylhexane, were not found in BG evaporative emissions. Shared constituents accounted for 85% (by mass) of BG evaporative emissions and 96% of E0 emissions. These differences may be attributable to the different procedures for generating the headspace emissions: BG evaporative emissions were created by heating and enclosing a vehicle in a shed, according to the then-current test protocol, whereas 2011 evaporative emissions were developed by a new EPA-approved method, an evaporative emissions generator.
- Compared to the 1997 fuel containing 10% ethanol, BG+E, evaporative emissions of E10 and E15 contain far fewer components. The components of E15 headspace were present in BG+E evaporative emissions, with the exceptions of propane, propyne, and ethane, present in E15 headspace in small amounts. Shared constituents accounted for 84% (by mass) of BG+E evaporative emissions and approximately 97% of E15 emissions.
- The 1997 Tier 1 literature searches for gasoline (evaporative and exhaust emissions) and diesel exhaust addressed all but two components of E15 headspace vapors. These two components were also present in evaporative emissions of E10 and E0.

Engine exhausts for the various fuels, both with and without engine after-treatment, were analyzed for specific hydrocarbons, aldehydes, alcohols, ethers (collectively, "hydrocarbons" herein), PAHs, and NPAHs, as well as regulated emissions such as CO. Comparisons of the data sets indicated the following:

- Catalyzed exhausts of E0, E10, and E15 easily met current standards for regulated emissions.
- Compared to regulated emissions from the engine using E10, use of E15 sometimes increased rates of emissions of regulated species in the presence of catalytic conversion but reduced emissions when after-treatment did not occur.
- The hydrocarbon profiles of catalyzed E15 and E10 exhausts were highly similar overall. Catalyzed E15 exhaust contained two hydrocarbons not detected in E10 exhaust, methanol and crotonaldehyde. However, these two species were not consistently detected in E15 exhaust. Emission rates of speciated hydrocarbons in catalyzed E15 exhaust tended to be somewhat higher than in E10 exhaust but lower than in E0 exhaust.
- Catalyzed E15 exhaust contained the same suite of PAHs and NPAHs as did E10 exhaust. Total emission rates for the various species (vapor and particulate phases) were

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generally greater in E15 exhaust than in E10 exhaust but much lower than in BG or BG+E exhaust.

- Uncatalyzed E15 exhaust contained several hydrocarbons not detected in uncatalyzed E10 exhaust, namely 1,2,4,5-tetramethylbenzene, cis-2-octene, hexanaldehyde, methyl isobutyl ketone (MIBK), tert-1-but-3,5-dimethylbenzene, and undecane. These compounds were never detected in E10 exhaust but were quantified once in E15 exhaust (twice, for MIBK). Overall, however, the two profiles were highly similar, qualitatively and quantitatively.
- Uncatalyzed E15 and E10 exhausts contained the same suite of PAHs and NPAHs. Total emission rates for detected species were generally lower for E15 than E10, and frequently lower for E15 than E0.
- Exhausts of current fuels, catalyzed or not, contained far fewer species, in smaller quantities, than the 1997 exhausts.
- All hydrocarbon components of catalyzed E15 exhaust were detected in 1997 catalyzed BG+E exhaust with the exceptions of 2-methylhexane (also present in E10 exhaust) and methanol. One component, methacrolein, was not sought in the 1997 exhaust.
- Uncatalyzed E15 exhaust contained 15 compounds not detected in BG+E exhaust. All of them were found in uncatalyzed E10 exhaust, however.
- The great majority of species in catalyzed E15 exhaust were included in the 1997 Tier 1 literature search.
- About 75% of the species in uncatalyzed E15 exhaust were included in the 1997 Tier 1 literature search or exempted by regulation. Structurally similar compounds were addressed for many of the remaining 25%.

Evaporative and exhaust emissions of E15 are thus highly similar to emissions of E0 and E10, approved fuels. Evaporative E15 emissions are also quite similar to evaporative emissions of 1997 BG+E and are essentially a subset of that emissions profile. Combustion emissions of E15 and BG+E are less similar but their profiles still overlap considerably. Differences among evaporative and exhaust profiles, from 1997 and 2011 fuels, are likely due to differences in baseline gasoline composition, test methods for evaporative emissions, improvements in analytical sensitivity, and perhaps other factors such as engine oils.

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2. *Introduction*

Sections 211(b)(2) and 211(e) of the Clean Air Act require that fuels and fuel additives be registered with U.S. EPA and undergo Tier 1 and Tier 2 testing, as outlined in 40 CFR 79.52-53. This submission evaluates Tier 1 data for E15, that is, gasoline containing 15% ethanol, in support of the registration effort for E15. Exhaust and evaporative emissions data for E0 (the baseline fuel), E10, and E15 were developed by SwRI. The analyses herein consider these 2011 data for E0, E10, and E15 in light of data developed over the last 15 or so years by the Section 211(b) Research Group for baseline gasoline (BG) and baseline gasoline with 10% ethanol (BG+E). The Research Group has already submitted to U.S. EPA the Tier 1 data for exhaust and evaporative emissions characterizations and the literature search for these 1997 fuels, as well as most of the required Tier 2 data[2], namely the health effects testing for baseline gasoline (most of the required tests) and baseline gasoline with 10% ethanol (all required tests).

The following analyses are presented:

- Internal comparison of evaporative emissions profiles for E0, E10, and E15;
- Comparison of evaporative emissions of E0 and BG;
- Comparison of evaporative emissions of E10, E15, and BG+E;
- Comparison of evaporative emissions of E15 to compounds in the atmospheres of animal test chambers during health effects testing of BG+E vapor condensate by the Section 211(b) Research Group;
- Comparison of speciated evaporative emissions of E15 to compounds addressed in the 1997 Tier 1 Literature Search submitted by the Section 211(b) Research Group; and
- Similar analyses for catalyzed and uncatalyzed exhausts of each fuel.

The following sources of information are used in this report:

Southwest Research Institute (1997). *Emissions Characterization of Baseline Gasoline and Gasoline/Oxygenate Blends Under Tier 1 of the CAA 211(b) Fuels and Fuel Additives Registration Regulations*. Prepared for the Section 211(b) Research Group in care of the American Petroleum Institute.[3]

Southwest Research Institute (2011a). *Evaporative Emissions Characterization of E0, E10, and E15 in Support of the Fuel and Fuel Additive Registration of E15*. SwRI Project No. 03.15812. Prepared for Renewable Fuels Association and Growth Energy.

Southwest Research Institute (2011b). *Combustion Emission Characterization of E0, E10, and E15 in Support of the Fuel and Fuel Additive Registration of E15*. SwRI Project No. 03.15812. Prepared for Renewable Fuels Association and Growth Energy.

[2] Tier 2 health effects testing requirements were modified by U.S. EPA in November 1998. The revised program is called Alternative Tier 2 Testing.
[3] API administered the research and testing programs for the Section 211(b) Research Group.

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EA Risk Sciences and Management (1997a). *Final Report: CAA 211(b) Literature Search and Summary Information for Diesel Exhaust, Gasoline Evaporative Emissions, and Gasoline Exhaust*. Prepared for the Section 211(b) Research Group in care of the American Petroleum Institute.

EA Risk Sciences and Management (1997b). *Final Report: CAA 211(b) Summary Information on Diesel Exhaust, Gasoline Evaporative Emissions, and Gasoline Exhaust from In-House Literature of Participating Petroleum Manufacturers and Associations*. Prepared for the American Petroleum Institute.

Huntingdon Life Sciences (2010). *Gasoline Ethanol Vapor Condensate: A 13-Week Whole-Body Inhalation Toxicity Study in Rats with Neurotoxicity Assessments and 4-Week in vivo Genotoxicity and Immunotoxicity Assessments: Appendix S, Analytical Report*. Study Number 00-6127. Submitted to the American Petroleum Institute.

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3. Evaporative emissions profile of E15

Southwest Research Institute used an evaporative emissions generator (40 CFR 79.57) to develop headspace samples of E0, E10, and E15 as described in the accompanying report (SwRI, 2011a). Approximately 30 of more than 200 target compounds were detected in each atmosphere, as shown in Table 1. The evaporative emissions profile for E15 and a comparison of 1997 and 2011 data are the focus of this chapter. Note that all charts presented in this chapter list, on the x-axis, the entire suite of compounds detected in the E0, E10, and/or E15 headspace analyses, in decreasing order of prevalence in E10 emissions; that the y-axis uses a log scale; and that non-detected compounds are arbitrarily shown as 0.001% to accommodate the log scale. Constituents are also listed in tables in order of decreasing prevalence in E10 headspace. Data are the percentage of each compound in total speciated emissions.

3.1. Are the headspace profiles of E0, E10, and E15 reasonably comparable?

The three headspace profiles are highly similar, as shown in Figure 1. The greatest number of compounds, 33, was present in the E0 headspace. With the addition of ethanol to the baseline fuel, only one new compound, ethanol itself, was found in headspace vapor of E10 or E15, compared to E0. Three analytes present at low concentrations in E0 headspace were not detected in E10 headspace: 2,2,3-trimethylpentane, trans-2-butene, and unidentified C_6 compounds. Seven analytes present at low concentrations in E0 headspace were not detected in E15 headspace vapor: 2-methylhexane, 2,3,4-trimethylpentane, decane, isobutylbenzene, 2,4-dimethylhexane, 2,2,3-trimethylpentane, and unidentified C_6. The loss of E0 compounds from the E10 and E15 profiles is presumably due to dilution of vapor by ethanol and the difficulty in measuring these compounds at such small concentrations.

As shown in Table 1 and Figure 1, all components of E15 headspace vapor were present in E10 headspace vapor, with the exception of trans-2-butene, which was inconsistently detected. (This compound was also inconsistently detected in E0 headspace.) The largest difference between the E10 and E15 profiles is the proportion of ethanol. Some trace compounds in E10 vapor were not detected in E15 vapor. Overall, however, the two profiles are highly similar.

3.2. Are the profiles for E0 and BG reasonably comparable?

The 2011 E0 and 1997 BG fuels are both considered "baseline" (reference) fuels for data development, but were blended to meet different specifications. Significant changes to federal regulations since 1997 precipitated the changes in fuel characteristics.

Evaporative emissions of E0 (and E10 and E15) were generated by a headspace method (see SwRI, 2011a report), whereas BG (and BG+E) evaporative emissions were generated using the diurnal heat-build and hot-soak loss sections of the Federal Test Protocol (SwRI 1997 report, submitted to U.S. EPA by the Section 211(b) Research Group). In the 1997 procedure, a vehicle was enclosed in a shed and evaporative emissions collected there. In the 2011 procedure, no

vehicle was used, and evaporated fuel species were collected directly from the evaporative emissions generator. These different methods do not allow comparison of emission rates of specific hydrocarbons. Therefore, assessments will compare proportions of hydrocarbons in the total evaporated material.

Thirty-three compounds were identified in the E0 headspace vapor (as described above), whereas more than 100 compounds were identified in BG evaporative emissions by the Section 211(b) Research Group. The compounds in BG evaporative emissions that also occur in E0, E10, or E15 headspace vapors are listed in Table 2, and the overlap in emissions profiles is shown in Figure 2. Despite the differences in fuels and evaporative emissions methods, the profiles overlap significantly and are reasonably comparable. The suite of compounds common to both E0 and BG evaporative emissions accounts for approximately 85% by mass of the speciated BG evaporative emissions and 96% of the speciated E0 emissions.

Four constituents of E0 headspace vapor were not reported to be present in BG evaporative emissions: propane, propyne, ethane, and 2-methylhexane. These constituents account for less than 5% of E0 emissions. Numerous compounds detected in BG evaporative emissions were not found in E0 headspace vapor: these were largely olefins, branched alkanes, and alkylbenzenes and included such species as benzene, toluene, ethyl benzene, and xylenes (BTEX).

3.3. Are the profiles for E10, E15, and BG+E evaporative emissions reasonably comparable?

As noted above, 2011 and 1997 vapors were generated by different methods using different base fuels. More than 100 constituents of BG+E evaporative emissions were identified by SwRI for the Section 211(b) Research Group, compared to 31 in E10 headspace and 27 in E15 in the current effort. Despite these differences, the evaporative emissions overlap extensively. Table 2 specifies the components of BG+E evaporative emissions that also occur in E0, E10, and/or E15 headspace vapors, and Figure 3 shows the overlap of the E10, E15, and BG+E profiles.

Of the 31 species found in E10 headspace, 27 also occurred in the BG+E evaporative emissions. The remaining species, propane, propyne, ethane, and 2-methylhexane, account for less than 4% of the speciated E10 vapor — these are the same constituents of E0 not present (or, at least, not detected) in BG evaporative emissions. The 27 co-occurring species account for approximately 85% of the BG+E evaporative emissions and 97% of E10 vapor. Ethanol was present at 7.7% in E10 vapor and 7.0% in BG+E emissions

As stated above, all species in E15 headspace were also present in E10 headspace, except for trans-2-butene. (Trans-2-butene was present in E0.) Twenty four of the 27 constituents of E15 evaporative emissions were present in BG+E evaporative emissions, as determined by SwRI in 1997 for the Section 211(b) Research Group. The three exceptions are propane, propyne, and ethane, which collectively account for less than 3% of mass of the E15 evaporative emissions. (These three compounds were absent in evaporative emissions of both BG and BG+E, but again were present in both the E0 and E10 evaporative emissions.) The species occurring in both emissions profiles account for approximately 84% by mass of the BG+E evaporative emissions

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and more than 97% of the E15 evaporative emissions. The balance of the BG+E evaporative emissions comprises numerous compounds, including BTEX, present in very small amounts. As mentioned, the different specifications for the baseline fuels used in 1997 and 2011, and/or the different methods for generating evaporative species, presumably account for the differences in vapor profiles. Ethanol was present at 12.4% in E15 vapor and 7.0% in BG+E.

3.4. Are E15 evaporative emissions reasonably comparable to GEVC used in Tier 2 health effects testing?

GEVC, used for health effects testing by the Section 211(b) Research Group, was generated (in very large quantities) from BG+E using a still and approximates the BG+E evaporative emissions developed during Tier 1. During the subchronic toxicity study in rats, the atmospheres of inhalation chambers were regularly tested for hydrocarbon and ethanol composition, as described in Huntington Life Sciences (2010), Appendix S. The atmospheric composition of GEVC in the animal chambers was not exhaustively determined: only 18 species were reported, accounting for about 98% of the mass. The constituents of GEVC also found in E0, E10, or E15 headspace vapors are listed in Table 2.

E15 evaporative emissions are reasonably comparable to GEVC. Twelve of the 27 constituents of E15 evaporative emissions were detected in GEVC atmospheres during exposures of rodents. These 12 species account for approximately 95% by weight of the E15 emissions and approximately 86% by weight of the GEVC in air. The 15 components of E15 evaporative emissions not sought in GEVC could have been present at no more than 2% of total chamber mass. Five compounds detected in GEVC were not found in E15 evaporative emissions: toluene (3.13%), trans-2-pentene (2.23%), benzene (2.14%), isooctane (1.97%), and 2,3-dimethylpentane (1.56%). The chemical analyses performed in 2011 of E15 (and other) emissions did not include "isooctane." Some isomers of octane did occur in E15 evaporative emissions, such as 2,2,4-trimethylpentane, but at very low concentrations. Ethanol constituted 12.4% of E15 emissions and 8.0% of GEVC. Figure 4 shows the E15 evaporative emissions profile and the overlap with GEVC.

3.5. Were the constituents of E15 evaporative emissions included in the 1997 Tier 1 literature search?

Setting aside generic analytes such as "unidentified C_8 compounds," all but two of the constituents of the E15 evaporative emissions profile were included in the evaporative and/or exhaust portions of the 1997 Tier 1 Literature Search for gasoline. The exceptions are 2,2-dimethylpropane and 2,2-dimethylpentane — however, both were present in trace quantities in the 1997 evaporative emissions. Note that 2,2-dimethylpentane co-elutes with another compound and could not be precisely quantified.

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3.6. Is a Tier 1 literature search for E15 evaporative emissions warranted?

No, it is not. The E15 species included in the 1997 Tier 1 Literature Search (EA Risk Sciences and Management, 1997a, 1997b) account for more than 99% of the weight of E15 evaporative emissions. While 2,2-dimethylpropane and 2,2-dimethylpentane were not explicitly included in the literature search, both compounds were present in the E0 and E10 emission profiles and the BG and BG+E evaporative emissions characterized by SwRI for the Section 211(b) Research Group. The search for the parent emission, "gasoline evaporative," should thus have captured relevant literature. These compounds have been reviewed by other groups, for example, ACGIH (as pentane, all isomers, and as heptane, all isomers.) The enormous literature search submitted by the Section 211(b) Research Group clearly covers, thoroughly, the compounds present in E15 emissions.

3.7. Is 211(b) Alternative Tier 2 health effects testing of evaporative emissions of E15 warranted?

No, it is not. Given the high degree of compositional overlap between E15 headspace vapors and both BG+E evaporative emissions (developed in Tier 1 by the Section 211(b) Research Group) and GEVC (used in Tier 2 health effects testing by the Research Group), and the specific composition of E15 headspace vapors, such a health effects testing program for E15 evaporative emissions is not warranted.

- All components of E15 headspace vapor also occur in E0 and E10 headspace vapors, with the exception of trans-2-butene, which was detected at low levels once in both E0 and E15 headspace but not in E10. As expected, ethanol was not present in E0 vapor.
- While E15 headspace vapors contain a much smaller suite of constituents than does BG+E (and presumably also GEVC, although it was incompletely analyzed), the overlaps in composition are considerable and in each case account for the great majority of the mass of emissions. Trans-2-butene was measured in evaporative emissions of both BG and BG+E.
- E15 vapors are, in essence, a subset of BG+E evaporative emissions, minus known toxic species such as benzene, toluene, ethylbenzene, and xylenes and numerous olefins, branched alkanes, and alkylbenzenes.
- No cancer slope factors are available in Oak Ridge National Laboratory's Risk Assessment Information System (http://rais.ornl.gov/) or the Toxicology for Excellence in Risk Assessment database (http://www.tera.org/ITER/) for the compounds present in E15 evaporative emissions (see Table 1) with the possible exception of commercial hexane, for which a provisional value is given in RAIS. E15 evaporative emissions contain proportionally less hexane than do BG+E evaporative emissions and GEVC.
- Non-cancer toxicity values are available in RAIS for several constituents of E15 evaporative emissions (n-pentane, hexane (n- and commercial), cyclohexane, methylcyclohexane, and methylcyclopentane). With the exception of (n-)pentane, all of these constituents were at least as prominent in BG+E evaporative emissions as in E15 headspace. N-pentane was a larger component of GEVC than of E15 headspace vapors.
- The three compounds detected in E15 evaporative emissions but not BG+E evaporative emissions, ethane, propane, and propyne (allylene; methyl acetylene) are simple gases far

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more likely to be simple asphyxiants than toxic hazards. A literature review found no ready information suggesting a carcinogenic hazard. The literature regarding these gases has been reviewed previously, by ACGIH, for example.

- Differences in ethanol's percentage contribution to the total suite of evaporative emissions for E15, BG+E, and GEVC are smaller than for some other components such as isobutane, isopentane, and butane.
- Health effects of isobutane, isopentane, and butane were recently reviewed by ACGIH as aliphatic hydrocarbon gases (alkanes, C_1 to C_4) and pentane (all isomers). U.S EPA's Office of Pesticide Programs reviewed the toxicology for isobutane in early 2011 (*Fed. Reg. 76(22)*).

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Table 1 Headspace profiles of E0, E10, and E15

Compound	E0 (mass %)	E10 (mass %)	E15 (mass %)
2-METHYLBUTANE (ISOPENTANE)	49.91	53.77	50.90
2-METHYLPROPANE (ISOBUTANE)	18.03	16.80	16.32
ETHANOL	0.00	7.67	12.40
PENTANE	6.45	6.55	6.53
BUTANE	15.15	6.44	6.33
PROPANE	3.32	2.55	2.41
2-METHYLPENTANE	0.95	1.07	0.91
2,3-DIMETHYLBUTANE	0.83	0.89	0.77
2,2,4-TRIMETHYLPENTANE	1.03	0.64	0.45
HEXANE	0.50	0.62	0.49
3-METHYLPENTANE	0.44	0.51	0.43
PROPYNE	0.38	0.42	0.36
2,2-DIMETHYLPROPANE (NEOPENTANE)	0.37	0.38	0.37
2,2-DIMETHYLBUTANE	0.26	0.26	0.25
CYCLOPENTANE	0.24	0.24	0.23
2,4-DIMETHYLPENTANE	0.24	0.18	0.12
CYCLOHEXANE	0.19	0.18	0.06
ETHANE	0.29	0.13	0.15
METHYLCYCLOPENTANE (NOTE A)	0.14	0.13	0.10
2,2-DIMETHYLPENTANE (NOTE A)	0.13	0.11	0.09
UNIDENTIFIED C9-C12+	0.33	0.08	0.10
2-METHYLHEXANE	0.20	0.08	0.00
3-METHYLHEXANE	0.05	0.07	0.04
UNIDENTIFIED C8	0.02	0.05	0.04
METHYLCYCLOHEXANE	0.10	0.04	0.02
UNIDENTIFIED C7	0.04	0.04	0.09
2-METHYLPROPENE (ISOBUTYLENE)	0.02	0.04	0.03
2,3,4-TRIMETHYLPENTANE	0.07	0.01	0.00
DECANE (NOTE B)	0.02	0.01	0.00
ISOBUTYLBENZENE (NOTE B)	0.02	0.01	0.00
2,4-DIMETHYLHEXANE	0.02	0.01	0.00
2,2,3-TRIMETHYLPENTANE	0.01	0.00	0.00
TRANS-2-BUTENE	0.02	0.00	0.01
UNIDENTIFIED C6	0.20	0.00	0.00

Note A: 2,2-Dimethylpentane and methylcyclopentane co-elute. GC peak area was split equally between the two compounds.

Note B: Decane and isobutylbenzene co-elute. GC peak area was split equally between the two compounds.

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Table 2 Constituents of evaporative emissions of BG, BG+E, and GEVC[4] also found in E0, E10, or E15

Compound	BG (mass %)	BG+E (mass %)	GEVC (mass %)
2-METHYLBUTANE (ISOPENTANE)	26.21	18.22	37.24
2-METHYLPROPANE (ISOBUTANE)	1.74	1.15	2.28
ETHANOL	0.00	6.98	7.99
PENTANE	3.67	4.07	7.95
BUTANE	38.99	38.71	11.87
PROPANE	0.00	0.00	Not sought
2-METHYLPENTANE	4.99	5.68	5.74
2,3-DIMETHYLBUTANE	1.73	1.92	1.97
2,2,4-TRIMETHYLPENTANE	0.47	0.50	Not sought
HEXANE	0.81	0.97	3.05
3-METHYLPENTANE	2.41	2.75	3.51
PROPYNE	0.00	0.00	Not sought
2,2-DIMETHYLPROPANE (NEOPENTANE)	0.25	0.18	Not sought
2,2-DIMETHYLBUTANE	1.03	1.12	Not sought
CYCLOPENTANE	0.15	0.16	Not sought
2,4-DIMETHYLPENTANE	0.35	0.44	1.34
CYCLOHEXANE	0.06	0.06	Not sought
ETHANE	0.00	0.00	Not sought
METHYLCYCLOPENTANE (NOTE A)	0.25	0.29	1.57
2,2-DIMETHYLPENTANE (NOTE A)	0.26	0.30	Not sought
UNIDENTIFIED C9-C12+	0.08	0.03	Not sought
2-METHYLHEXANE	0.00	0.00	1.5
3-METHYLHEXANE	0.61	0.75	1.76
UNIDENTIFIED C8	0.03	0.04	Not sought
METHYLCYCLOHEXANE	0.09	0.12	Not sought
UNIDENTIFIED C7	0.08	0.07	Not sought
2-METHYLPROPENE (ISOBUTYLENE)	0.03	0.03	Not sought
2,3,4-TRIMETHYLPENTANE	0.12	0.09	Not sought
DECANE (NOTE B)	0.0004	0.0003	Not sought
ISOBUTYLBENZENE (NOTE B)	0.0004	0.0003	Not sought
2,4-DIMETHYLHEXANE	0.09	0.07	Not sought
2,2,3-TRIMETHYLPENTANE	0.08	0.06	Not sought
TRANS-2-BUTENE	0.16	0.11	Not sought
UNIDENTIFIED C6	0.11	0.12	Not sought

Note A: 2,2-Dimethylpentane and methylcyclopentane co-elute. GC peak area was split equally between the two compounds.

Note B: Decane and isobutylbenzene co-elute. GC peak area was split equally between the two compounds.

[4] Data property of the Section 211(b) Research Group.

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Figure 1 Evaporative emissions profiles of E0, E10, and E15

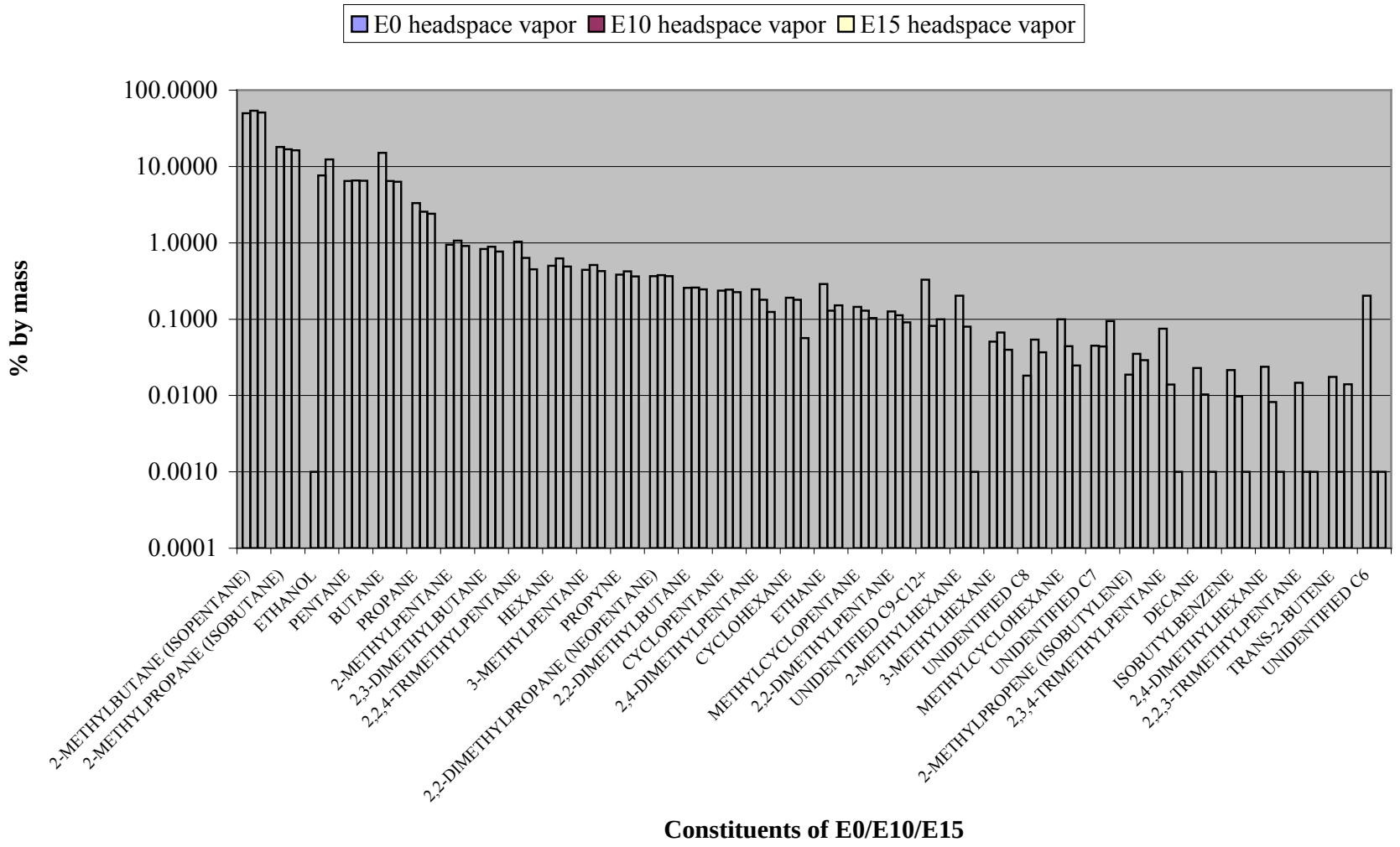

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Figure 2 Evaporative emissions of E0 and BG

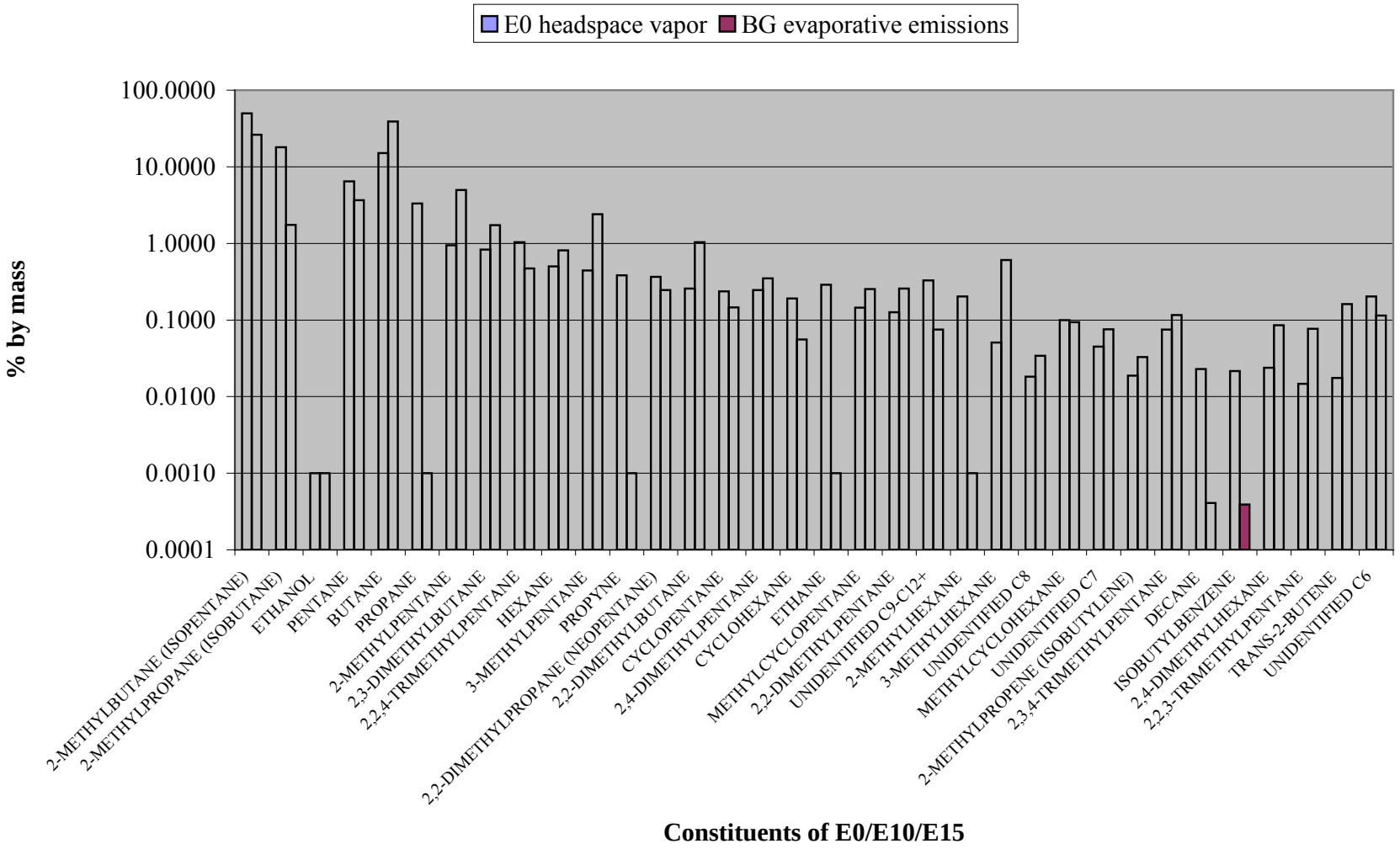

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Figure 3 Evaporative emissions of E10, E15, and BG+E

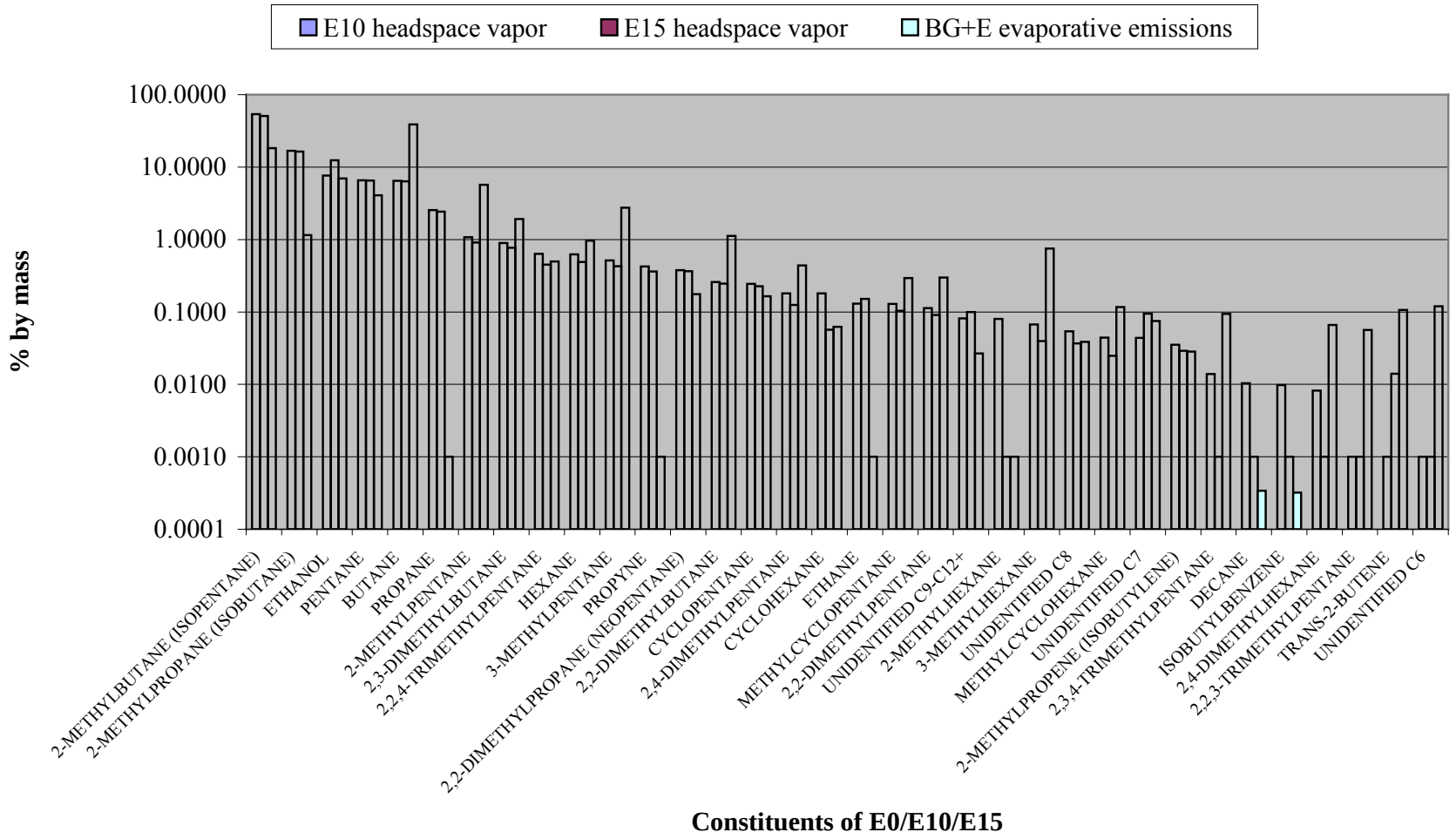

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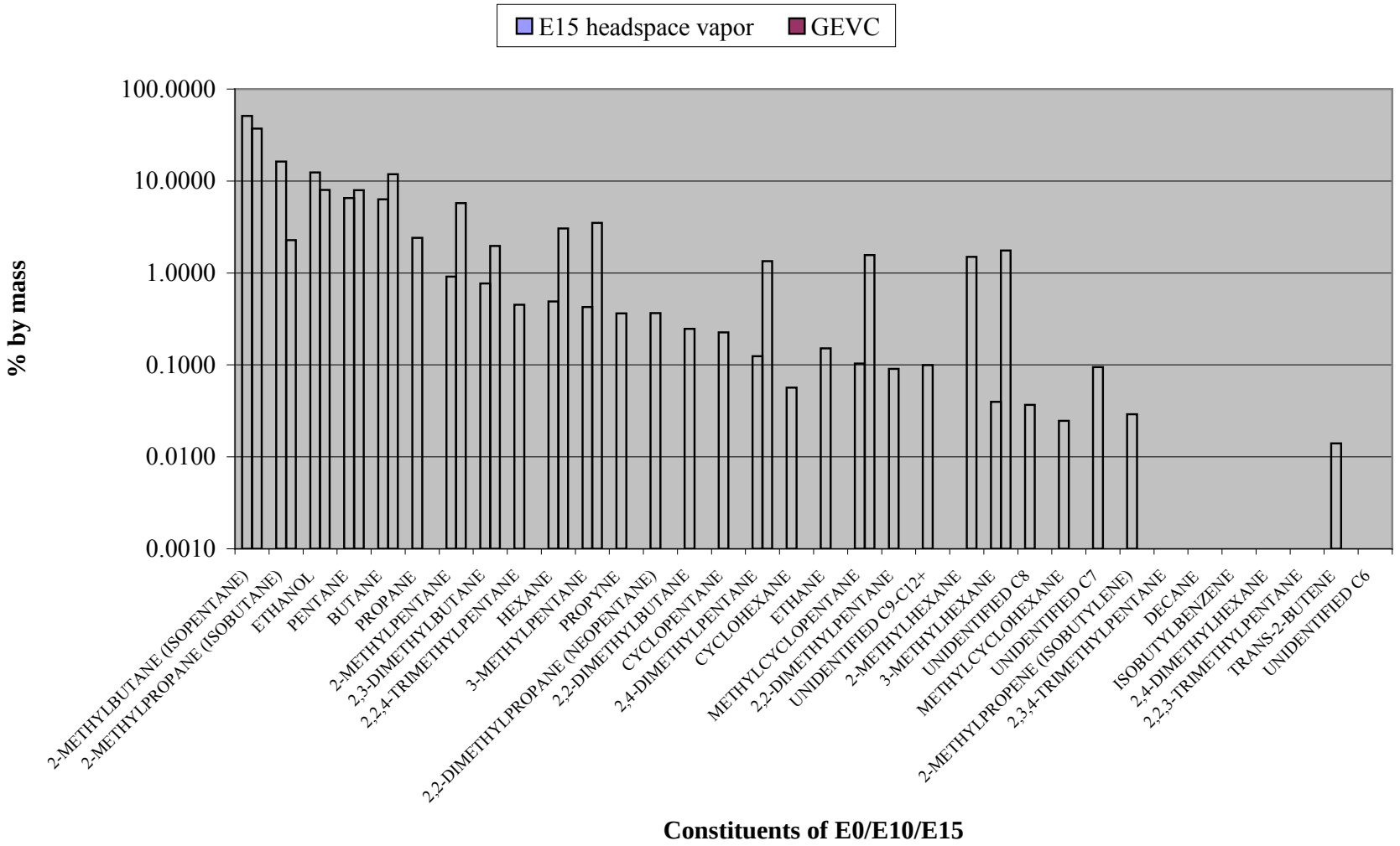
Figure 4 Evaporative emissions of E15 and GEVC



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4. Exhaust emissions profile of E15

Southwest Research Institute developed exhaust emissions profiles for E0, E10, and E15, with and without engine after-treatment, as described in SwRI (2011b). Note that all exhaust protocols were conducted in triplicate. This chapter evaluates the profiles for consistency across these fuel types and also compares them to exhaust emissions profiles developed for the Section 211(b) Research Group by SwRI in 1997 for BG and BG+E (Southwest Research Institute, 1997). In both studies, SwRI provided data on regulated emissions (such as CO), hydrocarbons, alcohols, aldehydes, and ethers (collectively, "hydrocarbons") and polycyclic aromatic hydrocarbons (PAHs) and nitrated PAHs (NPAHs) present in exhaust gases.

4.1. Are the exhaust emissions profiles of E0, E10, and E15 reasonably similar?

4.1.1. Regulated emissions

All emission rates for regulated pollutants in catalyzed fuels were well below standards. Addition of ethanol to baseline fuel generally reduces emission rates of regulated species (see Table 3). Some statistically significant differences (two-tailed, $p<0.05$) are noted between emissions from combustion of E0 and either E10 or E15, and some are noted between E10 and E15. In the latter case, use of E15 increases emission rates of some species when engine after-treatment is in place but generally reduces emission rates when after-treatment is not present.

4.1.2. Speciated hydrocarbon emissions

Many compounds were not detected in any test: these are listed in Table 4. Some additional compounds were not detected in any test using after-treatment: these are listed in Table 5. The remainder of this section addresses exhaust constituents that were present at least once in trace or measurable quantities. In calculations of mean emission rates (for those constituents measured in a fuel's exhaust at least once), both "ND" and "trace" were arbitrarily set to zero. For ease of display, compounds not quantified in any test of a fuel (but detected at least once in other fuel exhausts) were given an emission rate of 0.001 mg/mile. In the Figures, constituents are ordered by prevalence in E10 exhaust.

4.1.2.1. Catalyzed exhaust

Emission rates of hydrocarbons, alcohols, and aldehydes (hereafter called "hydrocarbons") generated in measurable quantities by combustion of E0, E10, and E15 with after-treatment are presented in Table 6 and displayed in Figure 5. Addition of 10% ethanol to the baseline fuel generally reduces emission rates of detected species, whereas an additional 5% ethanol (for a total of 15%) generally leads to emission rates between those generated by E0 and E10 fuels. A few compounds appearing in measurable quantities in E10 and/or E15 exhaust were not detected in E0 exhaust, namely ethanol (as expected), methacrolein (quantified once in E10 exhaust and present in trace quantities in most other tests), 2,2-dimethylpropane (neopentane) (quantified in all E15 exhaust tests and present in trace quantities in all other tests), and methanol (quantified once at low concentration in E15 exhaust and present at trace levels once in E0 exhaust). Approximately one dozen compounds present in either trace or measurable amounts in catalyzed E0 exhaust were not measurably present in either E10 or E15 exhausts.

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Qualitatively, the hydrocarbon profiles of catalyzed E10 and E15 exhaust are highly similar. Compared to catalyzed E10 exhaust, E15 exhaust contained two additional species: methanol and crotonaldehyde. In each case, the compound was never detected in E10 exhaust, detected or quantified once in E15 exhaust, and also detected once in E0 exhaust. Apparent differences between catalyzed E10 and E15 exhausts may reflect analytical challenges in reliably identifying and quantifying trace compounds rather than significant differences in composition. Several compounds detected at trace quantities in E10 exhaust were present at measurable levels in E15 exhaust: 2,2-dimethylpentane, 2,2-dimethylpropane, 2-methylpropane, 3-methylhexane, cis-2-butene, and methylcyclopentane. All of these were measurably present in E0 exhaust, except for 2,2-dimethylpropane which was present only in trace amounts. Finally, the only compounds reliably increasing in concentration with the addition of ethanol were ethanol and acetaldehyde.

4.1.2.2. Uncatalyzed exhaust

Considerably more hydrocarbons were identified in exhausts not passing through a catalytic converter. Emission rates of exhaust hydrocarbons generated by combustion of E0, E10, and E15 without after-treatment are presented in Table 7 and displayed in Figure 6 through Figure 8. Compounds are ordered by emission rate in E10 exhaust. Differences between emission rates for E10 and E15 are generally small.

A few compounds appearing in measurable quantities in E10 and/or E15 exhaust were not detected in E0 exhaust, namely ethanol (as expected), methanol (quantified in all E10 and E15 tests but reported only in trace quantities in E0 exhaust), trans-2-heptene (quantified only once, in E10, and at trace levels in most other exhausts), 1,2,4,5-tetramethylbenzene (identified only once, in an E15 test), methyl isobutyl ketone (identified only in two E15 tests), and tert-1-but-3,5-dimethylbenzene (identified in one E15 test). Nine compounds were measured in uncatalyzed E0 exhaust but not in E10 or E15 exhaust: 1,2-diethylbenzene, 1,3-dimethyl-2-ethylbenzene, 1,4-dimethyl-2-ethylbenzene, 1-methyl-2-n-propylbenzene, 1-trans-2-cis-3-trimethylcyclopentane, 2,4-dimethyloctane, 2-methyl-2-hexene, 3-methyl-1-butene, and propyne.

The profiles of uncatalyzed exhaust hydrocarbons for E10 and E15 are highly similar overall. However, compared to uncatalyzed E10 exhaust, E15 exhaust contained several additional species in minute amounts: 1,2,4,5-tetramethylbenzene, cis-2-octene, methyl isobutyl ketone (MIBK), tert-1-but-3,5-dimethylbenzene, and undecane. These compounds were never detected in E10 exhaust but were quantified once in E15 exhaust (twice, for MIBK). Three of these were never detected in uncatalyzed E0 exhaust: 1,2,4,5-tetramethylbenzene, MIBK, and tert-1-but-3,5-dimethylbenzene. Two additional compounds measured in trace levels in uncatalyzed E10 exhaust were quantified at least once in E15 (and E0) exhaust: cis-3-hexene and hexanaldehyde. Apparent differences between uncatalyzed E0, E10, and E15 exhausts may reflect analytical challenges in reliably identifying and quantifying trace compounds rather than significant differences in composition.

Emitted compounds appearing to increase with ethanol content in the fuel are ethanol, acetaldehyde, methanol, 1,4-diethylbenzene, 3-methyl-1-pentene, methylpropylbenzene, and unidentified C7 compounds.

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4.1.3. Speciated PAH and NPAH

SwRI (2011) analyzed the PAH and NPAH content in both the volatile and particulate phases of catalyzed and uncatalyzed exhausts of E0, E10, and E15. All charts of PAH and NPAH data herein display only measured emission rates, that is, not findings of trace or non-detect.

4.1.3.1. Catalyzed exhaust

The PAH and NPAH data for catalyzed exhausts of E0, E10, and E15 are presented in Table 8 and displayed in Figure 9 to Figure 15. The same set of compounds is detected in exhaust of each fuel type. No NPAHs were detected at measurable levels in any 2011 catalyzed exhaust. The proportions of the two PAH phases are roughly similar across fuel type for each chemical. Total emissions of the different species are generally higher for E15 than for E10, but may be higher or lower than for E0.

4.1.3.2. Uncatalyzed exhaust

The PAH and NPAH data for uncatalyzed exhausts of E0, E10, and E15 are presented in Table 9 and displayed in Figure 16 to Figure 24. The same set of compounds is detected in uncatalyzed exhaust of each fuel, including two NPAHs. 2-Nitrofluorene was generally found in the volatile phase, and 1-nitropyrene in the particulate phase.

In almost every case, compounds are found primarily in the particulate phase of exhaust. Total emission rates of specific PAHs are lower in E15 exhaust than in E10 exhaust; emission rates are comparable for PAHs in E15 and E0 exhaust. In the case of NPAHs, emission rates are similar for all three fuels.

4.2. Are the exhaust emissions profiles of E0 and BG reasonably comparable?

4.2.1. Speciated hydrocarbon emissions

Hydrocarbons in the exhaust of BG were speciated by SwRI for the 211(b) Research Group (SwRI, 1997). The emission profile is here compared to that for E0 characterized in 2011.

4.2.1.1. Catalyzed exhaust

Emission rates of hydrocarbons from engines using E0 (2011 tests) or BG (1997 tests) are dramatically different, with total speciated emissions in 2011 emitted at approximately one-tenth of the 1997 rate. In addition, far fewer compounds were detected in catalyzed E0 exhaust, compared to catalyzed BG exhaust. Figure 25 shows, for both fuels, the emission rates of species measured in catalyzed E0 exhaust (the many other compounds only quantified in 1997 are not displayed). These species account for about 63% of speciated BG hydrocarbons. Three compounds were emitted at higher rates in catalyzed E0 exhaust than in BG exhaust: cis-2-butene, 2-methylhexane, and 2-methylpropane. Four compounds detected only at trace levels in E0 exhaust (not displayed in Figure 25) were either not detected or not sought in BG exhaust: 1-cis,2-trans,3-trimethylcyclopentane, 3-methyl-1-butene, methanol, and methacrolein.

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4.2.1.2. Uncatalyzed exhaust

Total speciated hydrocarbons in uncatalyzed 2011 E0 exhaust were emitted at about 60% of the rate for BG exhaust. Approximately 25 compounds in BG exhaust were not detected in E0 exhaust, while 20 compounds measured in E0 exhaust were either not detected or, in the case of methacrolein, not sought in BG exhaust. Eight compounds not detected in BG exhaust were emitted at a rate of at least 1.0 mg/mi in E0 exhaust: 2-methylhexane, styrene, 1,2,3-trimethylbenzene, 1-methyl-2-isopropylbenzene, 3-methyl-1-butene, 3-methyl-1-pentene, 2-butyne, and 1-methylcyclopentene. Emission rates of hydrocarbons detected in uncatalyzed E0 exhaust are displayed for both fuels in Figure 26 through Figure 28. (Compounds only detected in BG exhaust are not shown.) The final four compounds shown in Figure 28 were found only in trace amounts in E0 exhaust.

4.2.2. Speciated PAHs and NPAHs

Rates of emission of PAHs and NPAHs from exhausts of 1997 BG (and BG+E) were determined by SwRI for the 211(b) Research Group (SwRI, 1997) and are presented in Table 10 and Table 11.

4.2.2.1. Catalyzed exhaust

Figure 9 to Figure 15 display detected PAHs and NPAHs in the volatile and particulate phases of catalyzed E0 and BG exhaust. All compounds detected in E0 exhaust, including those reported only at trace levels, were also detected in BG exhaust. One NPAH, 1-nitropyrene, was present in measurable quantity in BG exhaust but not in E0 exhaust. PAHs were emitted at significantly lower rates in catalyzed E0 exhaust, approximately one-tenth the rate from catalyzed BG exhaust.

4.2.2.2. Uncatalyzed exhaust

Figure 16 to Figure 24 display detected PAHs and NPAHs in the volatile and particulate phases of uncatalyzed E0 and BG exhaust. 2-Nitrofluorene was quantified in the volatile phase of E0 exhaust at low levels but was reported only as a trace analyte in BG exhaust. The compound was measured in the particulate phase of both exhausts. Otherwise, all compounds measured in E0 exhaust were also reported in BG exhaust. NPAHs were only found, at most, at low levels in exhaust; PAH levels were much reduced in uncatalyzed E0 exhaust compared to BG exhaust.

4.3. Are the exhaust emissions profiles of E10, E15, and BG+E reasonably comparable?

4.3.1. Speciated hydrocarbon emissions

4.3.1.1. Catalyzed exhaust

Figure 29 shows emission rates of all species detected in E10 and/or E15 catalyzed exhaust compared to 1997 BG+E. BG+E species not detected in 2011 exhausts are not shown. The modern exhausts contain significantly fewer chemicals in significantly lesser quantities. The shared species account for approximately 57% of speciated BG+E hydrocarbons. Several of the compounds emitted at the greatest rates from catalyzed BG+E exhaust are not detected in E10 or E15 exhausts, including xylenes, ethylbenzene, 1,2,4-trimethylbenzene, and unidentified C9-12+ compounds. In general, compounds in E15 exhaust are emitted at slightly higher rates than in E10 exhaust but at considerably lower rates than in BG+E exhaust. One compound, 2,2-

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dimethylpropane, is emitted at higher rates from E15 exhaust than from BG+E exhaust. 2-Methylhexane, measured in both E10 and E15 exhaust, was not detected in BG+E exhaust. Methacrolein was not sought in BG+E exhaust.

4.3.1.2. Uncatalyzed exhaust

Emission rates for all compounds detected in uncatalyzed E10 or E15 exhaust are shown for all three exhaust types in Figure 30 to Figure 32. E10 and E15 exhaust are more similar to each other than to BG+E exhaust, containing fewer species and demonstrating lesser total emission rates overall. In general, specific hydrocarbons are emitted at the highest rate in uncatalyzed BG+E exhaust. Sixteen compounds not detected in BG+E exhaust were found in E10 exhaust, fifteen of which were also detected in E15 exhaust. Such compounds emitted at a rate of at least 1.0 mg/mi from E10 or E15 exhaust included 2-methylhexane, methanol, styrene, 1,2,3-trimethylbenzene, 1,3-diethylbenzene, 3-methyl-1-pentene, and 2-butyne. More than 30 other compounds were measured in BG+E exhaust but not in E10 or E15 exhaust.

4.3.2. Speciated PAHs and NPAHs

4.3.2.1. Catalyzed exhaust

No NPAHs were found in measurable amounts in exhaust from E10, E15, or BG+E, with the exception of a low amount of 2-nitropyrene in the particulate phase of BG+E (Table 8 and Table 10). Several NPAHs were reported at trace levels in BG+E but not detected in E10 or E15 exhaust. For those species measured, emission rates for E10 and E15 were comparable, and generally much lower than for BG+E (Figure 9 through Figure 15).

4.3.2.2. Uncatalyzed exhaust

With the possible exceptions of 2-nitrofluorene and 1-nitropyrene, all PAHs and NPAHs found in uncatalyzed BG+E exhaust were emitted at much lower rates in E10 and E15 exhaust (see Table 9 and Table 11, as well as Figure 16 through Figure 24). 2-Nitrofluorene was measured in the volatile phase of E10 and E15 exhaust but reported only as "trace" in BG+E exhaust in 1997. 1-Nitropyrene was emitted at comparable rates in all exhausts. NPAHs were generally found in very low quantities, if at all. PAHs were emitted from E15 at about half the rate for E10.

The emission rate of 2-nitrofluorene was not increased by the addition of ethanol to either the 2011 or 1997 baseline fuels. Appearance of this species in measurable amounts in the volatile fraction of all uncatalyzed 2011 exhausts may be due to differences in baseline fuels and/or lubricating oils, compared to those used in 1997.

4.4. Were the constituents of E15 exhaust emissions included in the 1997 Tier 1 Literature search?

Most emitted species in catalyzed E15 exhaust were included in the 1997 Tier 1 literature search. All but one of the eight compounds not covered in the search — methanol — were present in catalyzed E10 exhaust. Methanol was detected only once, at low concentration, in catalyzed E15 exhaust.

All compounds found in catalyzed E15 exhaust were also found in uncatalyzed E15 exhaust (as well as in uncatalyzed E10 exhaust). Of the additional compounds found in uncatalyzed E15

exhaust, thirty were not included in the 1997 Tier 1 literature search — however, structurally similar compounds were covered in most cases. Twenty-five of the 30 were present in uncatalyzed E10 exhaust: the exceptions were 1,2,4,5-tetramethylbenzene, cis-2-octene, methyl isobutyl ketone, tert-1-but-3,5-dimethylbenzene, and 1,1,3-trimethylcyclopentane. In addition, of these 30 compounds, 23 were previously detected in uncatalyzed BG+E exhaust. Six compounds were not detected at that time (2,2,4-trimethyl-2-pentene, 2-butyne, 3,4-dimethyl-1-pentene, cis-1,2-dimethylcyclohexane, cis-2-octene, and isopropylcyclopentane), and one was not sought (methyl isobutyl ketone). Therefore, only cis-2-octene and possibly methyl isobutyl ketone were found in uncatalyzed E15 exhaust but not uncatalyzed E10 or BG+E exhaust. A compound structurally similar to cis-2-octene was addressed in the 1997 literature search.

4.5. Is further Tier 1 or Tier 2 data collection for combustion emissions of E15 warranted?

No it is not. First, combustion emissions of E15 are highly similar to E10 emissions: no new species were consistently identified in E15 compared to E10, an approved fuel. Second, E15 exhaust emissions are reasonably comparable to the BG+E emissions characterized by the Section 211(b) Research Group. All hydrocarbon components of catalyzed E15 exhaust were detected in 1997 catalyzed BG+E exhaust with the exceptions of 2-methylhexane (also present in E10 exhaust) and methanol. (One component, methacrolein, was not sought in the 1997 exhaust.) Uncatalyzed E15 exhaust contained 15 hydrocarbon compounds not detected in BG+E exhaust. All of them were found in uncatalyzed E10 exhaust, however. Uncatalyzed E15 exhaust (as well as E10 and E0 exhausts) contained measurable 2-nitrofluorene, whereas this compound was present at only trace levels in BG+E exhaust.

The great majority of species in catalyzed E15 exhaust were included in the 1997 Tier 1 literature search. About 75% of the species in uncatalyzed E15 exhaust were included in the 1997 Tier 1 literature search or exempted by regulation. Structurally similar compounds were addressed for many of the remaining 25%. Differences between exhaust profiles, from 1997 and 2011 fuels, are likely due to changes in baseline gasoline composition, improvements in analytical sensitivity, and perhaps other factors such as engine oils.

E15 emissions are reasonably comparable to those of E10 and BG+E, approved fuels. The results of the Section 211(b) Research Group program should be sufficient for E15.

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Table 3 Emission rates of regulated species in E0, E10, and E15 exhausts

	Emission rate (g/mi)					
	THC	NMHC	CO	NO_x	PM	CO_2
2011 Baseline (E0), catalyst	0.031(0.006)[a]	0.027(0.006)	0.086(0.009)	0.024(0.003)	0.0013(0.001)	334.67(3.0082)
2011 E10, catalyst	0.016(0.003)*	0.013(0.004)*	0.064(0.009)*	0.022(0.004)	0.0010(0.0003)	334.34(2.72)
2011 E15, catalyst	0.020(0.004)	0.015(0.002)*	0.089(0.011)#	0.027(0.005)	0.0039(0.0010)*#	333.85(2.27)
2011 Baseline (E0), no catalyst	1.483(0.044)	1.434(0.043)	11.382(1.229)	3.774(0.006)	0.0031(0.0004)	317.38(2.89)
2011 E10, no catalyst	1.405(0.022)	1.355(0.023)*	13.048(0.844)	3.753(0.059)	0.0021(0.000)*	320.77(1.444)
2011 E15, no catalyst	1.327(0.014)*#	1.283(0.015)*#	8.553(0.188)*#	3.975(0.078)*#	0.0021(0.000)*	313.05(1.364)#

[a] Mean(standard deviation)

* Differs significantly from the E0 emission rate (t-test, two-tailed, 0.05)

\# E15 emission rate differs significantly from the E10 emission rate (t-test, two-tailed, 0.05)

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Table 4 Compounds never detected in 2011 catalyzed or uncatalyzed exhaust tests

1,1,2-TRIMETHYLCYCLOPENTANE	3-METHYL-TRANS-3-HEXENE
1,1,3-TRIMETHYLCYCLOHEXANE	4,4-DIMETHYLHEPTANE
1,1-DIMETHYLCYCLOHEXANE	4-ETHYLHEPTANE
1,1-DIMETHYLCYCLOPENTANE	4-METHYL-1-PENTENE
1,2,3,4-TETRAMETHYLBENZENE	4-METHYL-CIS-2-PENTENE
1,2,3,5-TETRAMETHYLBENZENE	4-METHYLHEPTANE
1,2,4-TRIETHYLBENZENE	4-METHYLOCTANE
1,2-DIMETHYL-3-ETHYLBENZENE	CIS-1,2-DIMETHYLCYCLOPENTANE
1,3,5-TRIETHYLBENZENE	CIS-1,3-DIMETHYLCYCLOHEXANE
1,3,-DIMETHYL-5-ETHYLBENZENE	CIS-3-HEPTENE
1,3-DIMETHYL-4-ETHYLBENZENE	CIS-3-NONENE
1-BUTYNE	DI-ISOPROPYL ETHER
1-DECENE	DODECANE
1-HEPTENE	ETBE
1-METHYL-3-N-PROPYLBENZENE	ETHYLCYCLOPENTANE
1-METHYL-4-ISOPROPYLBENZENE	HEXYLBENZENE
1-METHYL-4-N-PROPYLBENZENE	INDAN
1-OCTENE	MTBE
2,2,4-TRIMETHYLHEXANE	NAPHTHALENE
2,2-DIMETHYLHEPTANE	N-PENT-BENZENE
2,3,4-TRIMETHYLHEXANE	PROPADIENE
2,3-DIMETHYL-2-PENTENE	TERT-1-BUT-2-METHYLBENZENE
2,3-DIMETHYLHEPTANE	TERT-1-BUTYL-4-ETHYLBENZENE
2,4,4-TRIMETHYLHEXANE	TERT-AMYL METHYL ETHER
2,5-DIMETHYLHEXANE	TERT-BUTANOL
2,6-DIMETHYLHEPTANE	TERT-BUTYLBENZENE
2-METHYLBUTYLBENZENE (sec AMYLBENZENE)	TRANS-1,2-DIMETHYLCYCLOPENTANE
3,3-DIMETHYLHEPTANE	TRANS-1-METHYL-2-ETHYLCYCLOPENTANE
3,3-DIMETHYLHEXANE	TRANS-1-METHYL-3-ETHYLCYCLOPENTANE
3,4 DIMETHYLCUMENE	TRANS-2-NONENE
3,4-DIMETHYLHEPTANE	TRANS-3-HEPTENE
3-ETHYL-CIS-2-PENTENE	TRANS-3-HEXENE
3-ETHYLHEXANE	TRANS-3-NONENE
3-METHYL-1-HEXENE	TRANS-4-OCTENE
3-METHYLCYCLOPENTENE	UNIDENTIFIED C5 OLEFINS

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Table 5 Additional compounds never detected in 2011 catalyzed exhaust

1,1,3-TRIMETHYLCYCLOPENTANE	3,4-DIMETHYLHEXANE
1,2 DIETHYLBENZENE	3,5-DIMETHYLHEPTANE
1,2,3-TRIMETHYLBENZENE	3-METHYL-1-PENTENE
1,2,4,5-TETRAMETHYLBENZENE	3-METHYL-CIS-2-PENTENE
1,2-DIMETHYL-4-ETHYLBENZENE	3-METHYLHEPTANE
1,3,5-TRIMETHYLBENZENE	3-METHYLOCTANE
1,3-DIETHYLBENZENE	3-METHYL-TRANS-2-PENTENE
1,3-DIMETHYL-2-ETHYLBENZENE	4-METHYL-TRANS-2-PENTENE
1,4-DIETHYLBENZENE	CIS-1,2-DIMETHYLCYCLOHEXANE
1,4-DIMETHYL-2-ETHYLBENZENE	CIS-1,3-DIMETHYLCYCLOPENTANE
1-BUTENE	CIS-1-METHYL-2-ETHYLCYCLOPENTANE
1-HEXENE	CIS-1-METHYL-3-ETHYLCYCLOPENTANE
1-METHYL-1-ETHYL-CYCLOPENTANE	CIS-2-HEPTENE
1-METHYL-2-ETHYLBENZENE	CIS-2-HEXENE
1-METHYL-2-ISOPROPYLBENZENE	CIS-2-OCTENE
1-METHYL-2-N-PROPYLBENZENE	CIS-2-PENTENE
1-METHYL-3-ETHYLBENZENE	CIS-3-HEXENE
1-METHYL-3-ISOPROPYLBENZENE	CYCLOHEXENE
1-METHYL-4-ETHYLBENZENE	CYCLOPENTENE
1-METHYLCYCLOPENTENE	DECANE
1-NONENE	DIMETHYLBENZALDEHYDE
1-PENTENE	ETHYLCYCLOHEXANE
1-TRANS-2-CIS-3-TRIMETHYLCYCLOPENTANE	HEXANALDEHYDE
1-TRANS-2-CIS-4-TRIMETHYLCYCLOPENTANE	ISOBUTYLBENZENE
2,2,3-TRIMETHYLBUTANE	ISOPROPYLBENZENE (CUMENE)
2,2,3-TRIMETHYLPENTANE	ISOPROPYLCYCLOPENTANE
2,2-DIMETHYLHEXANE	m-& p-XYLENE
2,2-DIMETHYLOCTANE	METHYL ETHYL KETONE
2,3,5-TRIMETHYLHEXANE	METHYL ISOBUTYL KETONE
2,3-DIMETHYLHEXANE	METHYLPROPYLBENZENE (sec butylbenzene)
2,3-DIMETHYLPENTANE	NONANE
2,4,4-TRIMETHYL-1-PENTENE	n-PROPYLBENZENE
2,4,4-TRIMETHYL-2-PENTENE	OCTANE
2,4-DIMETHYLHEPTANE	o-XYLENE
2,4-DIMETHYLOCTANE	STYRENE
2,5-DIMETHYLHEPTANE	TERT-1-BUT-3,5-DIMETHYLBENZENE
2-BUTYNE	TRANS-1,2-DIMETHYLCYCLOHEXANE
2-METHYL-1,3-BUTADIENE	TRANS-1,3-DIMETHYLCYCLOHEXANE
2-METHYL-1-BUTENE	TRANS-1,3-DIMETHYLCYCLOPENTANE
2-METHYL-1-HEXENE	TRANS-1,4-DIMETHYLCYCLOHEXANE
2-METHYL-1-PENTENE	TRANS-2-HEPTENE
2-METHYL-2-HEXENE	TRANS-2-HEXENE
2-METHYL-2-PENTENE	TRANS-2-OCTENE
2-METHYLOCTANE	UNDECANE
3,3-DIMETHYL-1-BUTENE	UNIDENTIFIED C6
3,3-DIMETHYLPENTANE	UNIDENTIFIED C7
3,4-DIMETHYL-1-PENTENE	UNIDENTIFIED C8

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Table 6 Emission rates of hydrocarbons in catalyzed emissions

Compound	E0CAT (mg/mile)	E10CAT (mg/mile)	E15CAT (mg/mile)
METHANE	4.2E+00	3.6E+00	5.0E+00
TOLUENE	4.4E+00	1.7E+00	2.4E+00
2,2,4-TRIMETHYLPENTANE	2.3E+00	1.1E+00	1.7E+00
2-METHYLBUTANE (ISOPENTANE)	2.0E+00	1.1E+00	3.3E-02
ETHYLENE	2.2E+00	9.4E-01	1.2E+00
BENZENE	1.3E+00	6.3E-01	8.4E-01
PROPYLENE	1.7E+00	6.0E-01	6.9E-01
ETHANE	9.E-01	5.E-01	6.E-01
2-METHYLPROPENE (ISOBUTYLENE)	2.E-01	4.E-01	4.E-01
2,3,4-TRIMETHYLPENTANE	6.E-01	3.E-01	6.E-01
2,3,3-TRIMETHYLPENTANE	5.E-01	3.E-01	4.E-01
ACETYLENE	8.E-01	3.E-01	4.E-01
ACETALDEHYDE	1.E-01	2.E-01	4.E-01
PENTANE	5.E-01	2.E-01	6.E-01
2,4-DIMETHYLHEXANE	5.E-01	2.E-01	3.E-01
FORMALDEHYDE	3.E-01	2.E-01	2.E-01
HEXANE	2.E-01	1.E-01	2.E-01
2-METHYL-2-BUTENE	1.E-01	1.E-01	7.E-02
2-METHYLPENTANE	4.E-01	1.E-01	2.E-01
2-METHYLHEXANE	3.E-01	1.E-01	3.E-01
2,3-DIMETHYLBUTANE	2.E-01	9.E-02	1.E-01
2,4-DIMETHYLPENTANE	2.E-01	8.E-02	1.E-01
3-METHYLPENTANE	2.E-01	8.E-02	3.E-01
2,2,5-TRIMETHYLHEXANE	2.E-01	7.E-02	2.E-01
BENZALDEHYDE	8.E-02	7.E-02	6.E-02
BUTANE	1.E-01	7.E-02	7.E-02
CYCLOHEXANE	1.E-01	7.E-02	6.E-02
ETHANOL	1.E-03	7.E-02	6.E-01
PROPANE	1.E-01	7.E-02	1.E-01
ACETONE	7.E-02	7.E-02	3.E-02
METHYLCYCLOHEXANE	1.E-01	7.E-02	9.E-02
1,3-BUTADIENE	3.E-01	3.E-02	7.E-02
HEPTANE	7.E-02	3.E-02	5.E-01
METHACROLEIN	1.E-03	3.E-02	1.E-03
TRANS-2-BUTENE	1.E-01	3.E-02	7.E-02
1,2,4-TRIMETHYLBENZENE	8.E-02	1.E-03	1.E-03
2,2-DIMETHYLBUTANE	7.E-02	1.E-03	1.E-03
2,2-DIMETHYLPENTANE (NOTE A)	7.E-02	1.E-03	7.E-02
2,2-DIMETHYLPROPANE (NEOPENTANE)	1.E-03	1.E-03	1.E+00
2-METHYLHEPTANE	2.E-01	1.E-03	1.E-03

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Compound	E0CAT (mg/mile)	E10CAT (mg/mile)	E15CAT (mg/mile)
2-METHYLPROPANE (ISOBUTANE)	2.E-01	1.E-03	7.E-02
3-METHYLHEXANE	7.E-02	1.E-03	7.E-02
CIS-2-BUTENE	1.E+00	1.E-03	3.E-02
CYCLOPENTADIENE	9.E-02	1.E-03	1.E-03
ETHYLBENZENE	2.E-01	1.E-03	1.E-03
METHANOL	1.E-03	1.E-03	3.E-02
METHYLCYCLOPENTANE (NOTE A)	7.E-02	1.E-03	7.E-02
TRANS-2-PENTENE	3.E-02	1.E-03	1.E-03

Compounds in order of E10CAT prevalence. "1.E-03" indicates trace and/or non-detect.

Note A: 2,2-Dimethylpentane and methylcyclopentane co-elute. GC peak area was split equally between the two compounds.

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Table 7 Emission rates of hydrocarbons in uncatalyzed emissions

Compound	E0 (mg/mile)	E10 (mg/mile)	E15 (mg/mile)
TOLUENE	2.23E+02	2.26E+02	1.92E+02
2,2,4-TRIMETHYLPENTANE	9.17E+01	9.55E+01	8.34E+01
ETHYLENE	9.66E+01	9.01E+01	8.80E+01
2,2-DIMETHYLPROPANE	9.0E+01	7.9E+01	6.5E+01
PROPYLENE	8.42E+01	7.16E+01	6.75E+01
ETHANOL	1.0E-03	6.4E+01	7.3E+01
1,2,4-TRIMETHYLBENZENE	5.8E+01	6.3E+01	6.1E+01
ACETYLENE	6.43E+01	6.26E+01	5.38E+01
2-METHYLPROPENE	5.73E+01	4.93E+01	4.62E+01
FORMALDEHYDE	5.20E+01	4.42E+01	5.64E+01
1-METHYL-3-ETHYLBENZENE	4.2E+01	4.3E+01	3.9E+01
METHANE	4.80E+01	4.32E+01	4.11E+01
BENZENE	4.09E+01	4.23E+01	3.54E+01
2,3,4-TRIMETHYLPENTANE	3.06E+01	3.19E+01	2.82E+01
UNIDENTIFIED C9-C12+	2.1E+01	2.2E+01	2.1E+01
1,3,5-TRIMETHYLBENZENE	2.1E+01	2.1E+01	1.9E+01
2,3,3-TRIMETHYLPENTANE	1.48E+01	2.08E+01	1.74E+01
PENTANE	1.95E+01	2.01E+01	1.59E+01
ACETALDEHYDE	9.99E+00	1.90E+01	2.85E+01
1-METHYL-2-ETHYLBENZENE	1.8E+01	1.9E+01	1.6E+01
1-METHYL-4-ETHYLBENZENE	1.8E+01	1.9E+01	1.7E+01
m-& p-XYLENE	1.6E+01	1.6E+01	1.4E+01
ETHANE	1.58E+01	1.42E+01	1.27E+01
ETHYLBENZENE	1.4E+01	1.4E+01	1.2E+01
BENZALDEHYDE	1.7E+01	1.3E+01	1.7E+01
2,2,5-TRIMETHYLHEXANE	1.2E+01	1.3E+01	1.1E+01
o-XYLENE	1.2E+01	1.2E+01	1.1E+01
n-PROPYLBENZENE	1.2E+01	1.2E+01	1.1E+01
2,4-DIMETHYLHEXANE	1.11E+01	1.15E+01	1.12E+01
2,2,3-TRIMETHYLPENTANE	1.1E+01	1.1E+01	8.8E+00
2-METHYLPENTANE	9.1E+00	9.8E+00	8.3E+00
1,3-BUTADIENE	1.1E+01	9.6E+00	9.7E+00
HEXANE	8.4E+00	8.6E+00	7.3E+00
2-METHYLHEXANE	8.0E+00	8.5E+00	7.4E+00
METHANOL	1.0E-03	8.1E+00	1.1E+01
1-METHYL-3-ISOPROPYLBENZENE	5.6E+00	7.7E+00	4.1E+00
2,3-DIMETHYLBUTANE	7.1E+00	7.5E+00	6.4E+00
2-METHYLBUTANE	8.37E+00	7.24E+00	7.09E+00

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Compound	E0 (mg/mile)	E10 (mg/mile)	E15 (mg/mile)
1-BUTENE	8.7E+00	7.1E+00	7.0E+00
2,4-DIMETHYLPENTANE	5.8E+00	6.9E+00	6.0E+00
TRANS-2-BUTENE	7.7E+00	6.5E+00	6.1E+00
2-METHYL-1-BUTENE	6.5E+00	6.2E+00	5.0E+00
ACETONE	6.2E+00	6.1E+00	5.3E+00
METHYLCYCLOHEXANE	5.9E+00	6.1E+00	5.2E+00
STYRENE	5.2E+00	6.0E+00	5.9E+00
2-METHYL-2-BUTENE	6.0E+00	5.8E+00	5.3E+00
3-METHYLPENTANE	5.7E+00	5.7E+00	4.9E+00
2-METHYLPROPANE	6.2E+00	5.5E+00	5.6E+00
HEPTANE	3.6E+00	5.5E+00	3.3E+00
M/P-TOLUALDEHYDE	4.9E+00	5.4E+00	5.4E+00
CIS-2-BUTENE	5.4E+00	4.6E+00	4.3E+00
1,2,3-TRIMETHYLBENZENE	4.0E+00	4.5E+00	3.8E+00
2,3-DIMETHYLHEXANE	1.4E+01	4.5E+00	1.0E-03
TRANS-2-PENTENE	3.9E+00	4.1E+00	3.9E+00
DIMETHYLBENZALDEHYDE	4.9E+00	4.0E+00	4.4E+00
CYCLOHEXANE	3.6E+00	4.0E+00	3.1E+00
BUTANE	9.6E+00	3.7E+00	2.9E+00
1,4-DIETHYLBENZENE	3.1E+00	3.6E+00	4.5E+00
UNIDENTIFIED C6	3.3E+00	3.3E+00	2.9E+00
PROPIONALDEHYDE	4.4E+00	3.2E+00	3.6E+00
1,3-DIETHYLBENZENE	6.0E-01	3.2E+00	3.1E+00
1-PENTENE	4.1E-01	2.8E+00	1.6E+00
3-METHYLHEXANE	2.5E+00	2.7E+00	2.3E+00
ACROLEIN	3.9E+00	2.7E+00	4.5E+00
METHACROLEIN	3.3E+00	2.6E+00	4.1E+00
2,2-DIMETHYLPENTANE (NOTE A)	2.5E+00	2.6E+00	2.3E+00
METHYLCYCLOPENTANE (NOTE A)	2.5E+00	2.5E+00	2.2E+00
3-METHYL-1-PENTENE	2.0E+00	2.1E+00	2.6E+00
2,2-DIMETHYLBUTANE	1.9E+00	2.0E+00	1.8E+00
2,3,5-TRIMETHYLHEXANE	1.9E+00	1.9E+00	1.7E+00
ISOPROPYLBENZENE	2.5E+00	1.9E+00	5.7E-01
CYCLOPENTANE	1.9E+00	1.8E+00	1.7E+00
UNIDENTIFIED C7	1.6E+00	1.7E+00	2.6E+00
3,4-DIMETHYLHEXANE (NOTE B)	1.6E+00	1.7E+00	1.3E+00
2-METHYL-1,3-BUTADIENE	1.4E+00	1.6E+00	1.3E+00
PROPANE	1.5E+00	1.5E+00	1.1E+00
2-BUTYNE	1.6E+00	1.4E+00	6.6E-01
O-TOLUALDEHYDE	2.0E+00	1.4E+00	1.4E+00

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Compound	E0 (mg/mile)	E10 (mg/mile)	E15 (mg/mile)
1,2-DIMETHYL-4-ETHYLBENZENE	1.8E+00	1.3E+00	2.1E+00
METHYLPROPYLBENZENE	3.9E-01	1.3E+00	3.5E+00
OCTANE	1.1E+00	1.2E+00	1.0E+00
2-METHYLHEPTANE	1.0E+00	1.1E+00	1.0E+00
NONANE	1.0E+00	1.1E+00	9.9E-01
3,3-DIMETHYL-1-BUTENE	7.6E-01	9.4E-01	7.5E-01
1-NONENE	9.1E-01	9.3E-01	8.6E-01
CYCLOHEXENE	9.0E-01	9.3E-01	8.9E-01
CIS-2-PENTENE	7.5E-01	9.3E-01	7.0E-01
3-METHYLHEPTANE	8.1E-01	9.0E-01	7.1E-01
N-/ISOBUTYRALDEHYDE	1.0E+00	8.4E-01	9.7E-01
1-METHYL-1-ETHYL-CYCLOPENTANE	9.4E-01	7.9E-01	6.7E-01
CROTONALDEHYDE	3.0E+00	7.8E-01	2.4E+00
1-CIS,2-TRANS,3-TRIMETHYLCYCLOPENTANE	6.9E-01	7.7E-01	4.8E-01
CYCLOPENTENE	7.5E-01	7.4E-01	6.6E-01
2-METHYL-2-PENTENE	8.0E-01	7.1E-01	7.8E-01
TRANS-1,3-DIMETHYLCYCLOPENTANE	6.2E-01	7.0E-01	5.7E-01
3-ETHYLPENTANE	5.7E-01	7.0E-01	6.2E-01
METHYL ETHYL KETONE	5.7E-01	7.0E-01	6.3E-01
DECANE (NOTE F)	5.6E-01	6.7E-01	1.1E+00
ISOBUTYLBENZENE (NOTE F)	5.3E-01	6.3E-01	1.1E+00
2,3-DIMETHYLPENTANE	5.3E-01	6.3E-01	5.9E-01
2,2,3-TRIMETHYLBUTANE	1.7E+00	6.1E-01	7.7E-01
4-METHYL-TRANS-2-PENTENE	5.6E-01	6.0E-01	5.8E-01
1-HEXENE	5.4E-01	6.0E-01	5.3E-01
2-METHYL-1-PENTENE	6.5E-01	6.0E-01	5.3E-01
2,2-DIMETHYLOCTANE	8.3E-01	5.9E-01	1.6E-01
3-METHYL-TRANS-2-PENTENE	6.2E-01	5.9E-01	5.8E-01
CIS-1,2-DIMETHYLCYCLOHEXANE	5.1E-01	5.4E-01	4.4E-01
CIS-1,3-DIMETHYLCYCLOPENTANE	4.2E-01	5.0E-01	4.4E-01
CIS-2-HEPTENE	4.9E-01	5.0E-01	4.4E-01
2-METHYLOCTANE	4.1E-01	4.8E-01	3.1E-01
3,5-DIMETHYLHEPTANE (NOTE D)	4.7E-01	4.7E-01	3.9E-01
2,5-DIMETHYLHEPTANE (NOTE D)	4.7E-01	4.7E-01	3.9E-01
2,2-DIMETHYLHEXANE	4.0E-01	4.0E-01	3.3E-01
ISOPROPYLCYCLOPENTANE	4.3E-01	4.0E-01	3.6E-01
CYCLOPENTADIENE	2.0E-01	3.9E-01	2.9E-01
3,4-DIMETHYL-1-PENTENE	4.3E-01	3.9E-01	4.8E-01
1-TRANS-2-CIS-4-TRIMETHYLCYCLOPENTANE	3.2E-01	3.8E-01	3.4E-01
3-METHYL-CIS-2-PENTENE	3.6E-01	3.4E-01	3.4E-01

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Compound	E0 (mg/mile)	E10 (mg/mile)	E15 (mg/mile)
2,4-DIMETHYLHEPTANE	3.3E-01	3.3E-01	3.2E-01
3,3-DIMETHYLPENTANE	3.3E-01	3.3E-01	1.8E+00
TRANS-1,4-DIMETHYLCYCLOHEXANE	2.9E-01	3.2E-01	1.0E-03
TRANS-1,2-DIMETHYLCYCLOHEXANE	2.8E-01	3.0E-01	2.3E-01
2,4,4-TRIMETHYL-1-PENTENE	2.6E-01	2.9E-01	2.3E-01
TRANS-1,3-DIMETHYLCYCLOHEXANE (NOTE C)	3.2E-01	2.8E-01	2.8E-01
2,4,4-TRIMETHYL-2-PENTENE	3.9E-01	2.5E-01	1.7E-01
1-METHYLCYCLOPENTENE	1.5E+00	2.4E-01	2.5E-01
CIS-2-HEXENE	2.1E-01	2.2E-01	1.0E-01
3-METHYLOCTANE	1.9E-01	2.2E-01	1.3E-01
TRANS-2-HEXENE	2.2E-01	2.0E-01	2.2E-01
TRANS-2-OCTENE	1.8E-01	1.7E-01	5.4E-02
UNIDENTIFIED C8	1.3E-01	1.0E-01	7.0E-02
VALERALDEHYDE	2.5E-01	1.0E-01	2.9E-01
1-METHYL-2-ISOPROPYLBENZENE	2.6E+00	7.0E-02	1.0E-03
ETHYLCYCLOHEXANE	3.3E-02	6.0E-02	2.1E-01
ISOVALERALDEHYDE	7.1E-01	3.3E-02	7.3E-01
TRANS-2-HEPTENE	1.0E-03	3.3E-02	1.0E-03
1,1,3-TRIMETHYLCYCLOPENTANE	1.0E-03	1.0E-03	1.0E-03
1,2 DIETHYLBENZENE	6.4E-01	1.0E-03	1.0E-03
1,2,4,5-TETRAMETHYLBENZENE	1.0E-03	1.0E-03	3.3E-02
1,3-DIMETHYL-2-ETHYLBENZENE	1.7E-01	1.0E-03	1.0E-03
1,4-DIMETHYL-2-ETHYLBENZENE	1.8E+00	1.0E-03	1.0E-03
1-METHYL-2-N-PROPYLBENZENE	3.6E+00	1.0E-03	1.0E-03
1-TRANS-2-CIS-3-TRIMETHYLCYCLOPENTANE	7.0E-02	1.0E-03	1.0E-03
2,4-DIMETHYLOCTANE	3.3E-02	1.0E-03	1.0E-03
2-METHYL-2-HEXENE	3.3E-02	1.0E-03	1.0E-03
3-METHYL-1-BUTENE	2.1E+00	1.0E-03	1.0E-03
CIS-2-OCTENE	7.0E-02	1.0E-03	3.3E-02
CIS-3-HEXENE	3.3E-02	1.0E-03	3.3E-02
HEXANALDEHYDE	4.8E-01	1.0E-03	2.7E-01
METHYL ISOBUTYL KETONE	1.0E-03	1.0E-03	5.7E-01
PROPYNE	1.1E+00	1.0E-03	1.0E-03
TERT-1-BUT-3,5-DIMETHYLBENZENE	1.0E-03	1.0E-03	3.3E-02
UNDECANE	1.7E-01	1.0E-03	1.3E-01

Compounds in order of E10 prevalence. "1.E-03" indicates trace and/or non-detect.
Note A - 2,2-Dimethylpentane and methylcyclopentane co-elute. GC peak area split equally between the two compounds. Note B - 3-Methyl-3-ethyl-pentane co-elutes with reported compound. Not reported separately. Note D - Cis-1,4-Dimethylcyclohexane co-elutes with reported compound. Not reported separately. Note E - 2,5-Dimethylheptane and 3,5-dimethylheptane co-elute. GC peak area split equally between the two compounds. Note F - Decane and isobutylbenzene co-elute. GC peak area split equally between the two compounds.

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Table 8 PAHs and NPAHs in catalyzed E0, E10, and E15 exhausts

	E0CAT (mean; ng/mi)	E10CAT (mean; ng/mi)	E15CAT (mean; ng/mi)
Volatile-phase Compounds			
2-Nitrofluorene	Trace	Trace	Trace
1-Nitropyrene	Trace	Trace	Trace
7-Nitrobenz(a)anthracene	ND [a]	ND	ND
6-Nitrochrysene	ND	ND	ND
6-Nitrobenz(a)pyrene	ND	ND	ND
Benzo(a)anthracene	28	36	95
Chrysene	23	23	60
Benzo(b)fluoranthene	4.3	2.8	5.8
Benzo(k)fluoranthene	1.3	0.9	2.0
Benzo(a)pyrene	1.1	0.5	1.0
Indeno(1,2,3-cd)pyrene	1.1	0.4	0.5
Dibenz(a,h)anthracene	0.3	0.2	0.3
Particulate-phase Compounds			
2-Nitrofluorene	Trace	ND	ND
1-Nitropyrene	Trace	Trace	Trace
7-Nitrobenz(a)anthracene	Trace	ND	ND
6-Nitrochrysene	ND	ND	ND
6-Nitrobenz(a)pyrene	ND	ND	ND
Benzo(a)anthracene	25	20	46
Chrysene	20	17	43
Benzo(b)fluoranthene	28	19	25
Benzo(k)fluoranthene	9.8	7.3	9.5
Benzo(a)pyrene	13	6.4	7.9
Indeno(1,2,3-cd)pyrene	14	4.6	4.8
Dibenz(a,h)anthracene	2.6	1.1	1.1

[a] Not detected

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Table 9 PAHs and NPAHs in uncatalyzed E0, E10, and E15 exhausts

	E0 (mean; ng/mi)	E10 (mean; ng/mi)	E15 (mean; ng/mi)
Volatile-phase Compounds			
2-Nitrofluorene	7.4	5.2	7.0
1-Nitropyrene	Trace	ND	Trace
7-Nitrobenz(a)anthracene	ND [a]	ND	ND
6-Nitrochrysene	ND	ND	ND
6-Nitrobenz(a)pyrene	ND	ND	ND
Benzo(a)anthracene	452	324	345
Chrysene	242	212	199
Benzo(b)fluoranthene	50	37	37
Benzo(k)fluoranthene	18	17	15
Benzo(a)pyrene	18	16	14
Indeno(1,2,3-cd)pyrene	11	9.9	7.7
Dibenz(a,h)anthracene	2.8	2.5	1.9
Particulate-phase Compounds			
2-Nitrofluorene	1.3	ND	ND
1-Nitropyrene	3.1	4.4	4.7
7-Nitrobenz(a)anthracene	ND	ND	ND
6-Nitrochrysene	ND	ND	ND
6-Nitrobenz(a)pyrene	ND	ND	ND
Benzo(a)anthracene	627	1564	663
Chrysene	386	1083	439
Benzo(b)fluoranthene	601	1136	456
Benzo(k)fluoranthene	176	390	177
Benzo(a)pyrene	234	471	187
Indeno(1,2,3-cd)pyrene	184	393	152
Dibenz(a,h)anthracene	31	89	46

[a] Not detected

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Table 10 PAHs and NPAHs in catalyzed BG and BG+E exhaust

	BG CAT (mean; ng/mi)[a]	BG+E CAT (mean; ng/mi)
Volatile-phase Compounds		
2-Nitrofluorene	Trace	Trace
1-Nitropyrene	Trace	Trace
7-Nitrobenz(a)anthracene	ND	Trace
6-Nitrochrysene	Trace	Trace
6-Nitrobenz(a)pyrene	ND	ND
Benzo(a)anthracene	267	442
Chrysene	267	454
Benzo(b)fluoranthene	48.0	28.0
Benzo(k)fluoranthene	41.0	26.0
Benzo(a)pyrene	39.0	16.0
Indeno(1,2,3-cd)pyrene	28.0	9.0
Dibenz(a,h)anthracene	11.0	2.0
Particulate-phase Compounds		
2-Nitrofluorene	Trace	Trace
1-Nitropyrene	2.0	3.0
7-Nitrobenz(a)anthracene	Trace	Trace
6-Nitrochrysene	Trace	Trace
6-Nitrobenz(a)pyrene	Trace	Trace
Benzo(a)anthracene	154	205
Chrysene	149	241
Benzo(b)fluoranthene	283	230
Benzo(k)fluoranthene	190.0	147.0
Benzo(a)pyrene	272.0	174.0
Indeno(1,2,3-cd)pyrene	314.0	185.0
Dibenz(a,h)anthracene	50.0	25.0

[a] Data property of the 211(b) Research Group.

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Table 11 PAHs and NPAHs in uncatalyzed BG and BG+E exhaust

	BG (mean; ng/mi)[a]	BG+E (mean; ng/mi)
Volatile-phase Compounds		
2-Nitrofluorene	Trace	Trace
1-Nitropyrene	ND	1.0
7-Nitrobenz(a)anthracene	Trace	Trace
6-Nitrochrysene	1.0	2.0
6-Nitrobenz(a)pyrene	ND	ND
Benzo(a)anthracene	2295	2753
Chrysene	2068	2337
Benzo(b)fluoranthene	362	295
Benzo(k)fluoranthene	258.0	213.0
Benzo(a)pyrene	271.0	221.0
Indeno(1,2,3-cd)pyrene	164.0	111.0
Dibenz(a,h)anthracene	36.0	18.0
Particulate-phase Compounds		
2-Nitrofluorene	1	Trace
1-Nitropyrene	4.0	5.0
7-Nitrobenz(a)anthracene	2	1
6-Nitrochrysene	ND	Trace
6-Nitrobenz(a)pyrene	4	1
Benzo(a)anthracene	3746	3411
Chrysene	3657	3456
Benzo(b)fluoranthene	2118	1940
Benzo(k)fluoranthene	1249	1115
Benzo(a)pyrene	1516	1360
Indeno(1,2,3-cd)pyrene	1159	1137
Dibenz(a,h)anthracene	254	265

[a] Data property of the 211(b) Research Group.

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Figure 5 Catalyzed exhaust: E0, E10, and E15

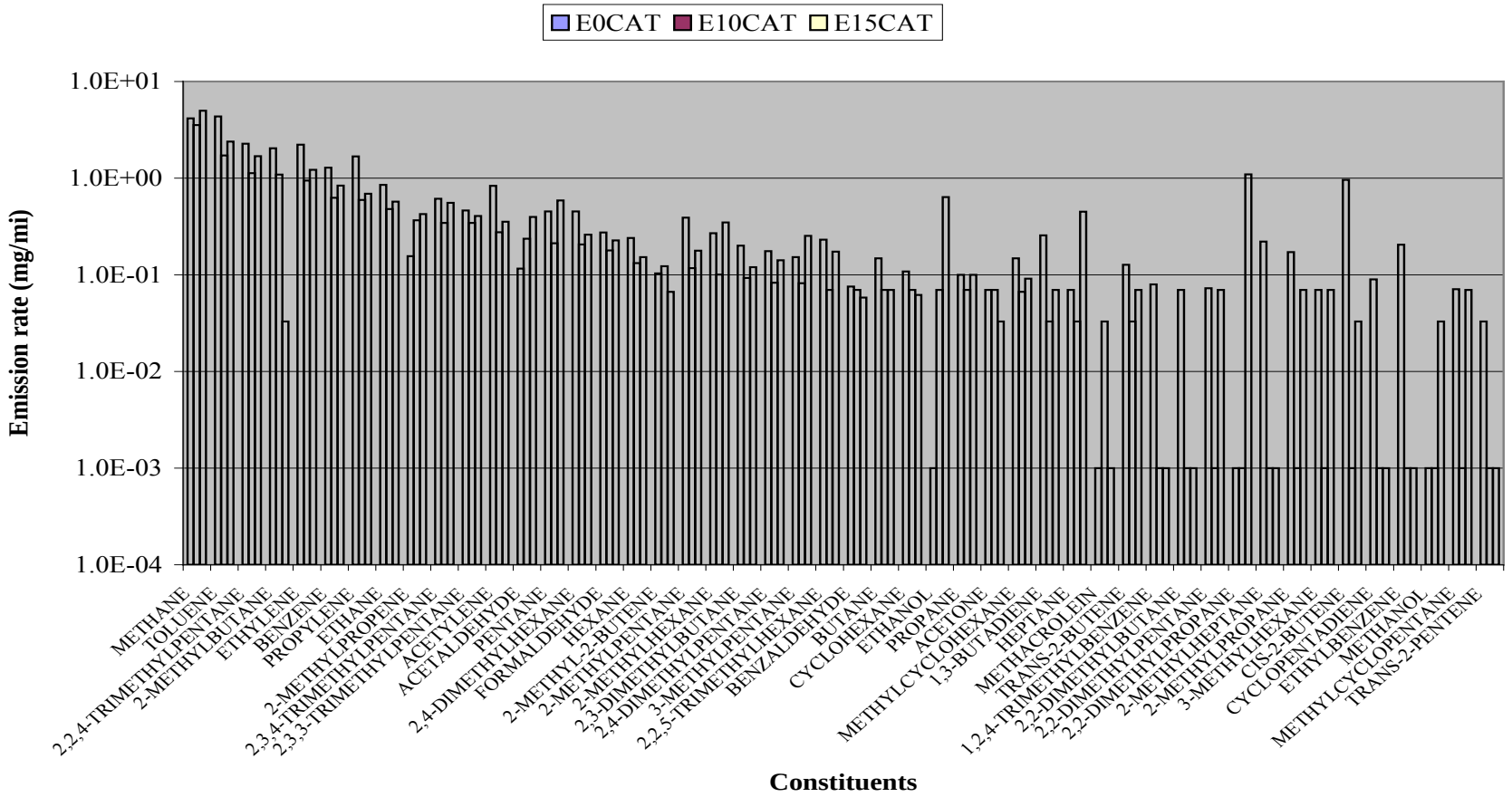

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Figure 6 Uncatalyzed exhaust 1: E0, E10, and E15

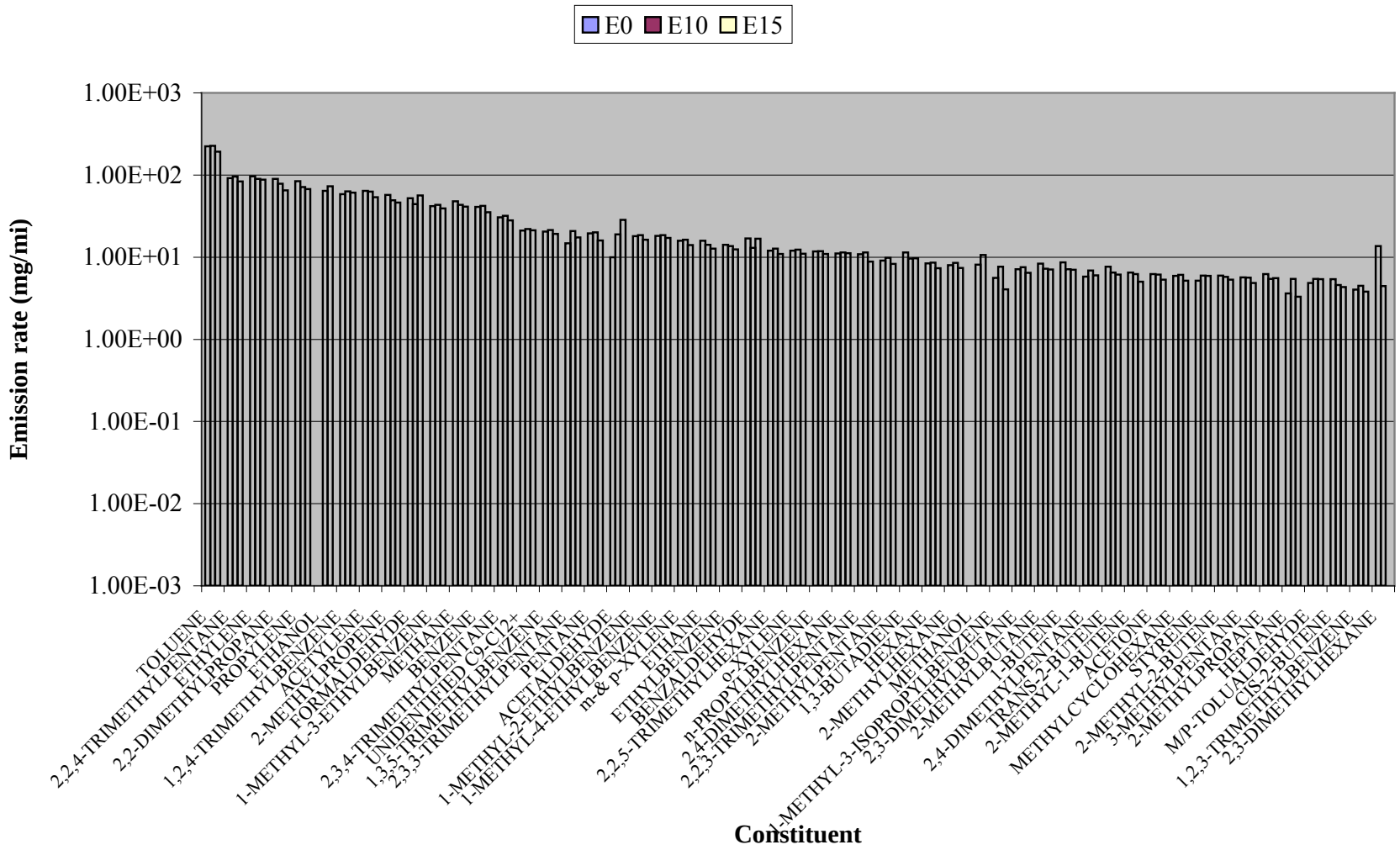

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Figure 7 Uncatalyzed exhaust 2: E0, E10, and E15

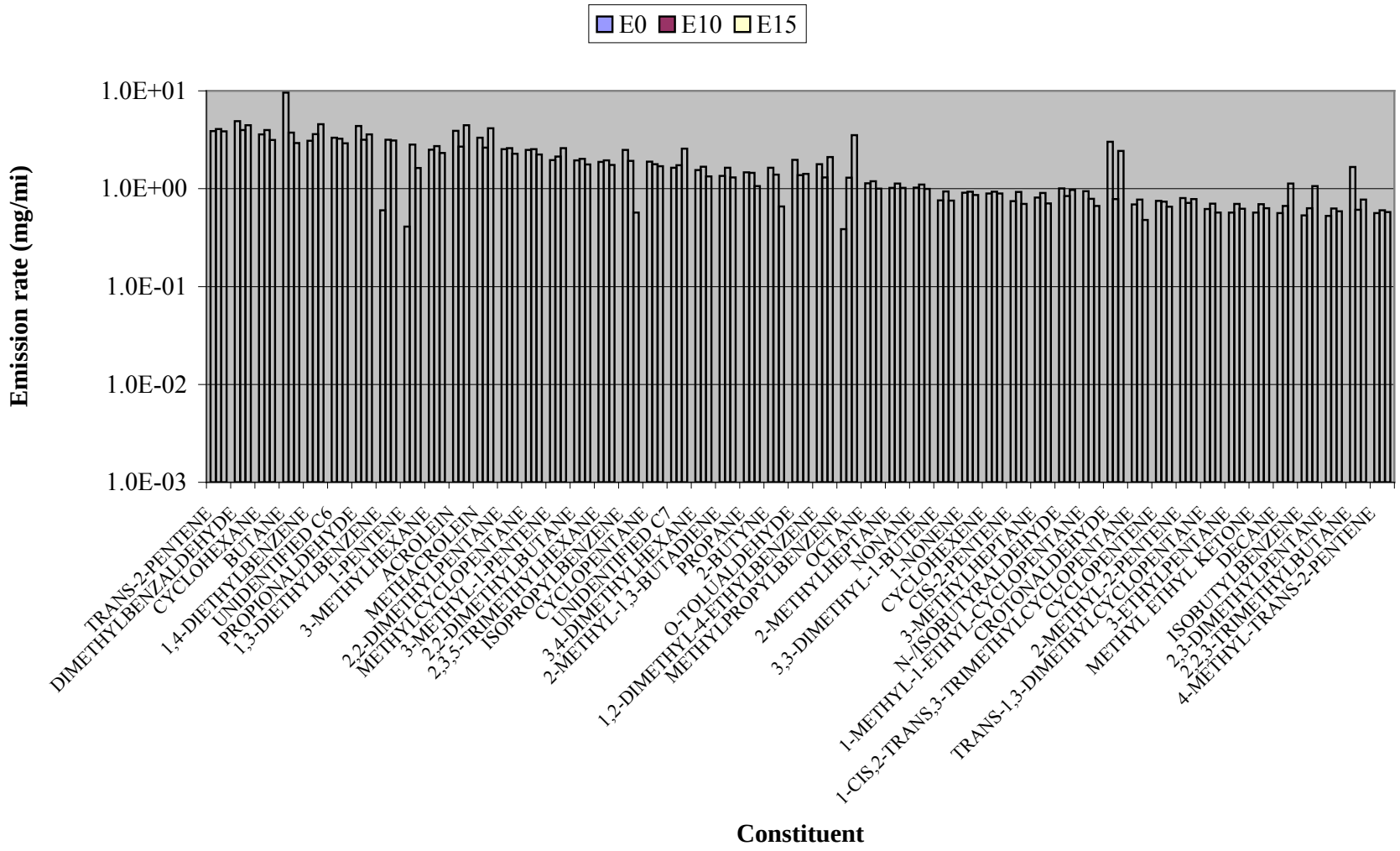

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Figure 8 Uncatalyzed exhaust 3: E0, E10, and E15

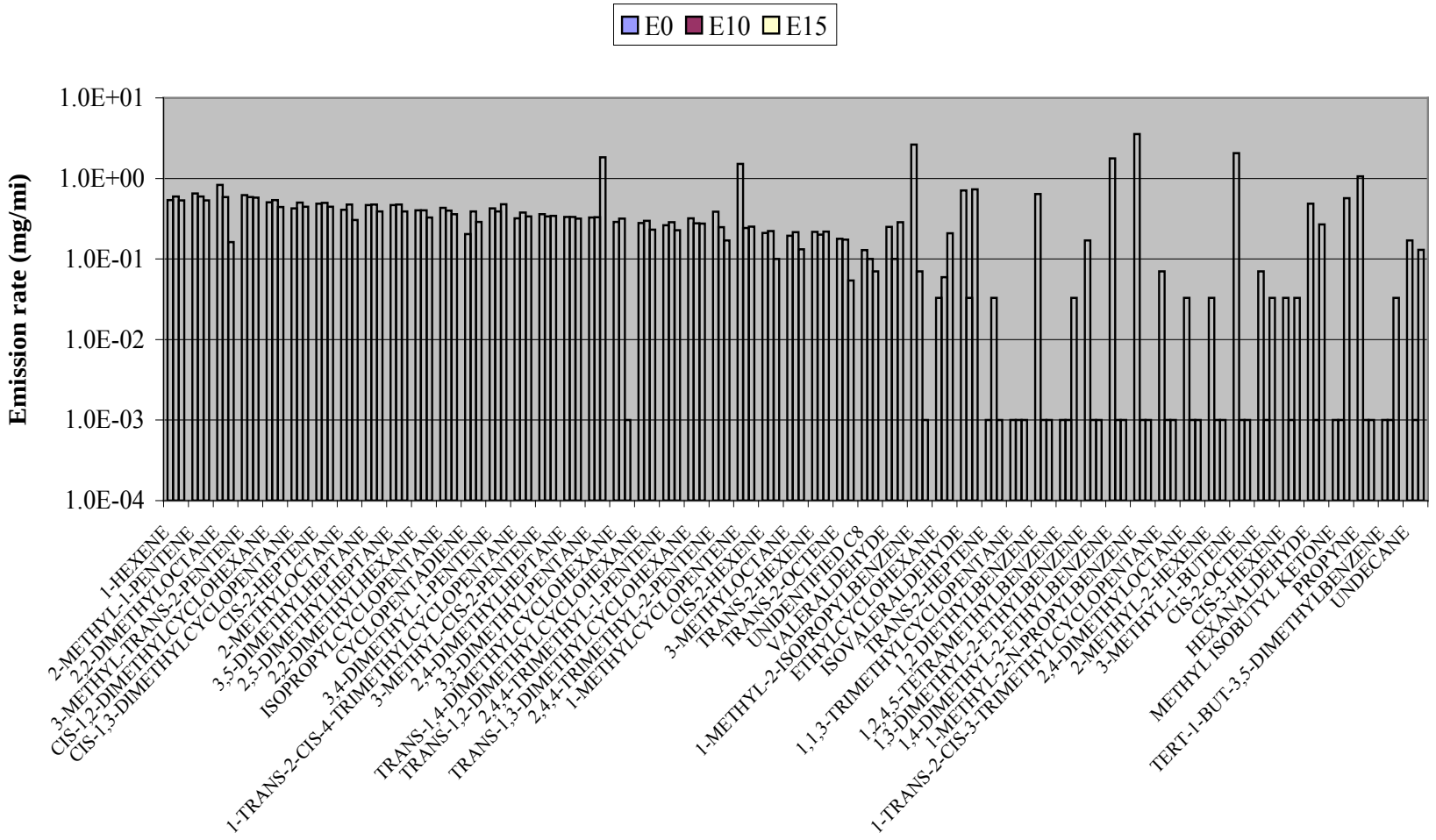

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Figure 9 Benzo(a)anthracene in catalyzed exhaust

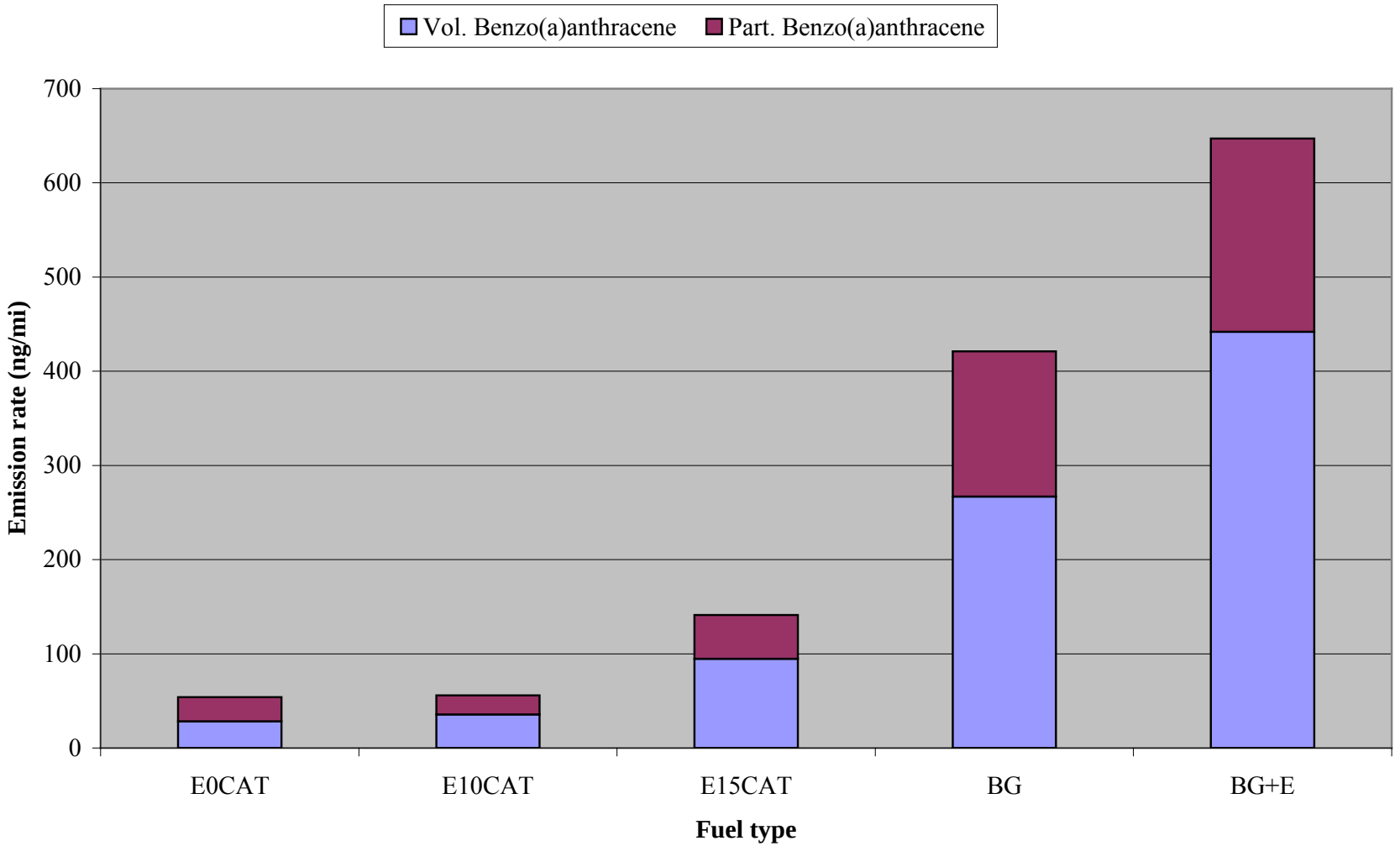

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Figure 10 Chrysene in catalyzed exhaust

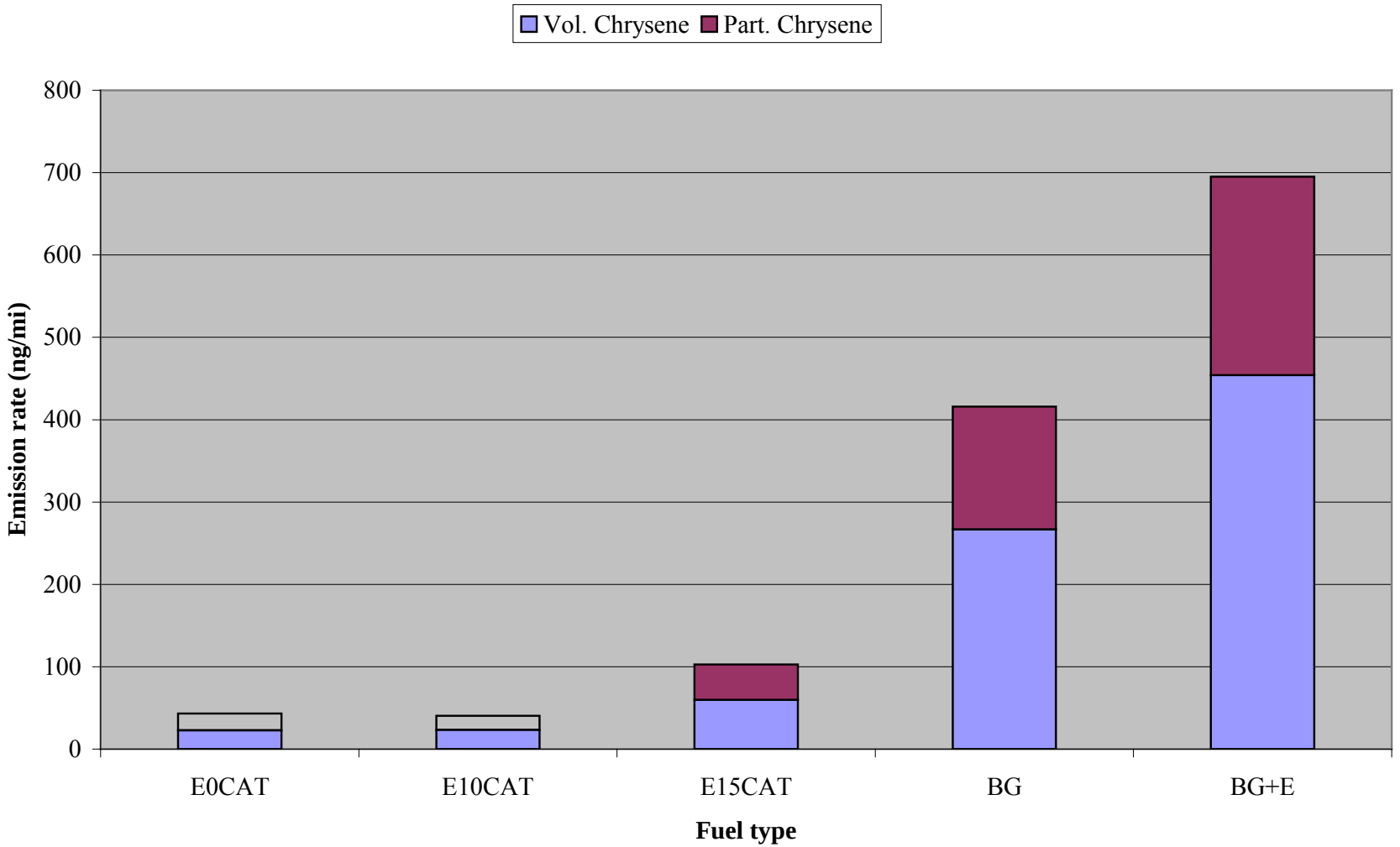

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Figure 11 Benzo(b)fluoranthene in catalyzed exhaust

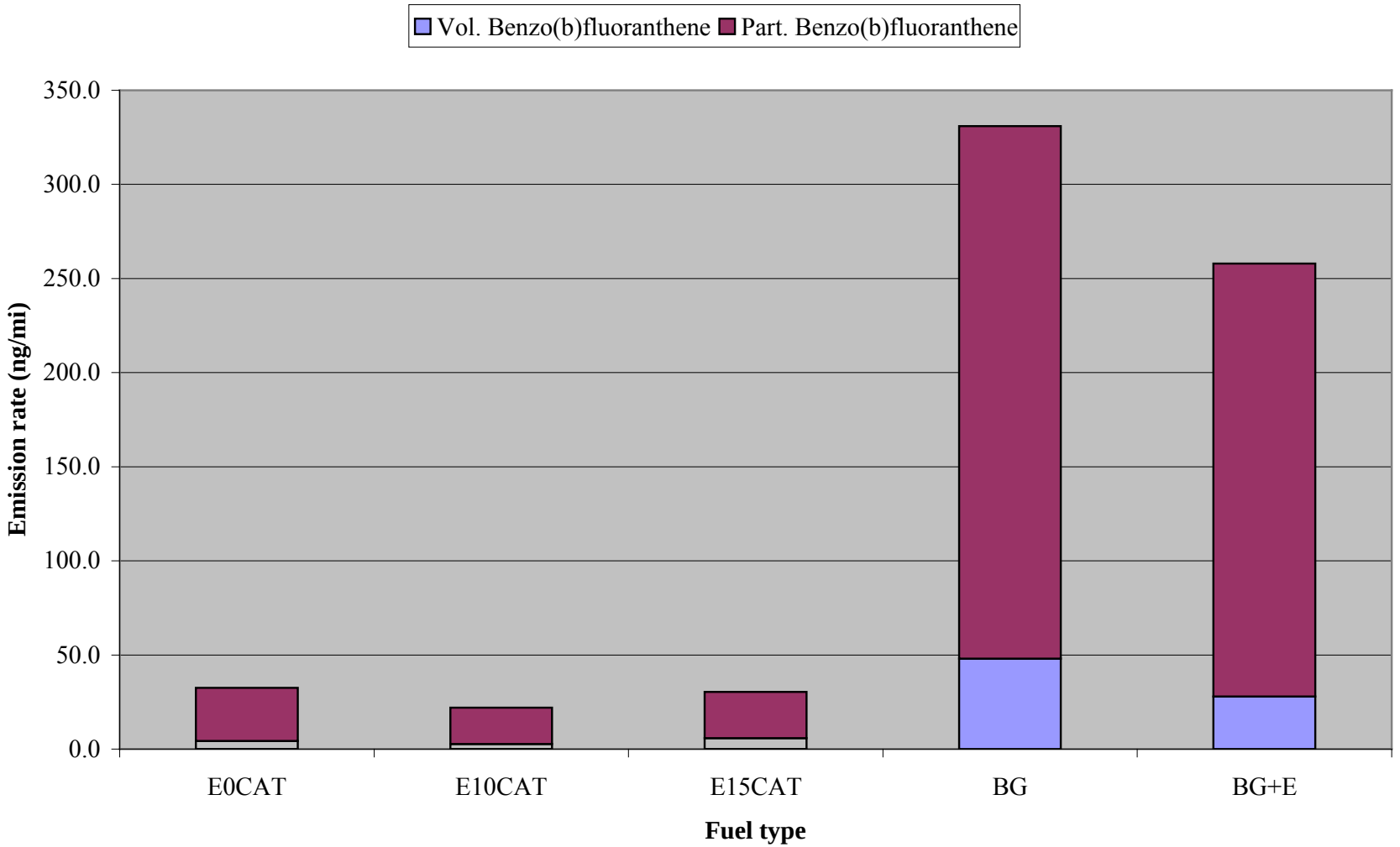

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Figure 12 Benzo(k)fluoranthene in catalyzed exhaust

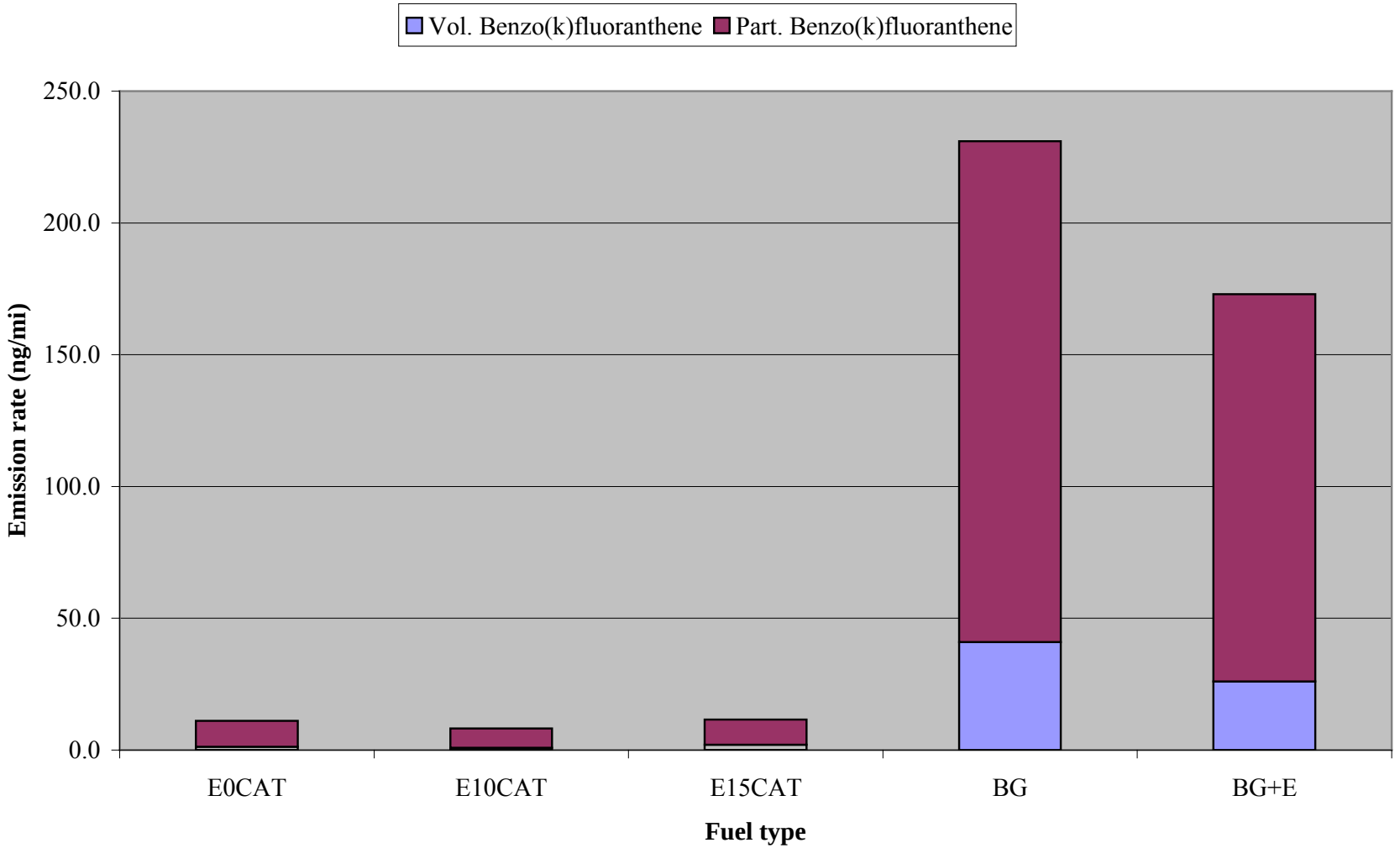

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Figure 13 Benzo(a)pyrene in catalyzed exhaust

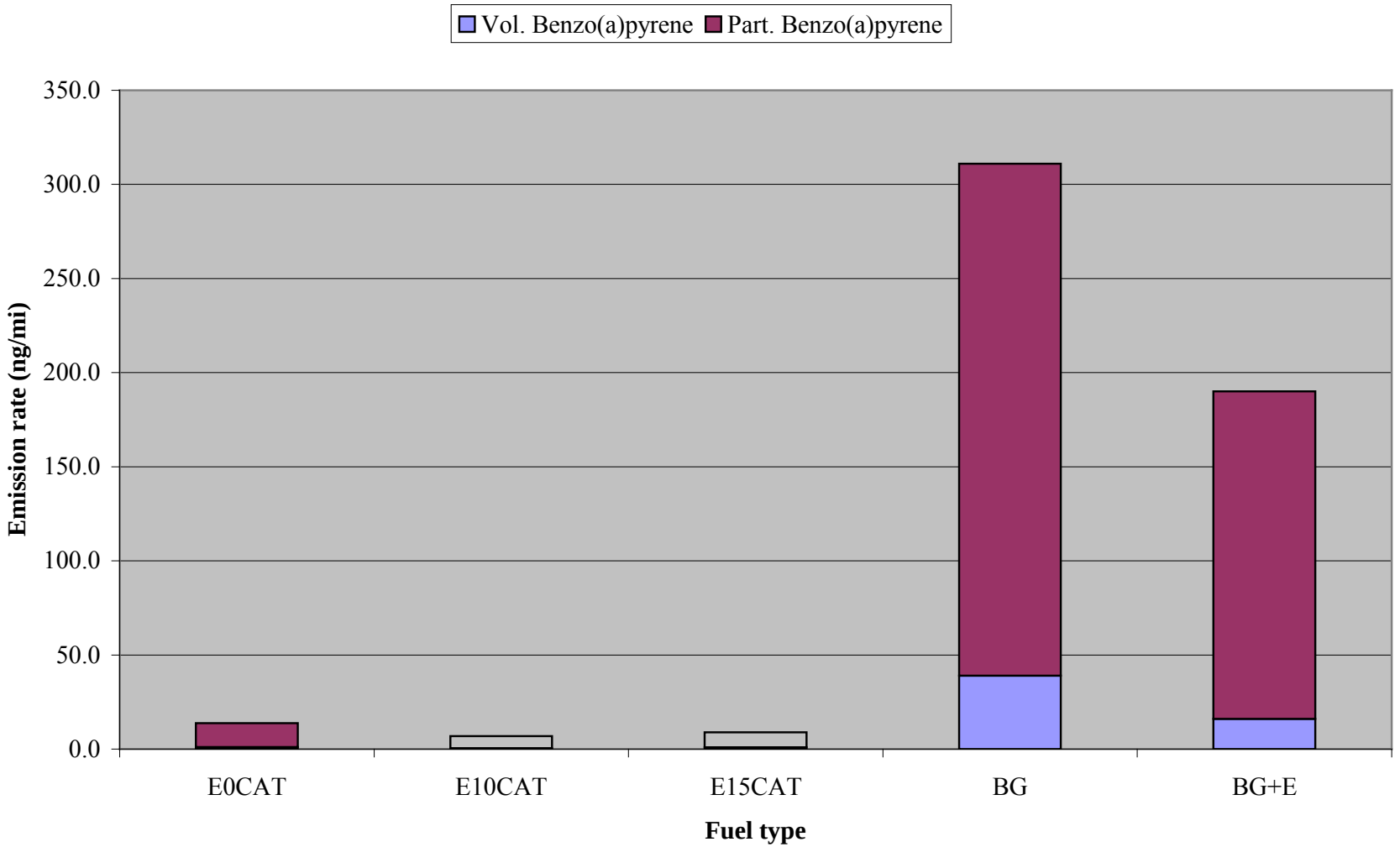

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Figure 14 Indeno(1,2,3-cd)pyrene in catalyzed exhaust

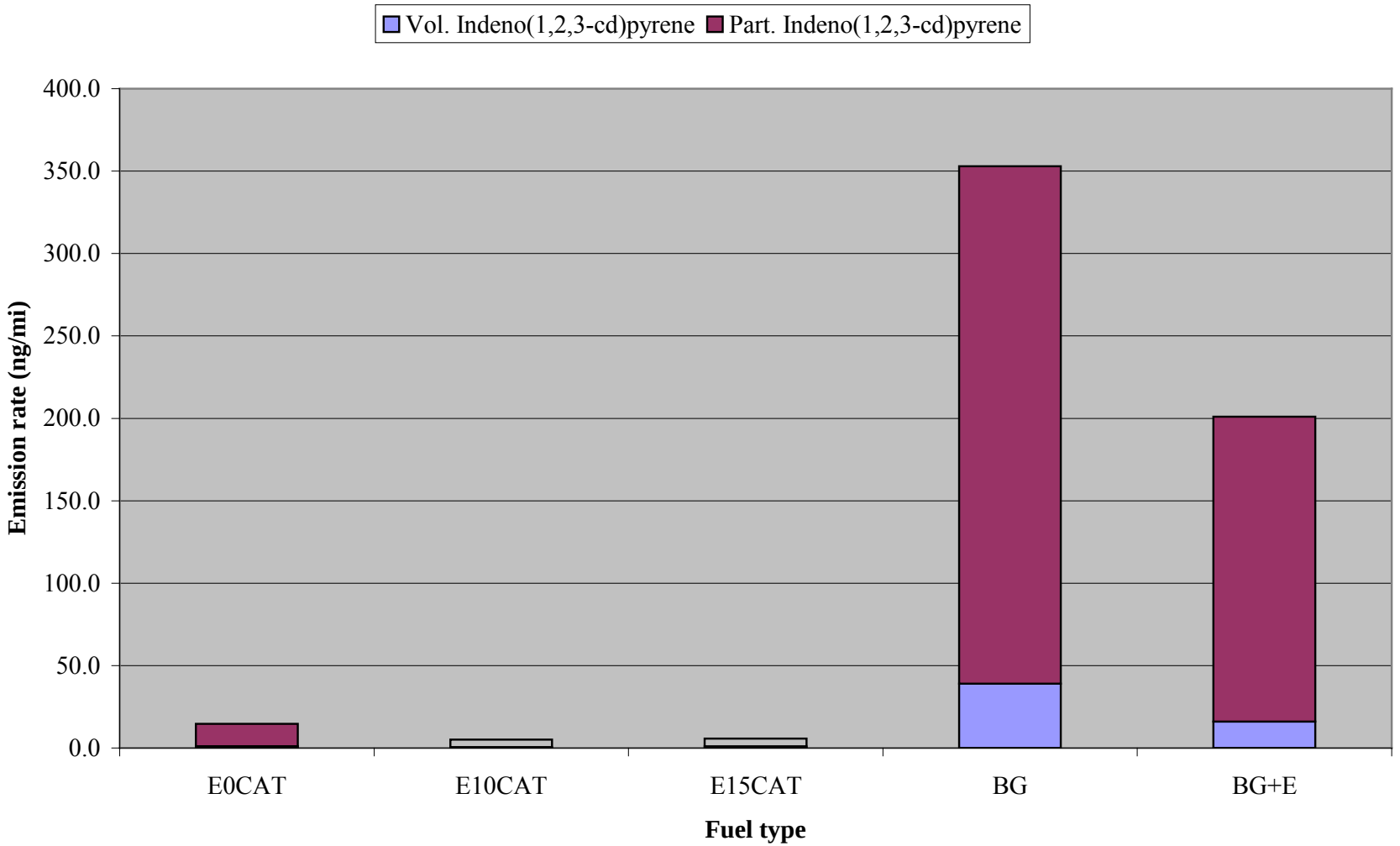

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Figure 15 Dibenz(a,h)anthracene in catalyzed exhaust

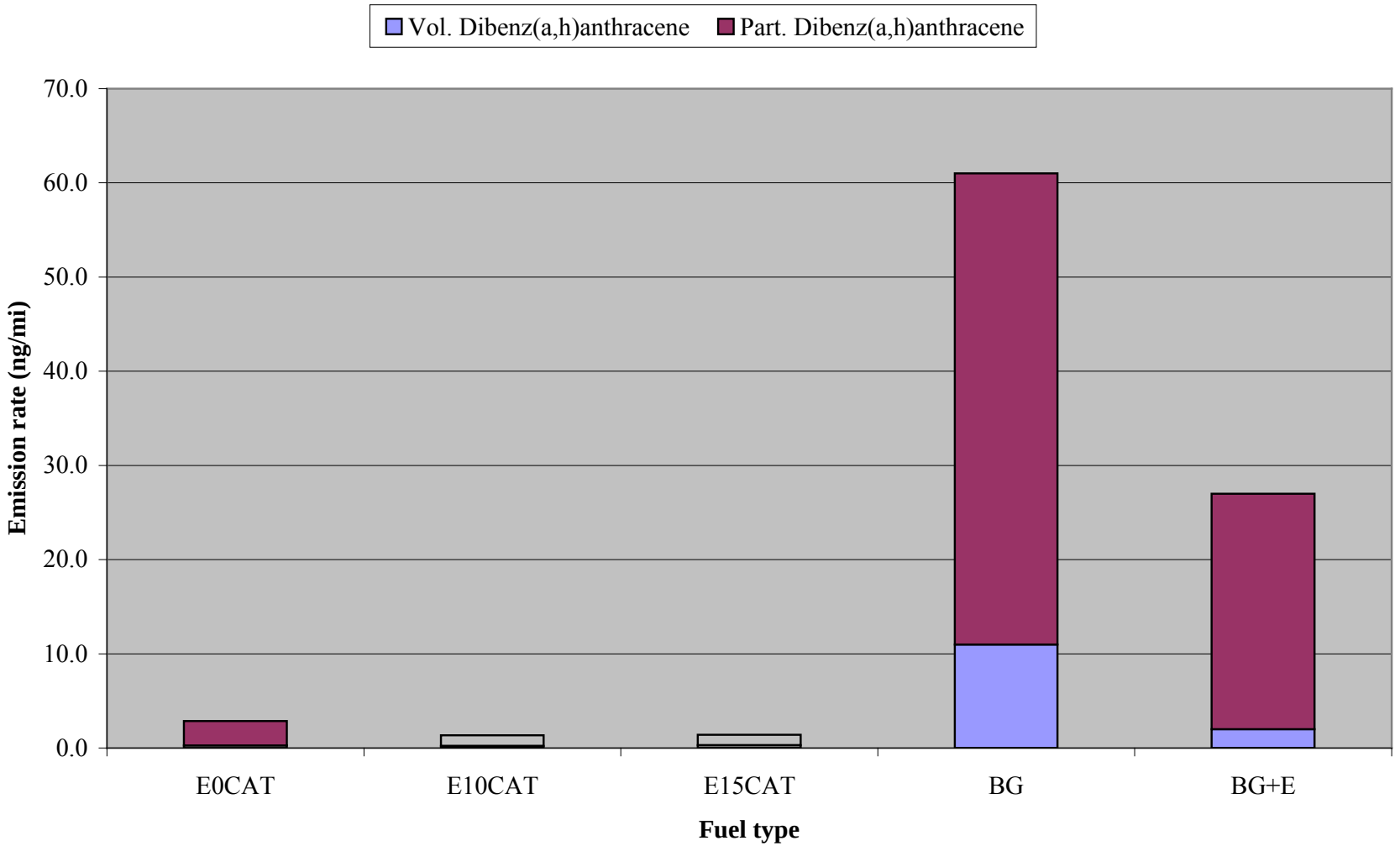

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Figure 16 2-Nitrofluorene in uncatalyzed exhaust

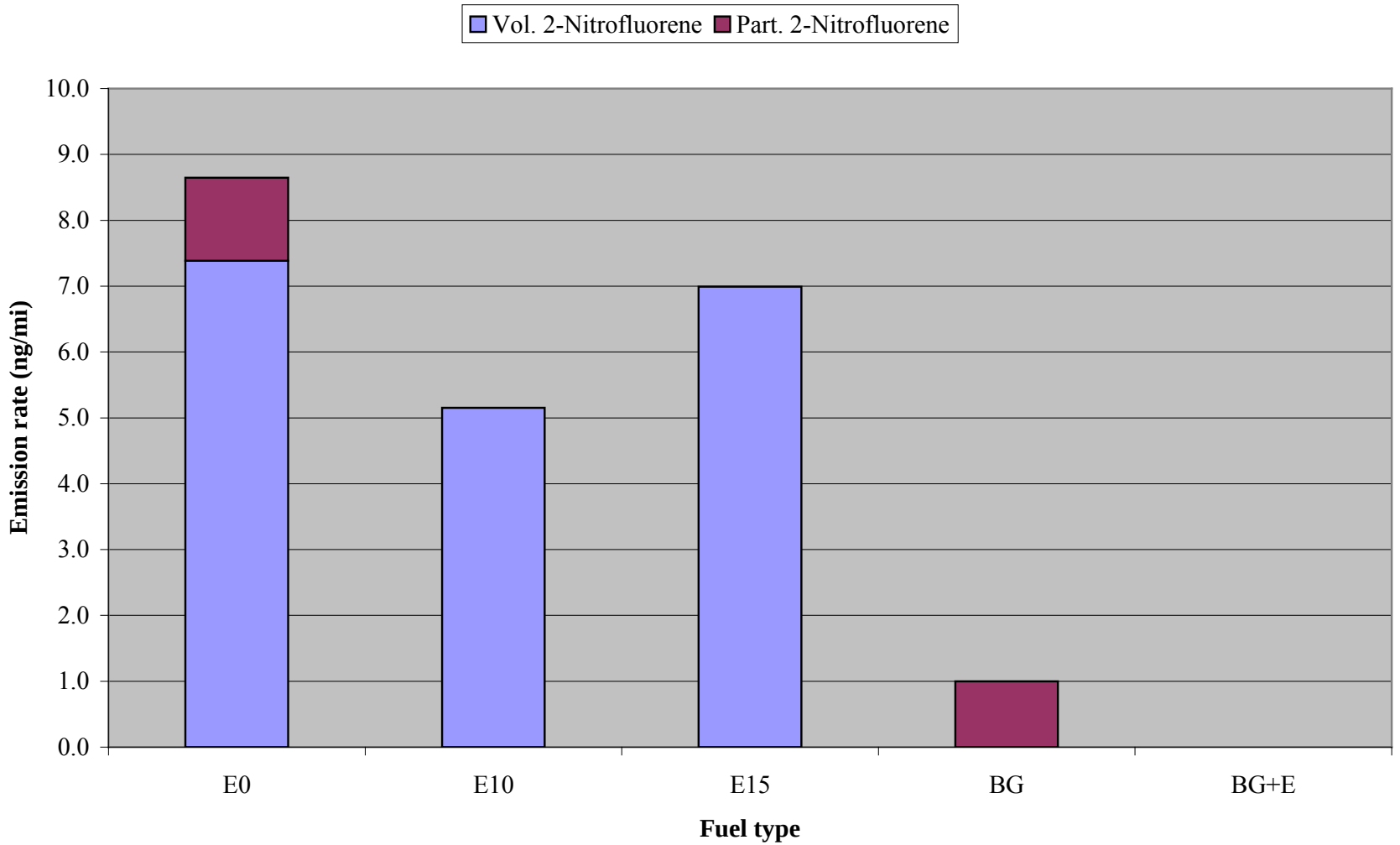

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Figure 17 1-Nitropyrene in uncatalyzed exhaust

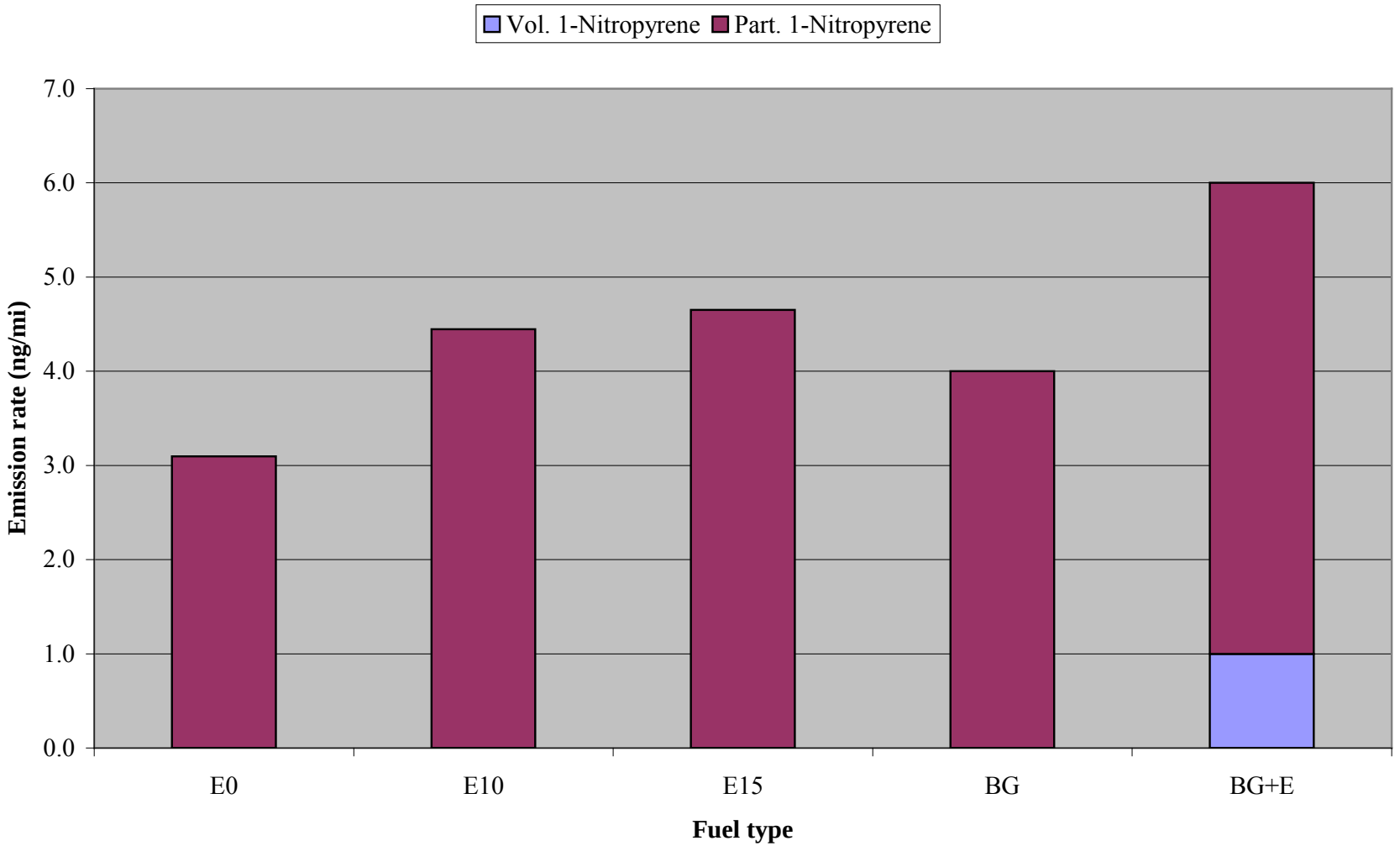

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Figure 18 Benzo(a)anthracene in uncatalyzed exhaust

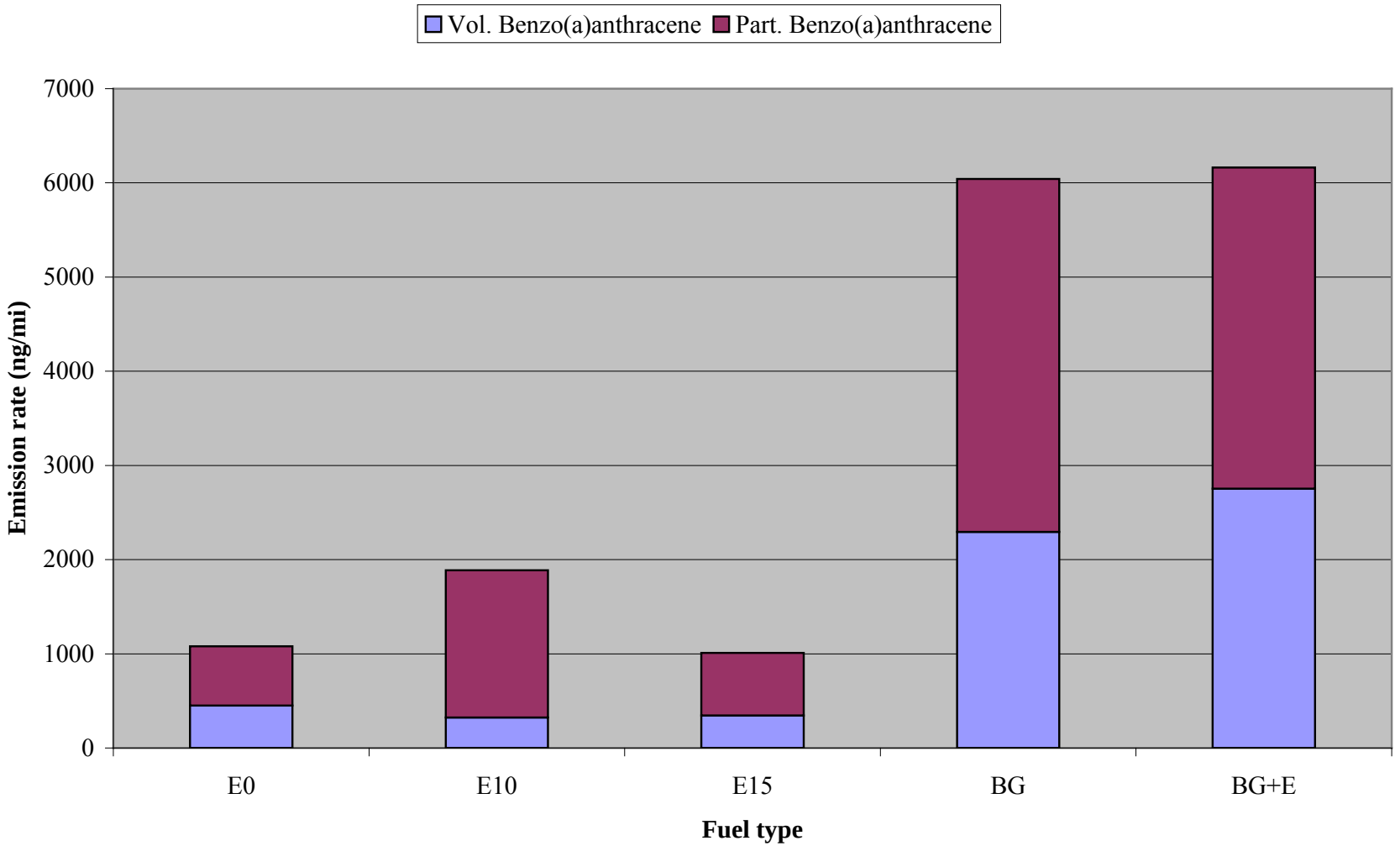

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Figure 19 Chrysene in uncatalyzed exhaust

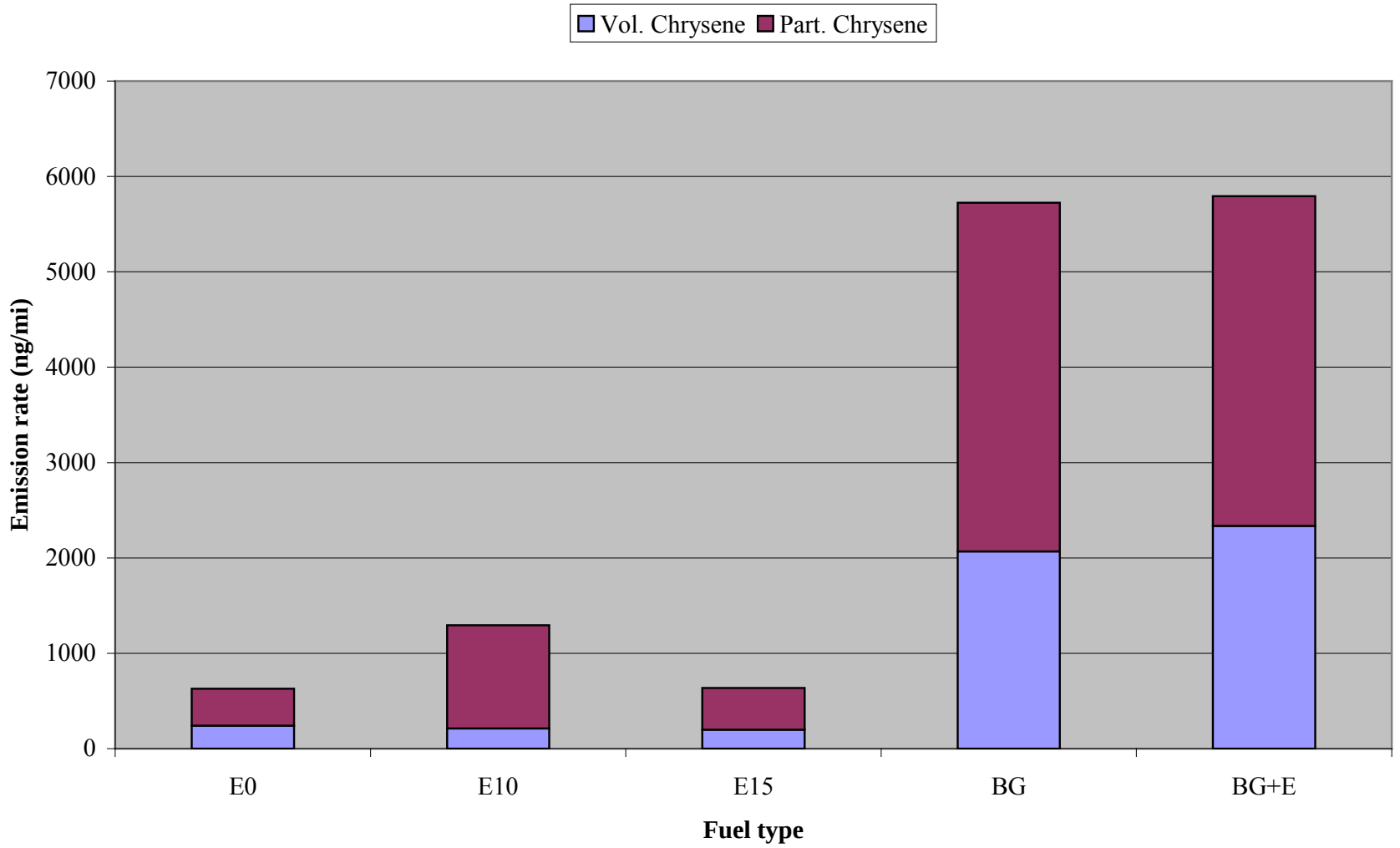

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Figure 20 Benzo(b)fluoranthene in uncatalyzed exhaust

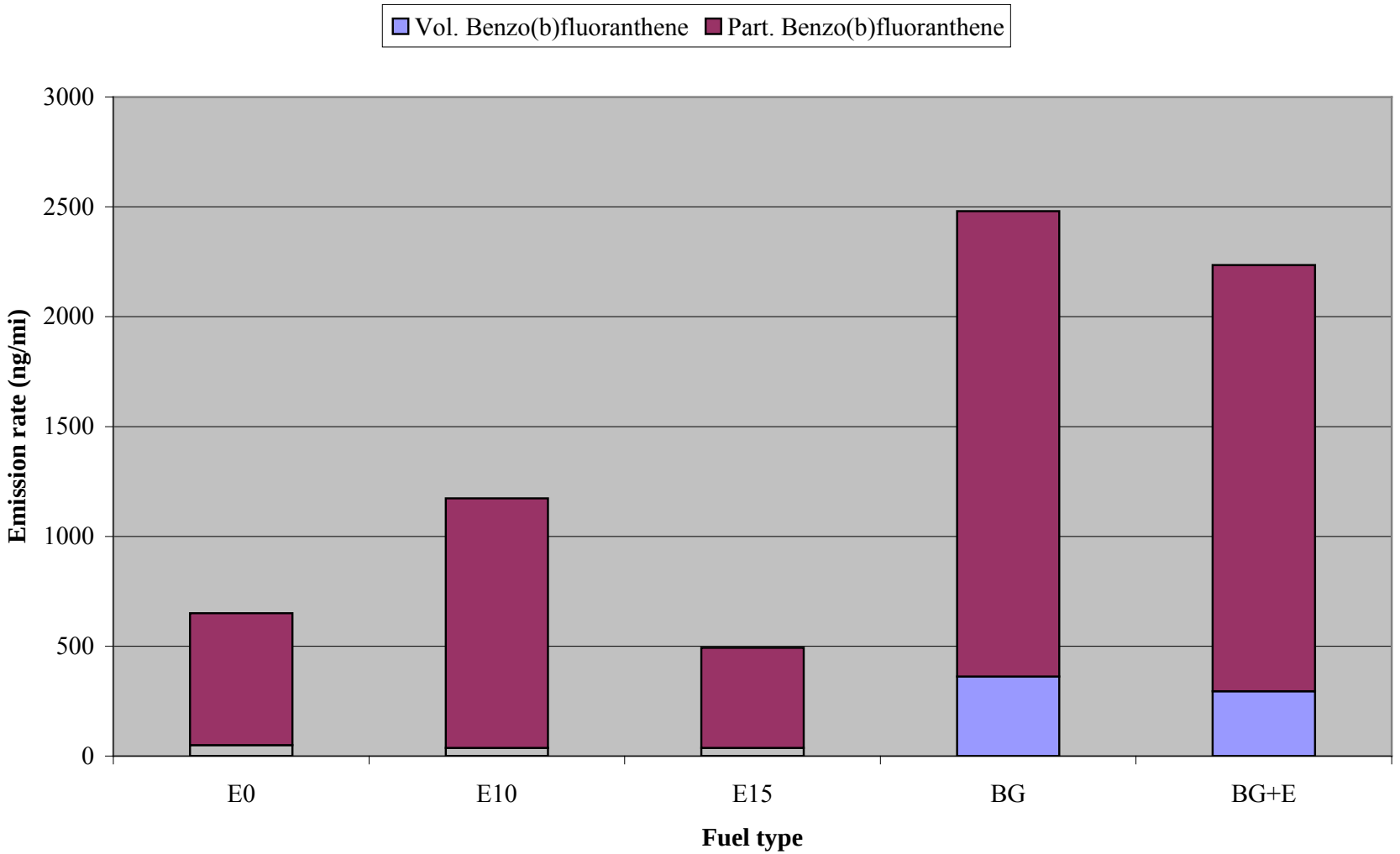

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Figure 21 Benzo(k)fluoranthene in uncatalyzed exhaust

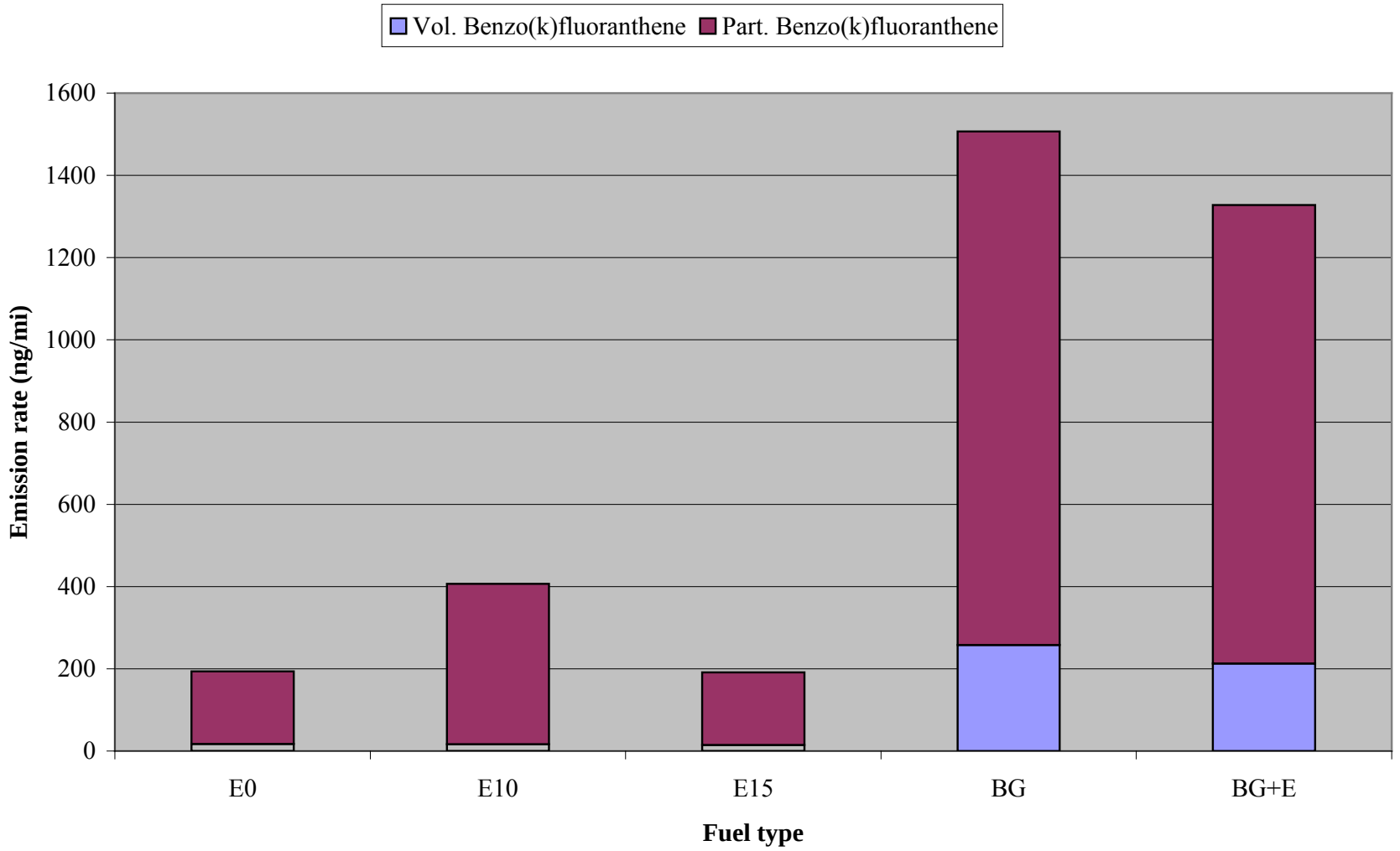

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Figure 22 Benzo(a)pyrene in uncatalyzed exhaust

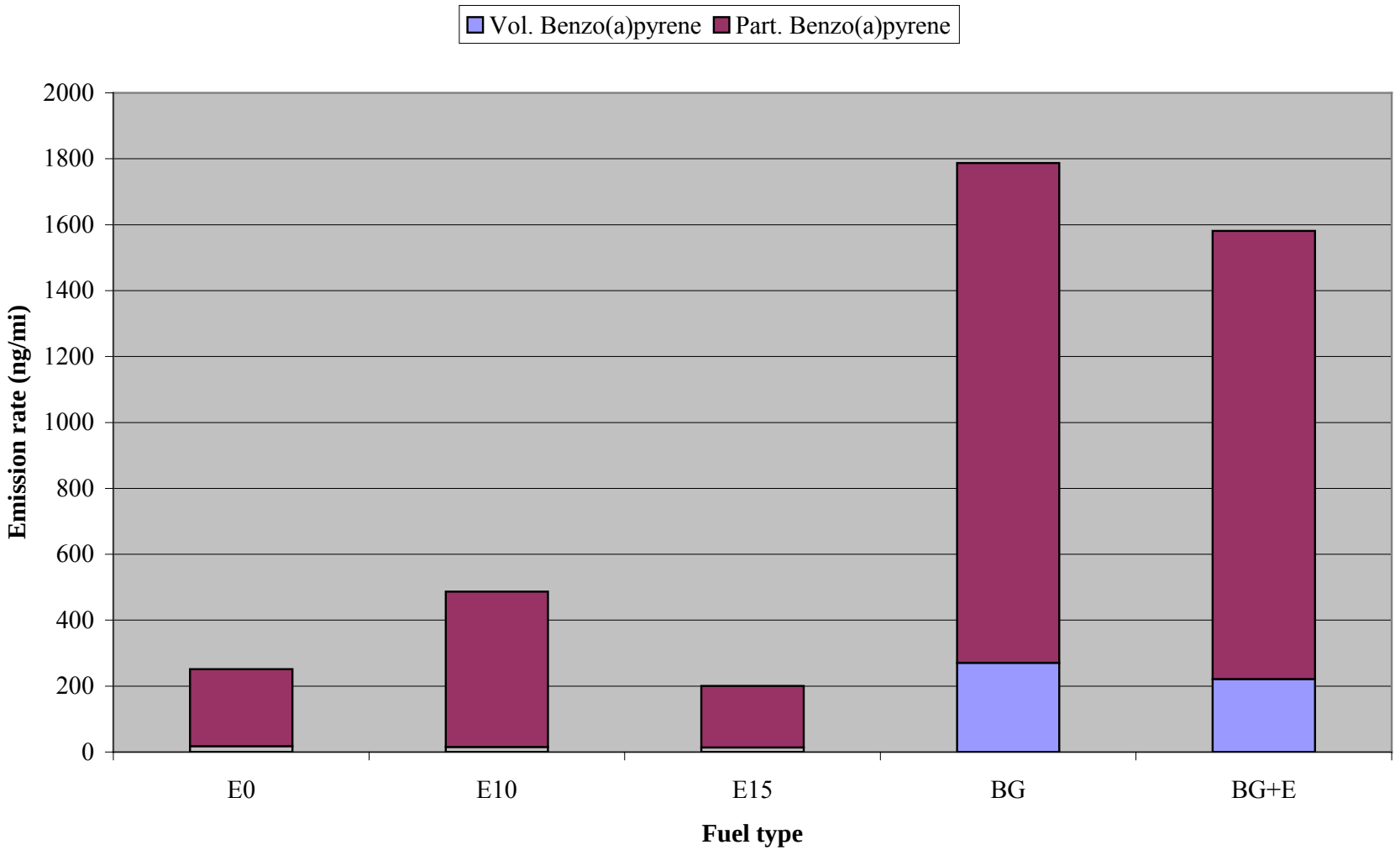

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Figure 23 Indeno(1,2,3-cd)pyrene in uncatalyzed exhaust

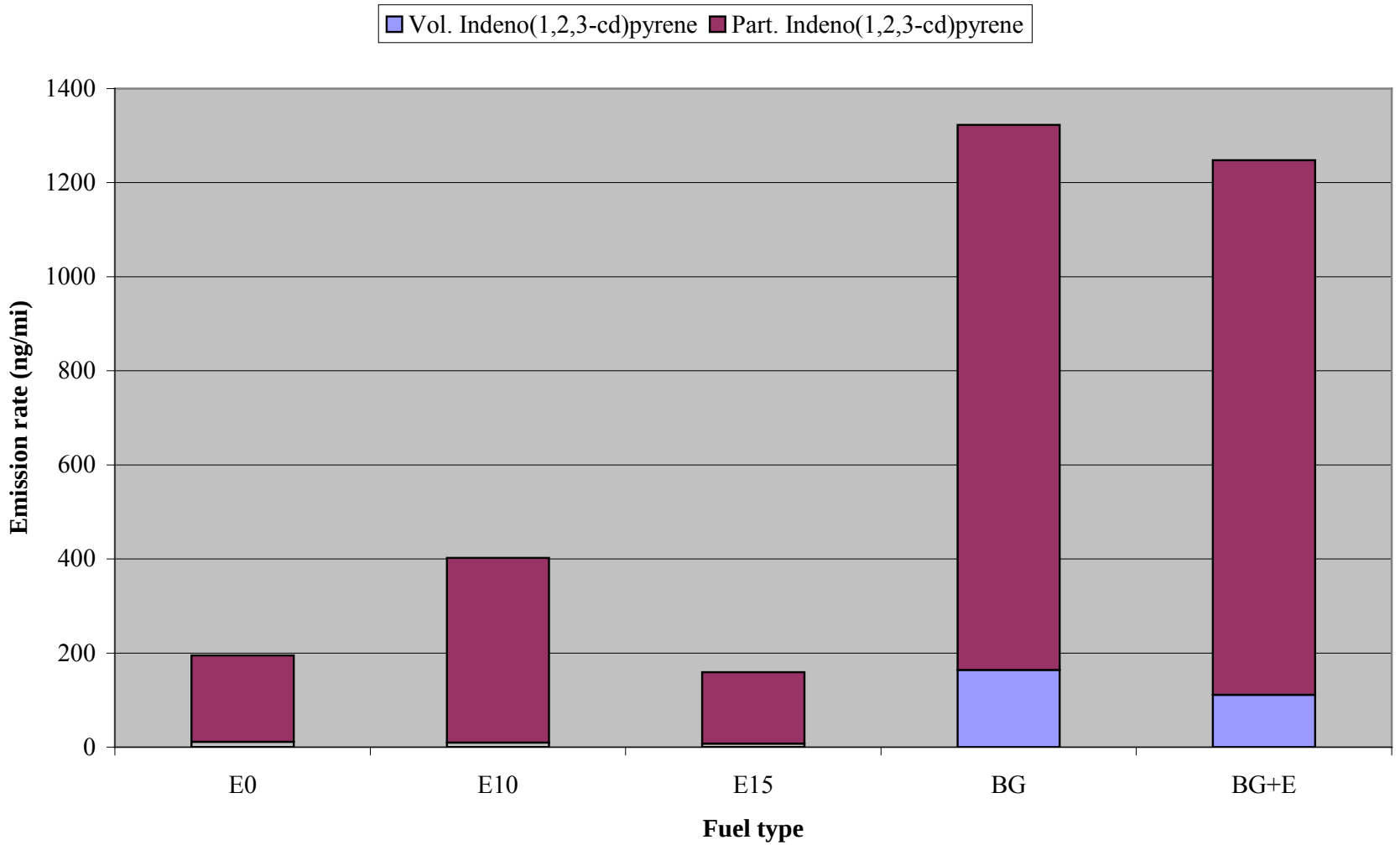

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Figure 24 Dibenz(a,h)anthracene in uncatalyzed exhaust

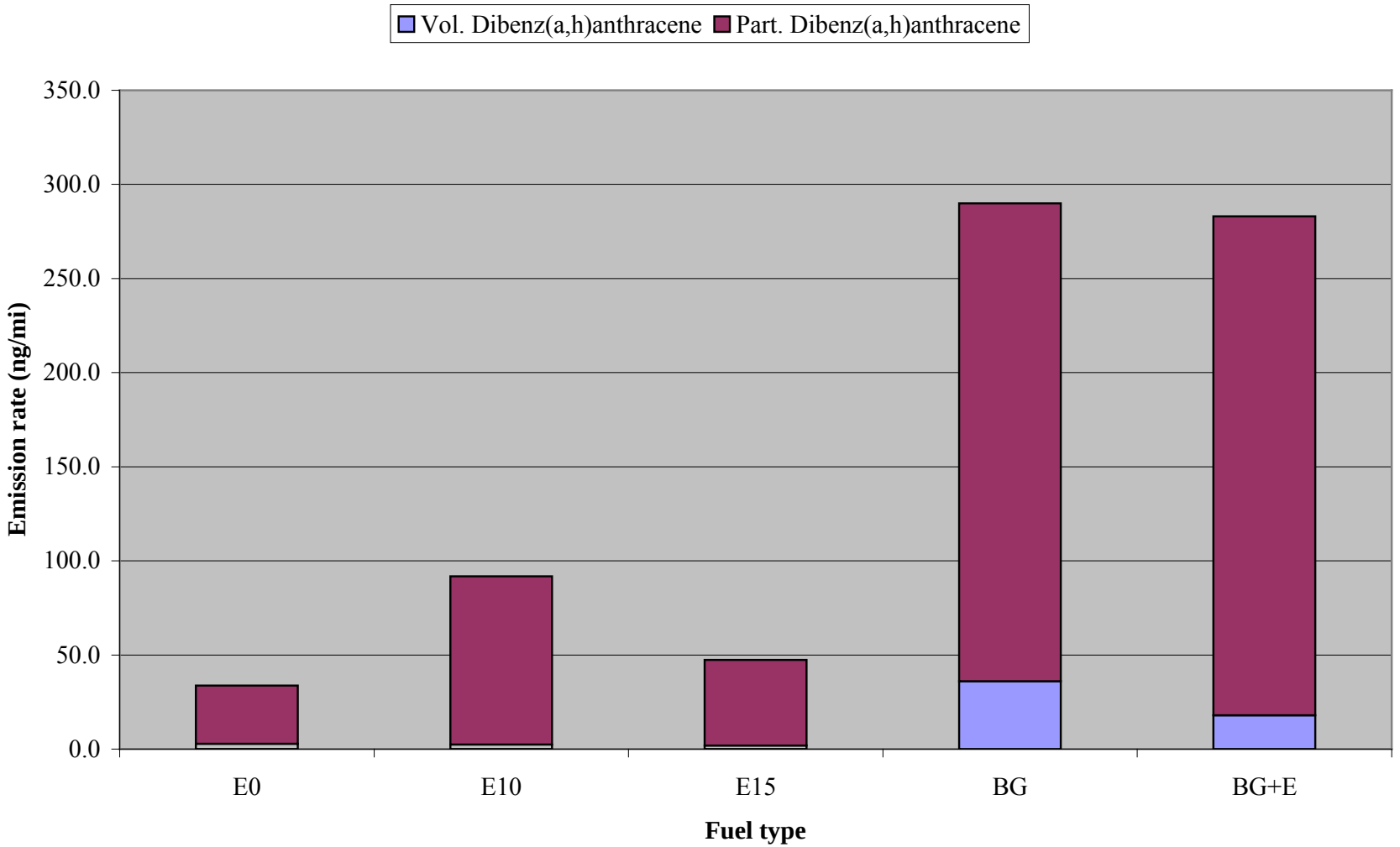

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Figure 25 Catalyzed exhaust: E0 vs. BG

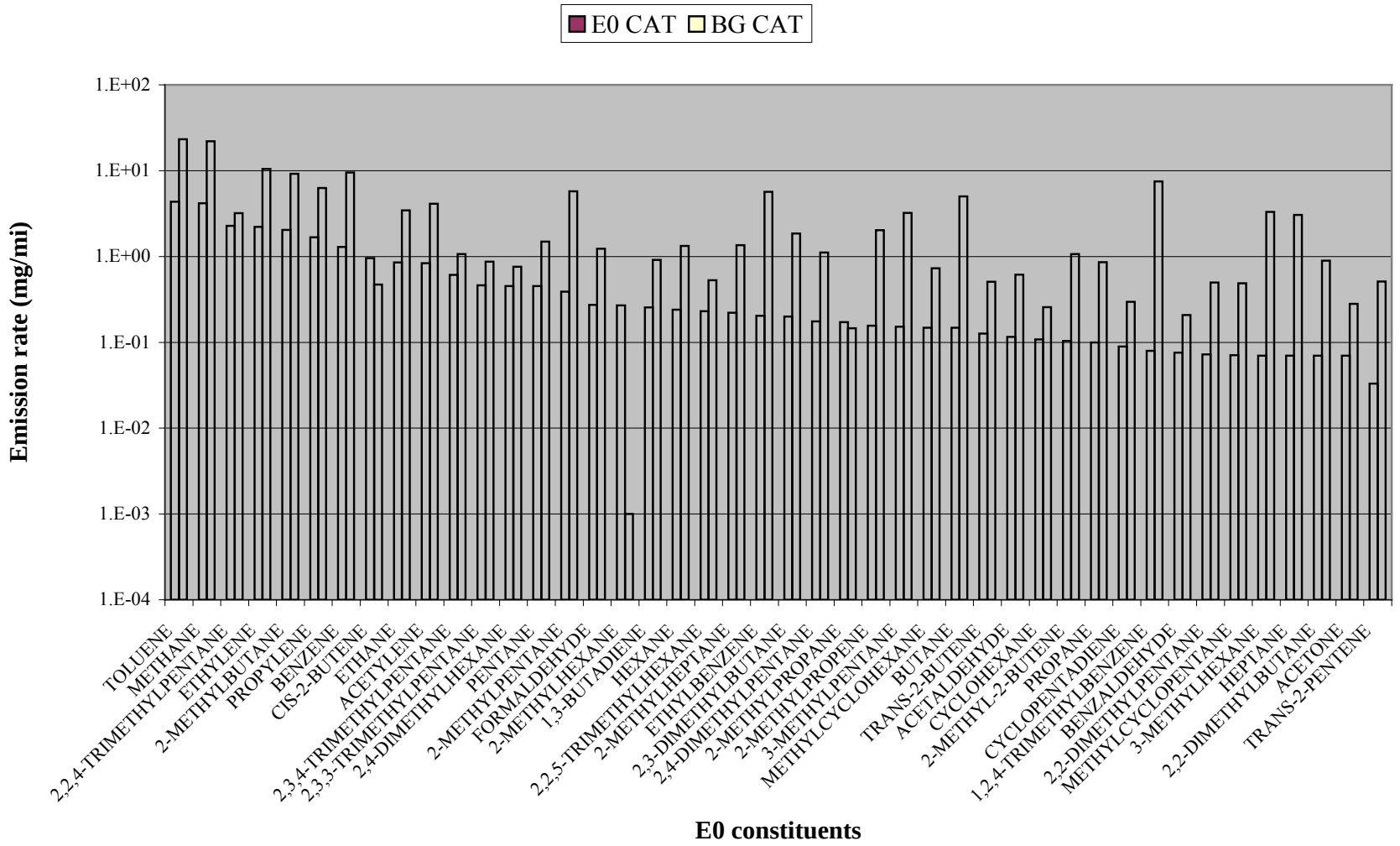

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Figure 26 Uncatalyzed exhaust 1: E0 and BG

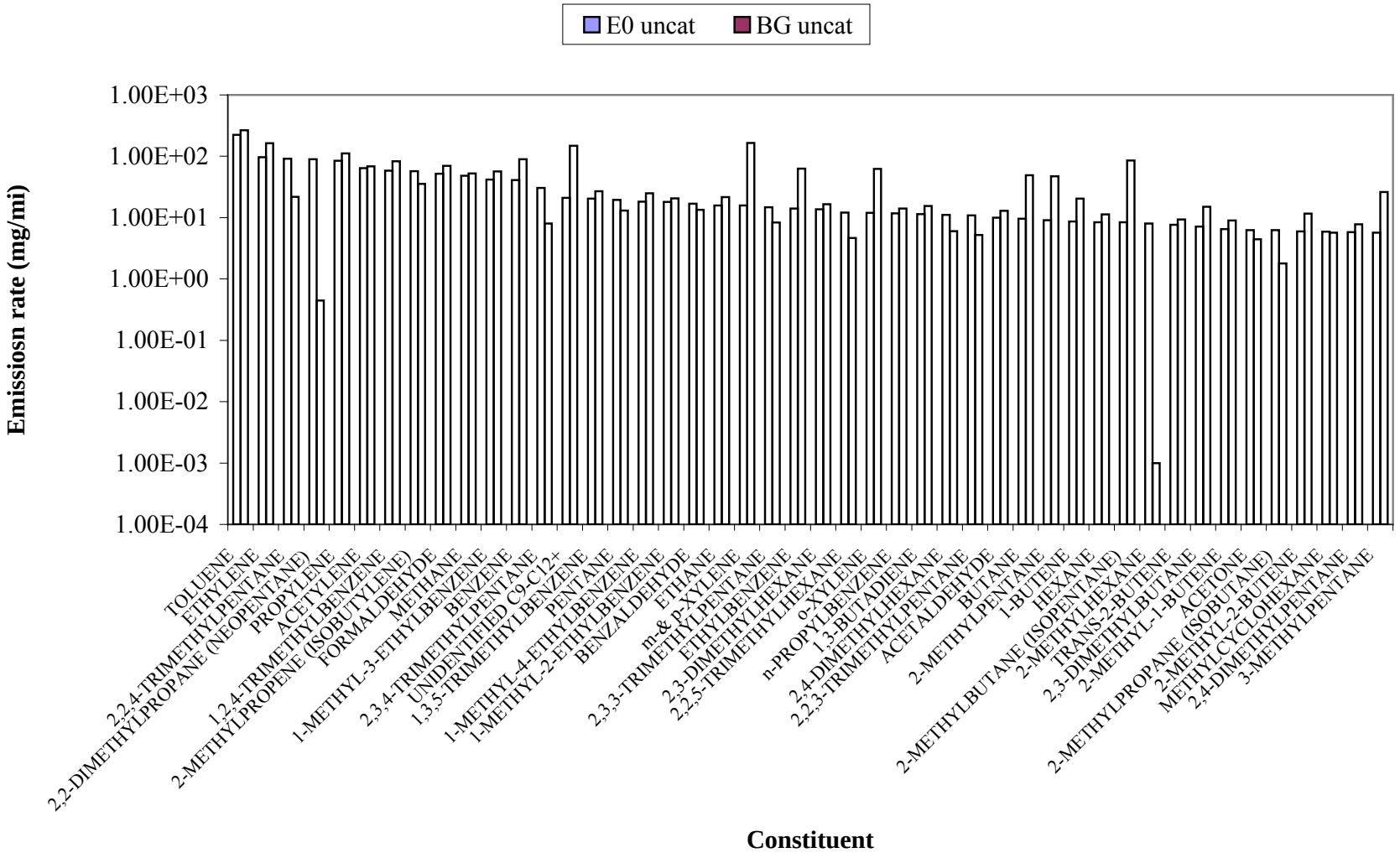

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Figure 27 Uncatalyzed exhaust 2: E0 and BG

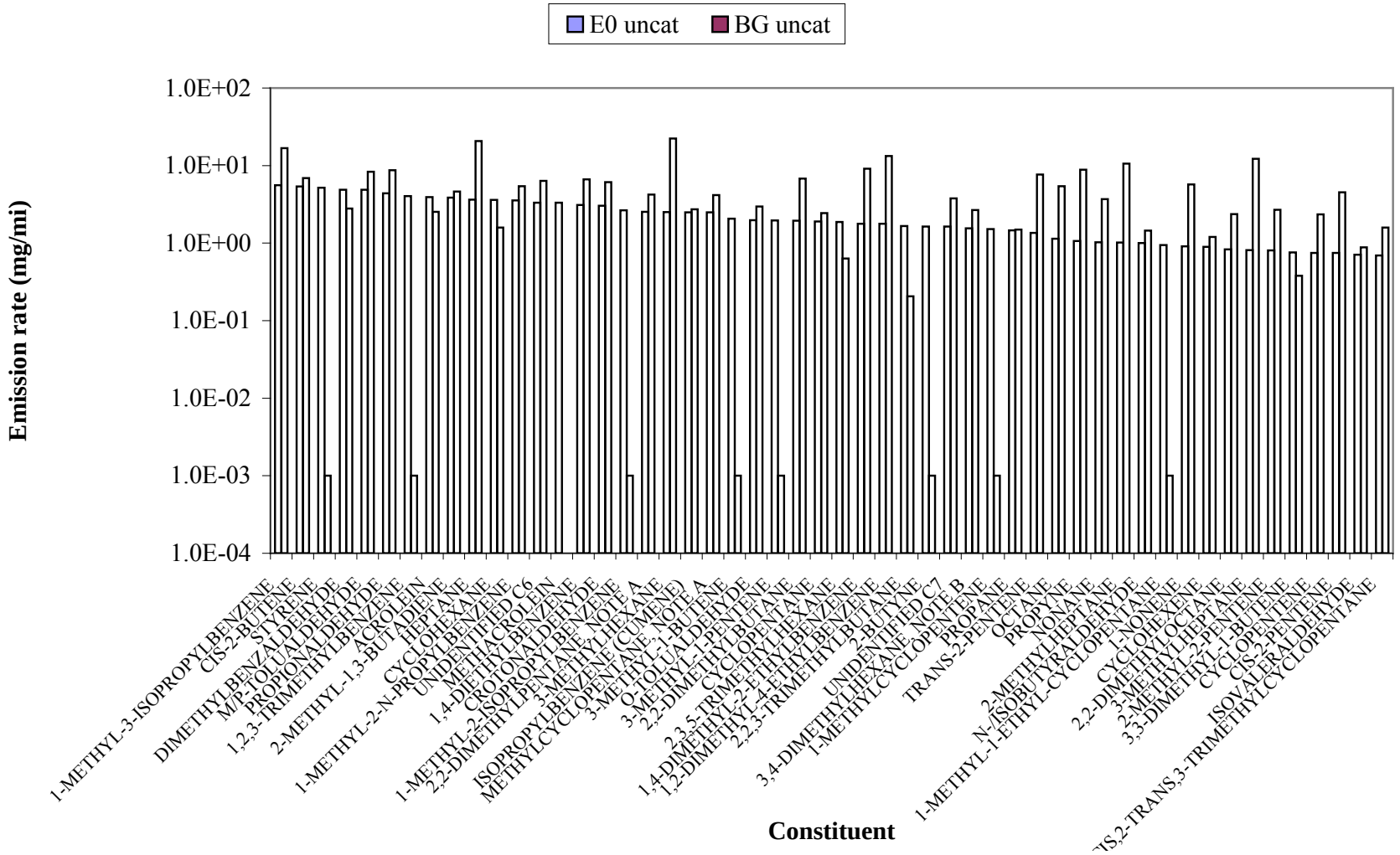

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Figure 28 Uncatalyzed exhaust 3: E0 and BG

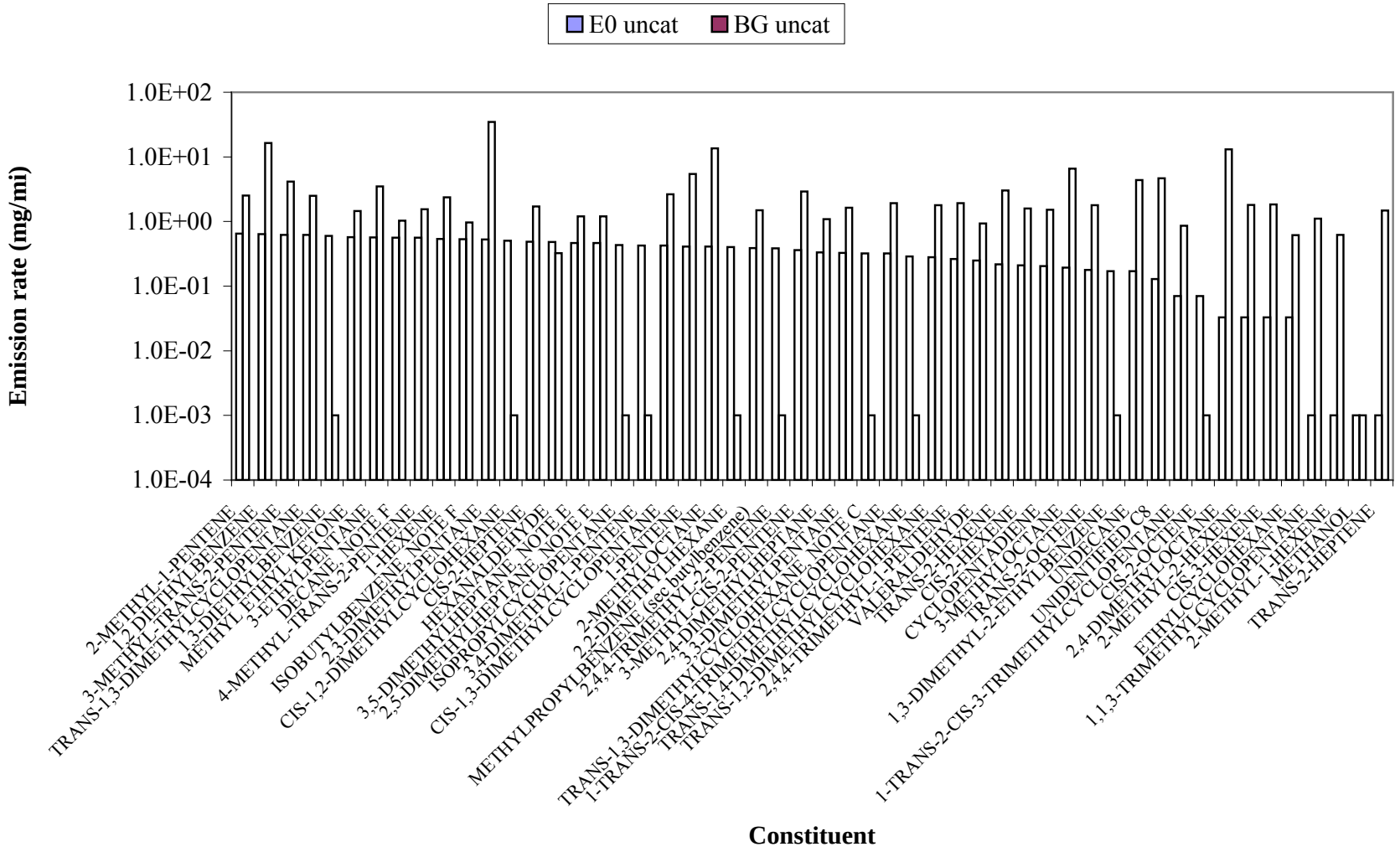

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Figure 29 Catalyzed exhaust: E10, E15, and BG+E

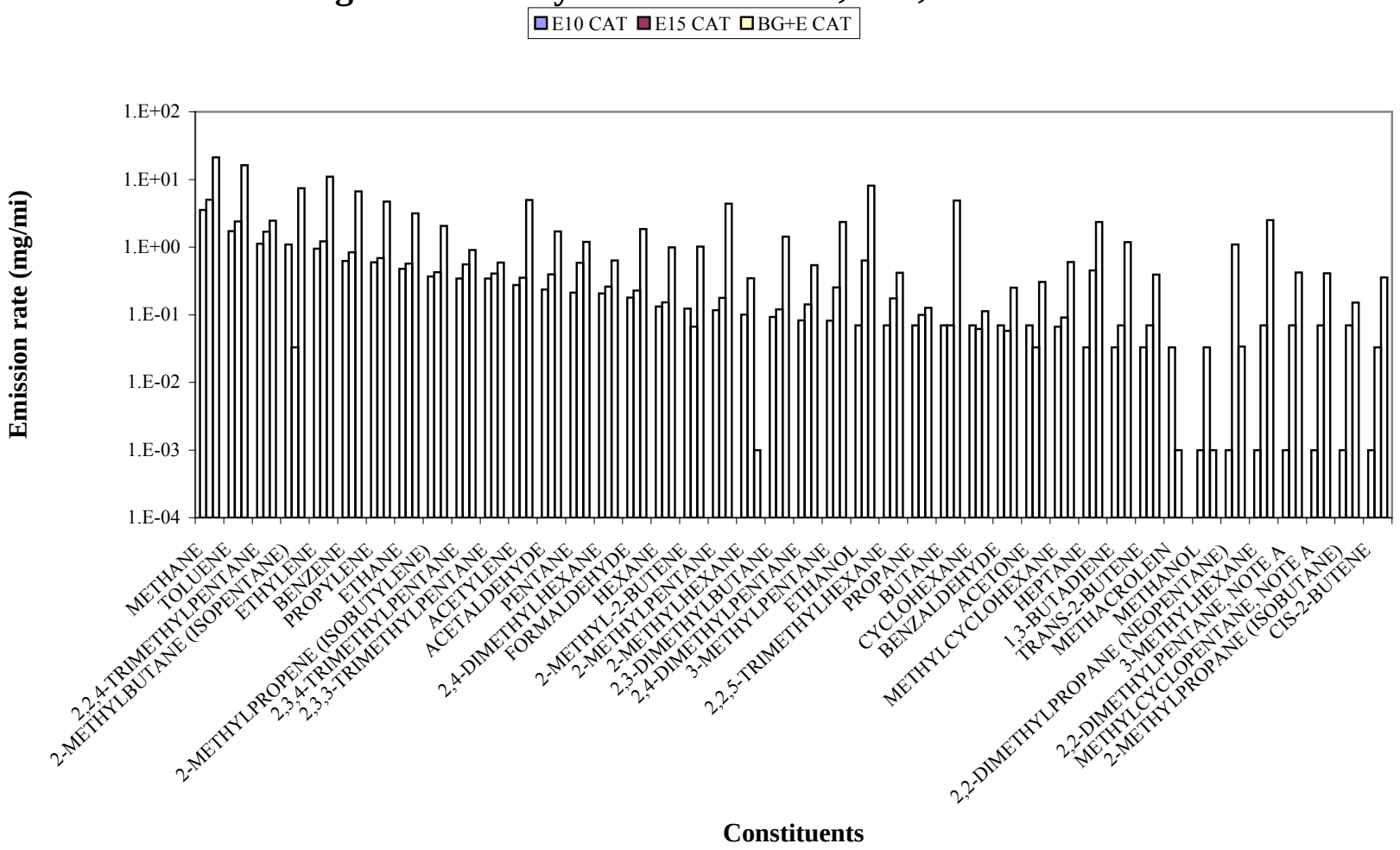

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Figure 30 Uncatalyzed exhaust 1: E10, E15, and BG+E

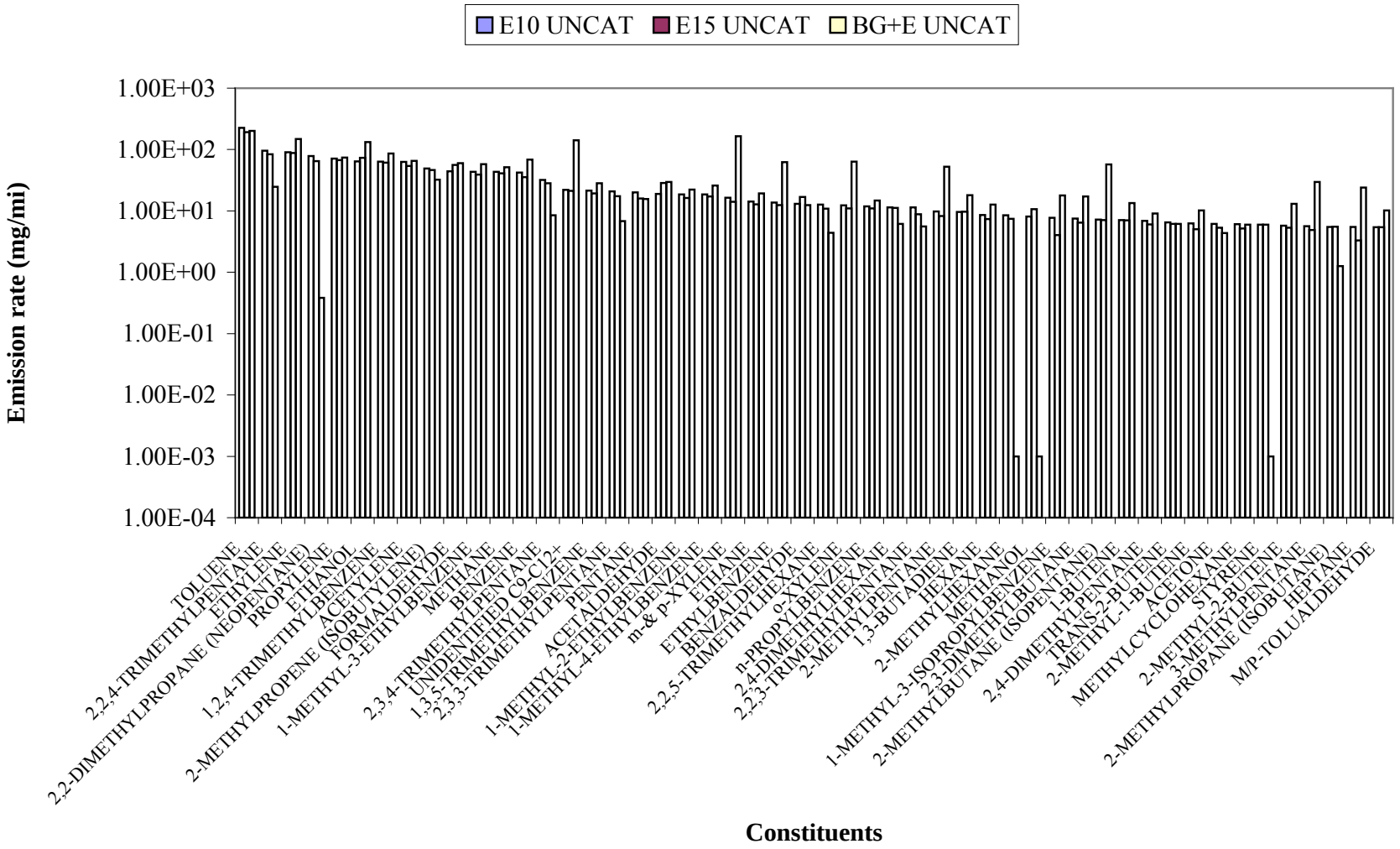

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Figure 31 Uncatalyzed exhaust 2: E10, E15, and BG+E

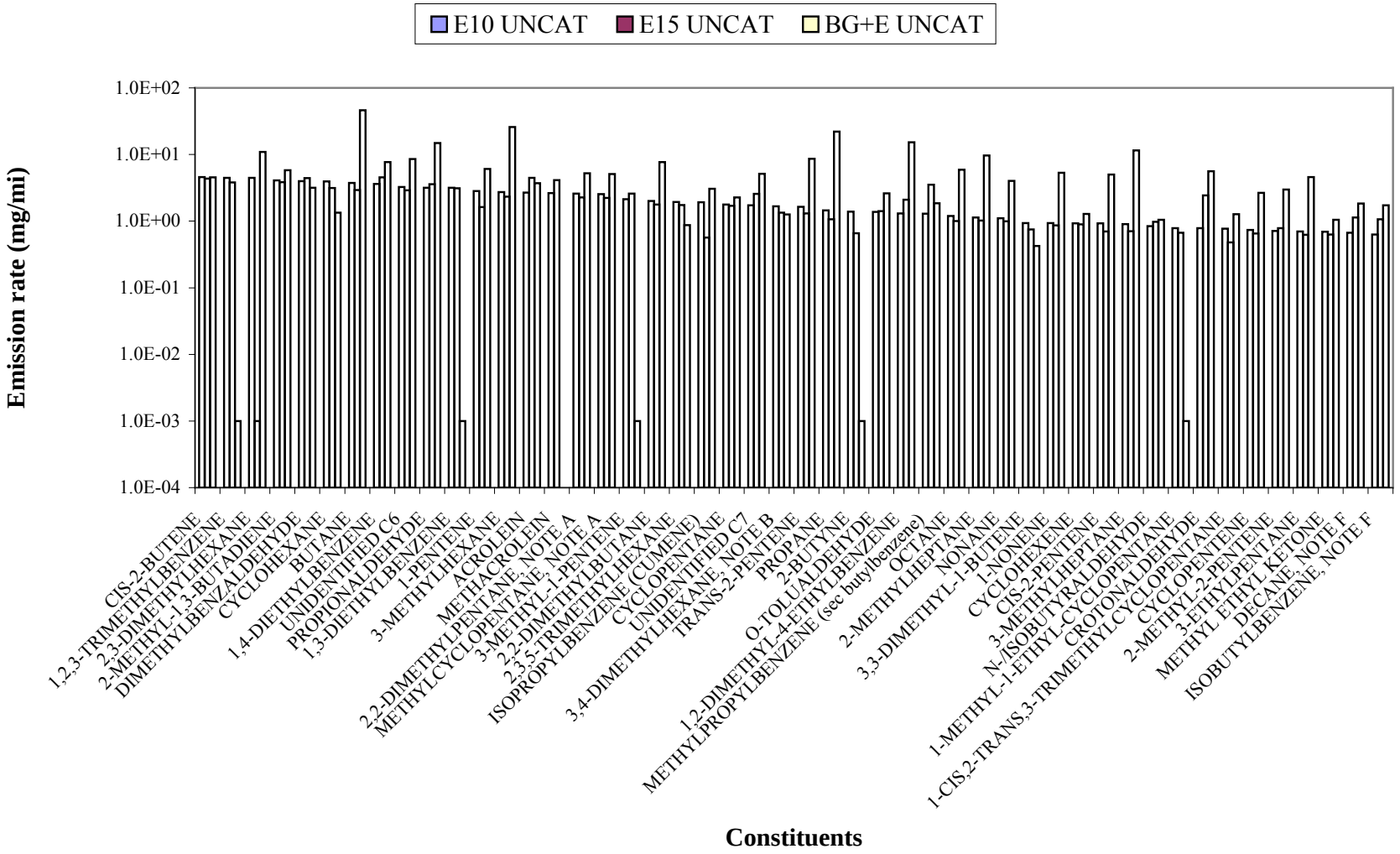

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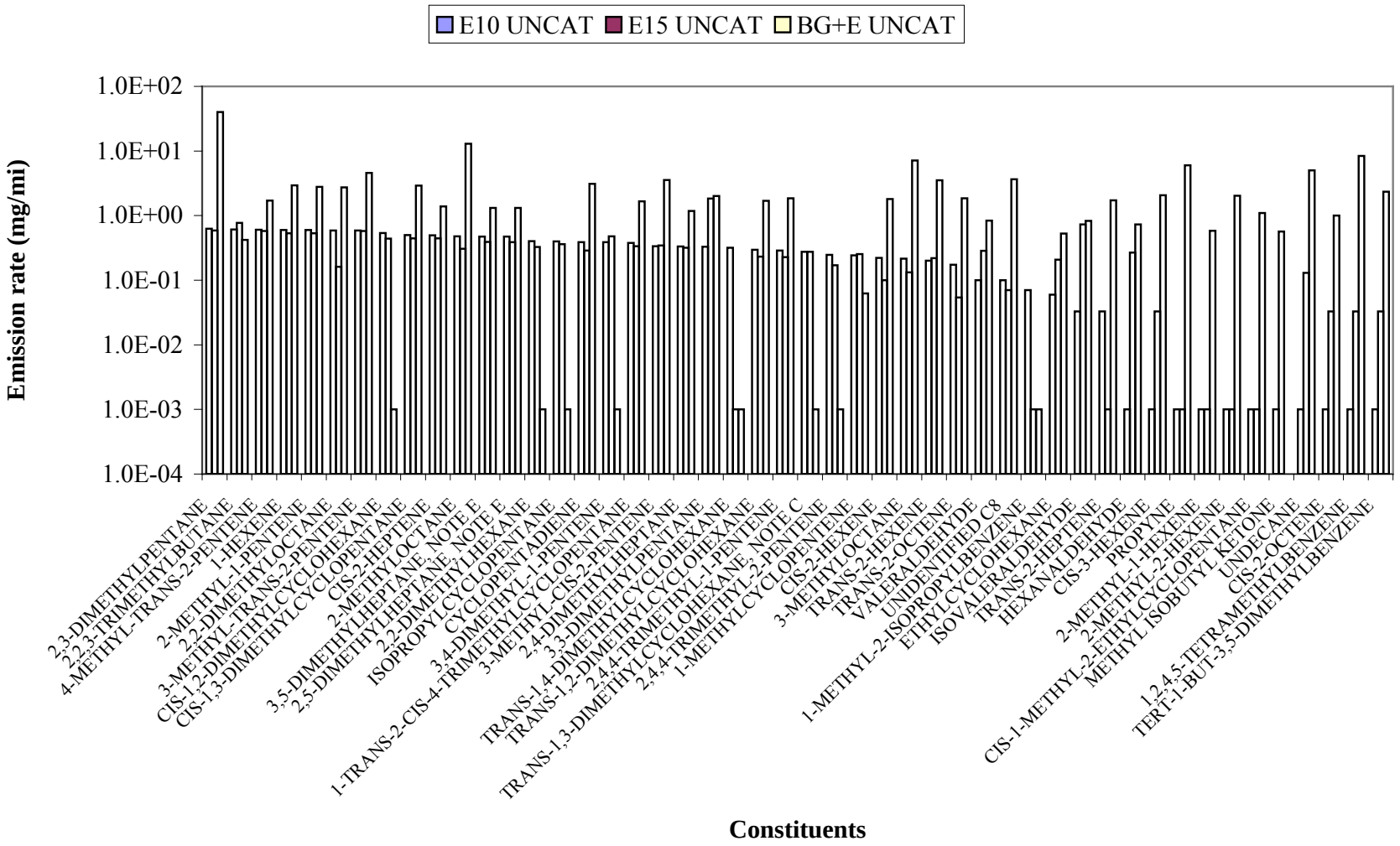
Figure 32 Uncatalyzed exhaust 3: E10, E15, and BG+E



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Addendum to: Characterization of 2011 E0, E10, and E15: Comparison to Data Developed by the Section 211(b) Research Group

Prepared for:
Growth Energy and the Renewable Fuels Association

Prepared by:
ENVIRON International Corporation
Tampa, Florida
Arlington, Virginia
Emeryville, California

Date:
February 3, 2012

Project Number:
24-23486C

Contents

1 Introduction and Purpose

This addendum was prepared to address a potential information gap in Cambridge Environmental, Inc.'s February 17, 2011 submission to EPA on behalf of the Renewable Fuels Association and Growth Energy entitled "Characterization of 2011 E0, E10, and E15: Comparison to Data Developed by the Section 211(b) Research Group" (Cambridge Environmental, Inc. 2011). In response to the ethanol industry's application to increase the allowed ethanol content that can be blended into gasoline from 10 percent (E-10) to 15 percent (E-15), Cambridge Environmental. Inc.'s submission served to fulfill requirements stipulated by EPA under Title 40, Section 79.52(d) that any new registration applications be accompanied by emissions characterization data and health impact information for the emissions. The Cambridge Environmental, Inc. submission addressed the former requirement, while a follow-on submission by ENVIRON entitled "Literature Review and Report: Ethanol-15 (E-15) Emission Product Health and Welfare Effects Studies Summary" (ENVIRON 2011) addressed the latter by evaluating, via a literature search, the health effects information for 18 emission compounds found in both unleaded certification fuel (E-0) and unleaded certification fuel with 15% ethanol (E-15).

An underlying assumption in ENVIRON's submission, although not expressly stated, was that E-10 and E-15 are identical or nearly identical fuels, or at a minimum are "reasonably comparable" in that health effects caused by E-15, which have not been specifically identified by studies of E-15, would be the same as those for E-10, which have specifically been identified through studies of this fuel through Alternative Tier 2 testing.[1] This addendum was prepared to validate the assumption of "reasonably comparable" such that Alternative Tier 2 testing of E-15 need not be conducted.

EPA regulations in 40 CFR 79.53(d) define "reasonably comparable" as it pertains to testing conducted on fuels:

> "After submission of all information and testing, EPA in its judgment shall determine whether previously conducted tests relied upon in the registration submission are adequately performed and documented and provide information reasonably comparable to that which would be provided by the tests described herein."

"Previously conducted tests" can refer to tests on E-10 fuel; "that which would be provided by the tests described herein" can mean Alternative Tier 2 tests conducted on E-15 fuel, if they were to be conducted.

Further, a presentation by Mr. Jim Caldwell of EPA's Office of Transportation and Air Quality at the SAE Government/Industry Meeting in Washington, D.C. on May 13, 2008 provided an interpretation of "reasonably comparable" as it relates to E-10 and higher ethanol fuels:

[1] In Alternative Tier 2 testing, laboratory animals are subjected to evaporative emissions of a fuel such as E-15 and screening toxicity tests are performed.

> "Based on a comparison of the E-10 Tier 1 speciation data and the higher level blend Tier 1 data, the E-10 [Alternative] Tier 2 health effects data . . . may be applicable to higher level blends of ethanol [such as E-15] . . . This would procedurally involve a submission of a scientific argument that the E-10 testing essentially covers the testing assigned to the higher level blend [such as E-15]."

This addendum serves as the "scientific argument" that the E-10 testing previously conducted has adequately characterized the health effects of E-15.

2 Studies of E-10 and the Basis for "Reasonably Comparable"

The basis for "reasonably comparable" is the assumption that, even though E-15 evaporative emissions showed an increase, compared to E-10, in ethanol concentration in the emissions profile, the health effects of this increase are demonstrated in the E-10 Alternative Tier 2 testing. In essence, the E-10 Alternative Tier 2 testing is still relevant to health impacts expected from any increases in exposure from the increased ethanol content of E-15.

E-10 has been extensively studied and the results of toxicity tests in laboratory animals (rats) are available in the EPA docket at http://www.regulations.gov/#!searchResults;rpp=10;po=0;s=EPA-HQ-OAR-2003-0065. This docket contains nearly 700 documents related to toxicity testing for E-10 and other fuel blends; however, four studies in particular are germane to E-10 toxicity and the issue of "reasonably comparable." These studies, conducted by the API (American Petroleum Institute) 211(b) Research Group, are as follows:

- "Gasoline Ethanol Vapor Condensate: A One-Generation Whole-Body Inhalation Reproduction Toxicity Study in Rats" dated March 18, 2009 (docket nos. EPA-HQ-OAR-2003-0065-0521 and EPA-HQ-OAR-2003-0065-0523);
- "Whole-Body Developmental Toxicity Study in Rats with Gasoline with Ethanol Vapor Condensate (MRD-00-714)" undated (EPA docket no. EPA-HQ-OAR-2003-0065-0487);
- "Gasoline Ethanol Vapor Condensate: A 13-Week Whole-Body Inhalation Toxicity Study in Rats with Neurotoxicity Assessments and 4-Week *In Vivo* Genotoxicity and Immunotoxicity Assessments" dated January 27, 2010 (docket nos. EPA-HQ-OAR-2003-0065-0558 through EPA-HQ-OAR-2003-0065-0561); and
- "Immunological Evaluation of Gasoline Ethanol Vapor Condensate in Female Sprague Dawley Rats Using the Plaque Forming Cell Assay" dated January 4, 2002 (EPA docket no. EPA-HQ-OAR-2003-0065-0189).

These studies are described in more detail below.

2.1 Reproduction Toxicity Study

Exposure of rats to 0 (control) 2,000, 10,000 and 20,000 mg/m^3 of gasoline-ethanol vapor condensate[2] (GEVC) produced no effect at any of the exposure levels on reproductive performance in the study, including mating, fertility, parturition, lactation and offspring survival.

2.2 Developmental Toxicity Study

The developmental toxicity of GEVC was evaluated in rats at concentrations of 0 (control), 2,000, 10,000, and 20,000 mg/m^3 in air. The animals were exposed daily for six hours from Gestation Day (GD) 5 through GD 20. The study showed that there were no exposure-attributable statistically significant differences for uterine implantation data, and external, skeletal, and visceral observations in the fetuses. Thus, exposure to GEVC at levels up to 20,000 mg/m^3 did not cause developmental toxicity. In addition, this study reported evidence of slight maternal toxicity in the 20,000 mg/m^3 target concentration-treated dams (females) as indicated by a statistically significant decrease in the GD 20-21 and GD 0-21 intervals body weight change intervals and statistically significant linear trends (decreases) in the GD 21 body weight and the body weight change data for the GD 8-11, GD 20-21, GD 5-21, GD 0-21, and GD 0-21C intervals. The decreases in body weight change, however, were noted by the authors to be a reflection of the statistically significant trends in decreased food consumption that were noted for the majority of the food consumption intervals. In addition, it is important to note that body weights of high-level and mid-level females treated with baseline gasoline vapor condensate (BGVC) – that is, vapor from fuel not containing ethanol – have been reported to be below control values (API 2004), indicating that the effect on maternal body weight may be due to components in GEVC other than ethanol.

2.3 13-Week Study Including Neurotoxicity

This study was designed to assess the potential inhalation toxicity of GEVC when administered via whole-body exposures to rats for 13 weeks followed by a 4-week recovery period. The assessments included routine toxicology parameters such as genotoxicity and immunotoxicity as well as detailed evaluations of neurotoxicity. The test substance was administered at target concentrations of 2,000, 10,000 and 20,000 mg/m^3 for 6 hours per day, 5 days per week for 13 weeks. In addition, an air control group received nitrogen-enriched air only while in chamber. Gasoline-ethanol vapor condensate did not show any evidence of immunotoxicity or genotoxicity. Neurotoxicity was assessed by measuring glial fibrillary acidic protein (GFAP) concentrations in the brain after inhalation exposure to gasoline-ethanol vapor condensate. The GFAP assay was performed on tissue from 9 areas of brain from rats (5 per sex per group) exposed to gasoline-ethanol vapor by inhalation at the above-listed concentrations.

Exposure to GEVC caused slight increases in GFAP in several brain regions in males; in general, these increases were not concentration-related. However, GFAP in the cerebellum region of the brain was statistically significantly increased at all exposure concentrations. The degree of GFAP increase (approximately 30%) was marginal, indicating minor gliosis (i.e., changes in glial cells in the brain). No signs of neurotoxicity were observed in any of the treated animals, and no neuropathological findings were evident. Statistically significant increases in GFAP were not detected in females.

[2] The gasoline-ethanol vapor condensate used in this and the other studies described below contained approximately 10% ethanol.

There are several reasons that the positive GFAP assay results are at best inconclusive and at worst not real. To begin with, given the large number of GFAP tests conducted – more than 500 (2 sexes of rats x 9 brain regions x 4 ethanol vapor concentrations including controls x 7 different fuels including E-10) – the observed positive GFAP results could simply be a result of statistical chance. In other words, the likelihood of a "false positive" result increases with an increase in the number of tests.

Second, the authors of the study reported that statistically significant increases in GFAP (compared to air controls) occurred in only 9 of the 27 tests with E-10 (9 brain regions x 3 test concentrations = 27 tests) – in 5 brain regions at the low dose (2,000 mg/m^3); in 3 regions at the mid-dose (10,000 mg/m^3); and in only 1 region at the highest dose (20,000 mg/m^3). In the 18 other tests, no increases in GFAP occurred. Such a non-systematic pattern of increase argues against a true effect of E-10 on GFAP.

Third, the study did not indicate an increase in GFAP in brain regions with increasing dose. As described above, most (5 of 9) of the increases occurred at the lowest dose; only 3 of 9 occurred at the mid-dose; only one occurred at the high dose. In other words, there occurred no increase in GFAP with increasing gasoline-ethanol vapor concentration – no dose-response effect – as might be expected if GFAP were in fact affected by treatment.

Fourth, increases in GFAP occurred only in males. No increases in GFAP occurred in females. Although there are examples of sex-dependent toxicity in the scientific literature (many are sex hormone-dependent), most toxicities observed occur in both males and females.

Fifth, it has been noted by the API 211(b) Research Group (Twerdok 2002) that although increased levels of GFAP can be interpreted as very early markers of potential neurotoxicity in rats, it is difficult to extrapolate increases in GFAP to overt neurotoxicity due to the many redundant neural pathways of the brain. In fact, as was noted in the 13-week study, no signs of neurotoxicity were observed in any of the treated animals, and no neuropathological findings were evident.

Sixth, ethanol given to rats via ingestion caused an increase in GFAP (Udomuksorn et al. 2011), thus establishing a positive GFAP response to ethanol in rats. In this study, GFAP was increased in all parts of the cerebral cortex following exposure to a high dose of ethanol after 21 days and to both high and low doses of ethanol after 3 months or 6 months of treatment, compared to those of age-matched control groups. In this study, rats treated with 2 to 5 g ethanol per kg body weight were found to have blood ethanol concentrations (BECs) from between 30 to 150 milligrams per deciliter (mg/dL). However, pharmacokinetic modeling (i.e., modeling performed to predict concentrations of substances in tissues, blood, urine, and other bodily fluids of laboratory animals and humans as a consequence of exposure to those substances) showed that the maximum or peak BECs that can be achieved in rats as a consequence of ethanol inhalation were of the order of 0.07 to 0.33 mg/dL, depending on the exposure concentration and exposure duration (Pastino et al. 1997).[3] Thus, BECs as a result of

[3] In this study, rats were exposed to either 600 ppm ethanol for 6 hours or to 200 ppm for 30 minutes. For the 6-hour exposure to 600 ppm, the maximum BEC was 71 microM, or 0.33 mg/dL. For the 30-minute

ingestion (the route of exposure in the Udomuksorn et al. 2011 study) are more than 400 times higher than the blood concentrations that can be achieved via the inhalation route of exposure (the most likely route of exposure to E-10 fuel).[4] As a consequence, it is highly unlikely that an increase in ethanol content from 10% (in E-10) to 15% (in E-15) – an increase of only 1.5 times – would be sufficient to increase blood levels to the point where neurotoxic effects, such as those demonstrated through an increase in GFAP, would manifest from inhalation exposure to E-15.

2.4 Immunotoxicity Study

The objective of the immunotoxicity study was to determine the potential effects of GEVC for its ability to affect the humoral (i.e., antibody-generating) immune component of the immune system, when evaluated in the antibody-forming cell response to the T-dependent antigen, sheep erythrocytes. Female Sprague Dawley rats were administered GEVC for 5 days per week for 4 weeks by inhalation via whole body exposure. Three exposure levels – 2,000, 10,000, and 20,000 mg/m^3 of GEVC – were used in the study.

In contrast to the developmental toxicity study (Section 2.2), in which decreases in maternal body weight were reported in the 20,000 mg/m^3 target concentration-treated females, exposure to GEVC resulted in no statistically significant changes in body weight for any exposure level. Exposure to GEVC did result in a statistically significant dose-related decrease in the IgM antibody-forming cell (AFC) response to the T-dependent antigen, sheep erythrocytes, when evaluated as either specific activity (AFC/10^6 spleen cells) or as total spleen activity (AFC/spleen). The decrease reached the level of statistical significance only at the high (20,000 mg/m^3) exposure concentration. At the mid-range test concentration (10,000 mg/m^3 GEVC), a decrease in the IgM AFC response was observed, but this decrease was not statistically significant. A LOAEL (lowest observed adverse effect level) for GEVC immunotoxicity in female rats was reported to be about 10,000 mg/m^3 based on an approximately 50% suppression of the IgM-mediated humoral response. The NOAEL (no observed adverse effect level) for GEVC immunotoxicity in female rats was approximately 2,000 mg/m^3. The authors concluded that, under the experimental conditions used, exposure to GEVC adversely affected the functional ability of the humoral immune component of the immune system.

This effect, or more precisely, the ethanol concentration causing this effect, requires context. The ethanol concentration reported to cause immunotoxicity in rats was between 200 mg/m^3 and 1,000 mg/m^3, assuming the ethanol concentration in GEVC in this study was 10%, and the

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exposure to 200 ppm, the peak BEC was approximately 15 microM, or 0.07 mg/dL. The maximum or peak BEC values reported for this study are applicable only to the exposure concentrations and exposure durations used in the study.

[4] The 400-times higher value was calculated by dividing the ingestion route BEC concentrations (30 to 150 mg/dL) by the inhalation route BEC concentrations (0.07 to 0.33 mg/dL). It is important to note that BECs in rats are dependent on both the concentration of and duration of exposure to ethanol, such that the ratio of BECs for the ingestion route compared to the inhalation route could be greater than or less than the ratio calculated herein. Other factors affecting BECs include the age, body weight, and strain of rat, as well as any ethanol tolerance that may develop in rats treated over relatively longer periods of time.

NOAEL and LOAEL for immunotoxicity are as reported in this study, or 2,000 and 10,000 mg/m^3, respectively (2,000 mg/m^3 x 10% = 200 mg/m^3; 10,000 mg/m^3 x 10% = 1,000 mg/m^3). If between 200 mg/m^3 and 1,000 mg/m^3 ethanol in an immunotoxicity study with E-10 causes an effect, then it is reasonable to assume that between 200 mg/m^3 and 1,000 mg/m^3 ethanol in an immunotoxicity study with E-15 also causes an effect, even though E-15 has not been specifically tested in a rat immunotoxicity study. Compared to human exposures to ethanol from 10% ethanol gasoline as reported in the so-called "Chicago Microenvironment" study (Desert Research Institute 2004), in which it was shown that the highest ethanol exposure in the study – nearly 120 parts per billion (ppb), or 0.23 mg/m^3 – occurred for persons engaged in outdoor refueling (see page 2-24 of the Desert Research Institute 2004 study), the immunotoxicity effects range for ethanol in rats is from approximately 900 (200 mg/m^3 vs. 0.23 mg/m^3) to more than 4,000 (1,000 mg/m^3 vs. 0.23 mg/m^3) times greater than human ethanol exposures. This means that the ethanol exposure concentrations in E-10 or E-15 gasoline that cause immunotoxicity in rats are substantially (up to more than 4,000 times) greater than measured ethanol exposures in humans from E-10 gasoline, thus calling into question the relevance, if any, of the immunotoxicity effects observed in rat studies.

3 Summary

This addendum was prepared to validate the assumption of "reasonably comparable" such that Alternative Tier 2 testing of E-15 need not be conducted, thus filling a potential information gap in ENVIRON's report entitled "Literature Review and Report: Ethanol-15 (E-15) Emission Product Health and Welfare Effects Studies Summary."

E-10 has been extensively studied and the results of available toxicity tests in laboratory animals showed negligible effects of E-10 but for neurotoxicity and immunotoxicity studies. For neurotoxicity, effects of E-10 were demonstrated by increased concentrations of GFAP in certain regions of the male rat brain. However, there are several reasons that the positive GFAP assay results are highly questionable:

- Given the large number of GFAP tests conducted, the observed positive GFAP results could simply be a result of statistical chance – a "false positive" result.

- Statistically significant increases in GFAP occurred in only 9 of the 27 tests with E-10. In the 18 other tests, no increases in GFAP occurred. This non-systematic pattern of increase argues against a true effect of E-10 on GFAP.

- There occurred no increase in GFAP in brain regions with increasing dose; i.e., no dose-response effect as might be expected if GFAP were in fact affected by ethanol treatment.

- Increases in GFAP occurred only in males. Most toxicities reported in the scientific literature occur in both males and females.

- Although increased levels of GFAP can be interpreted as early markers of potential neurotoxicity in rats, it is difficult to extrapolate these results to overt neurotoxicity due to the many redundant neural pathways of the brain.

- Since pharmacokinetic modeling shows that the ethanol blood levels that can be achieved in rats as a consequence of ethanol ingestion are more than 400 times higher than the blood concentrations that can be achieved via inhalation (the most likely route of exposure to E-10 fuel), it is highly unlikely that an increase in ethanol content from 10% (in E-10) to 15% (in E-15) would be sufficient to increase blood levels to the point where neurotoxic effects would occur from inhalation exposure to E-15.

Regarding immunotoxicity, effects of E-10 were demonstrated by a dose-related decrease in the IgM antibody-forming cell (AFC) response to sheep erythrocytes. However, the ethanol exposure concentrations in E-10 (or E-15) gasoline that caused immunotoxicity in rats were substantially (up to more than 4,000 times) greater than measured ethanol exposures in humans from E-10 gasoline, thus calling into question the relevance, if any, of the immunotoxicity effects observed in rat studies.

Thus, in can be demonstrated that E-10 and E-15 fuels are “reasonably comparable” such that Alternative Tier 2 testing of E-15 need not be conducted.

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Literature Review and Report: Ethanol-15 (E-15) Emission Product Health and Welfare Effects Studies Summary

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Growth Energy and the Renewable Fuels Association

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Contents

List of Tables – Health and Welfare Effects Summaries

List of Attachments – Literature Search Results

1 Executive Summary

An increase in ethanol content in gasoline from 10 percent (E-10) to 15 percent (E-15) requires the registration of ethanol as a fuel additive at increasing concentrations, and pursuant to Section 79.52(d), any new registration application must be accompanied by health impact information. Testing was conducted to identify the types and concentrations of emissions products of E-15 and per 40 CFR 79.52(d)(3), a literature search was performed for 18 emission products found for E-15 that were not found in the testing on base gasoline by the American Petroleum Institute testing consortium, which was submitted to EPA in 1997. Commercial databases were searched to identify the relevant health and welfare effects literature for ethanol and the 17 additional subject chemicals.

Reviews of the literature indicated that, for 10 of the 18 chemicals – 1-methyl-1-ethylcyclopentane, 2,2-dimethylhexane, 2,4,4-trimethyl-2-pentene, 2-methylhexane, 3,4-dimethyl-1-pentene, 3-methyl-1-pentene, cis-2-octene, isopropylcyclopentane, trans-1,3-dimethylcyclohexane, and trans-1,4-dimethylcyclohexane – there were no health or welfare effects studies, so an assessment of the health and welfare effects of these chemicals was not possible. These chemicals, however, are all short carbon chain substituted alkanes and alkenes that most likely produce relatively mild neurological effects (central nervous system depression) at high concentrations in air, similar to the non-substituted alkanes such as propane, butane, pentane, etc. Similarly, cis-1,3-dimethylcyclohexane, for which no health effects studies were found, is expected to be a mild central nervous system depressant at high concentrations. For the related short carbon chain alkyne 2-butyne, there was limited evidence in animals that it was toxic when administered via the oral route, and insufficient evidence that it was toxic in humans.

For the substituted benzenes – 1,2,3-trimethylbenzene (TMB) and 1,3-diethylbenzene – there was some evidence of toxic effects in laboratory animals but insufficient evidence of these effects in humans. Oral administration of 1,3-diethylbenzene did not appear to be neurotoxic in rats and there was no evidence in humans of potential toxic effects. There was limited information available on the welfare effects of TMB and 1,3-diethylbenzene.

Studies showed that exposure to high concentrations of ethanol via inhalation may result in some mild reversible, neurobehavioral, biochemical, hematological and histological effects. Studies also suggested that ethanol may be mildly genotoxic, and/or that it is ethanol‘s metabolite, acetaldehyde, that may be genotoxic. Carcinogenicity data suggested that exposure to ethanol via inhalation was not carcinogenic. Ethanol exposure via inhalation at concentrations experienced in the workplace is not expected to be carcinogenic in humans or result in any noticeable acute effects. The evidence from the welfare effects studies indicated that at certain concentrations, ethanol reduced fertility, effected development, and resulted in behavioral effects in aquatic organisms.

Methacrolein was shown to elicit respiratory effects in animals. There was only very limited evidence of toxicity in humans. There was limited information available on the welfare effects of methacrolein – a single study that showed trees served as a significant sink for methacrolein.

Methanol was shown to be acutely toxic in animals and humans exposed to high concentrations via inhalation and ingestion; however, genotoxicity and cytotoxicity studies indicated that methanol is not genotoxic and likely does not pose a carcinogenic risk to humans. Welfare effects studies indicated that methanol exhibited low ecotoxicity in aquatic species.

There was some evidence that styrene was toxic in laboratory animals and neurotoxic in humans; however, this effect in humans was observed in occupationally-exposed individuals who were exposed to high concentrations of styrene in air. Styrene was shown to be mutagenic/genotoxic in animals. There was some suggestive evidence of styrene carcinogenicity in animals; however, there was no evidence of this in humans. The evidence from the welfare effects studies indicated the styrene was moderately toxic to aquatic organisms.

It can be concluded from the available studies on 7 of the 18 emission compounds found in both unleaded certification fuel (E-0) and E-15 – i.e., 2-butyne, 1,2,3-trimethylbenzene, 1,3-diethylbenzene, ethanol, methacrolein, methanol, ands styrene – that sufficient health and welfare effects data exist such that further characterization of these effects through, for example, Alternative Tier 2 testing, is not warranted. For the remaining 11 emission compounds for which limited or no health or welfare effects data were found – i.e., 1-methyl-1-ethylcyclopentane, 2,2-dimethylhexane, 2,4,4-trimethyl-2-pentene, 2-methylhexane, 3,4-dimethyl-1-pentene, 3-methyl-1-pentene, cis-2-octene, isopropylcyclopentane, trans-1,3-dimethylcyclohexane, trans-1,4-dimethylcyclohexane, and cis-1,4-dimethylcyclohexane – further health and welfare effects testing likewise is not required since these chemicals are all short carbon chain substituted alkanes and alkenes that most likely display similar effects to the non-substituted alkanes and alkynes for which ample data exist.

2 Introduction and Purpose

Ethanol has been used in the U.S. as a fuel additive for more than 30 years to improve the air quality impacts from the combustion of petroleum-based motor fuels. The ethanol industry seeks to increase the allowed ethanol content that can be blended into gasoline. An increase in ethanol content into gasoline requires the registration of ethanol as a fuel additive at increasing concentrations. The registration of new fuel and fuel additives into the marketplace is regulated by EPA under Title 40, Section 79. Specifically, per Section 79.52(d), any new registration application must be accompanied by health impact information; the information in this report was developed in support of increasing the ethanol content in gasoline, specifically the registration of ethanol in E-15, a fuel blend of 15% ethanol and 85% gasoline, as a fuel additive.

The Renewable Fuels Association (RFA) and Growth Energy completed testing to identify the types and concentrations of emissions products per 40 CFR Section 79.52(d) and results for combustion and evaporative emissions were submitted to EPA on February 18, 2011. After review of these data, EPA requested a literature research be completed for 18 emission compounds found in both unleaded certification fuel (E-0) and unleaded certification fuel with 15% ethanol (E-15). As stated in the regulations, the literature search submittal to EPA must include results of searching appropriate commercially available chemical, toxicologic, and environmental databases using a minimum of CAS numbers, chemical names and common synonyms. The time period to be covered by the literature search is at least 30 years prior to February 18, 2011, the date the Tier 1 report and E-15 emission testing results were submitted to EPA, and ending no earlier than six months prior to that date.

The E-15 emission compounds of interest include the following 18 chemicals:

1,2,3-trimethylbenzene	1,3-diethylbenzene	1-methyl-1-ethyl-cyclopentane
2,2-dimethylhexane	2,4,4-trimethyl-2-pentene	2-butyne
2-methylhexane	3,4-dimethyl-1-pentene	3-methyl-1-pentene
cis-1,2-dimethylcyclohexane	cis-2-octene	ethanol
isopropylcyclopentane	methacrolein	methanol
styrene	trans-1,3-dimethylcyclohexane	trans-1,4-dimethylcyclohexane

The requirements for the literature search report are described at 40 CFR 79.59 (c)(4):

> ~~t~~he literature search shall include a text summary of the methods and results of the literature search, including the following: (A) Identification of person(s) performing the literature search, (B) Description of data sources accessed, search strategy used, search period, and terms included in literature search, (C) Documentation of all unpublished in-house and other privately-conducted studies, (D) Tables summarizing the protocols and results of all cited studies, (E) Summary of significant results and conclusions with respect to the effects of the emissions of the subject fuel or fuel additive on the public health and welfare, including references if used to support such results and conclusions, (F) Statement of the extent to which the literature search has produced adequate information comparable to that which would otherwise be obtained through the performance of applicable emission characterization requirements under §79.52(b)

and/or health effects testing requirements under §79.53, including justifications and specific references."

Section 3 of this report identifies the person performing the literature searches and provides a description of data sources accessed, search strategy used, search period, and terms included in literature search. Sections 4 through 21 summarize the health effects and welfare effects studies for each of the 18 subject chemicals. A summary and conclusions are provided in Section 22. A complete list of references is provided in Section 23. Tables 1 through 18 that appear after the references summarize the protocols and results of all cited studies for each of the 18 chemicals of interest, and literature search output for each chemical are provided in Attachments A through R.

3 Methods

This report was prepared by ENVIRON's Risk Assessment & Risk Management group, consisting of an international team of toxicologists, health scientists, and environmental scientists, which has over 25 years of experience in assessing human health and environmental risks. ENVIRON scientists have played leading roles in the development and application of many of the methodologies used to evaluate epidemiology and toxicology data and dose-response information for purposes of human health risk assessment.

All literature searches were performed by Suzanne Radford, MBA, of ENVIRON's Emeryville, CA office. Ms. Radford has 19 years of experience and possesses a Library Information and Technology Certificate and is a member of the Special Libraries Association, officer of the Environmental Resource & Management Division. Ms. Radford is proficient in using numerous searching engines and has extensive experience with selecting and using the most appropriate engines for a particular research topic and possesses the necessary indexing skills necessary for all aspects of Internet searching.

ENVIRON searched a number of commercial databases to identify the relevant health and welfare effects literature for ethanol and the 17 additional subject chemicals. The databases selected encompassed available relevant information from industry, government, and public sources. Databases searched included PubMed[1], Google Scholar, and ECOTOX[2]. A list of relevant keywords used to search each database for applicable information that ENVIRON has used in the past to successfully identify similar studies for fuel additive evaluations is provided in the following table:

[1] The National Library of Medicine website, from which the PubMed database is accessed, also provides access to the Toxicology Data Network which includes access to the following databases: ChemIDplus, HSDB, TOXLINE, CCRIS, DART, GENETOX, IRIS, ITER, LactMed, Multi-Database, TRI, Haz-Map, Household Products, and TOXMAP.

[2] Additional databases or websites that were accessed included Oak Ridge National Laboratory (ORNL), Risk Assessment Information System (RAIS), and Wildlife Ecological Risk Assessment Management (ERAM).

Keyword List			
air pollution	biological system	biota	case
clinical	disposal	distribution	ecology/ecological
emission	epidemiology	fate and transport	field study
global climate	health effect	*in vitro*	monitor
motor vehicle emission	occupational	odor	ozone
persist	pharmacokinetic	production	sensitive population
	toxic	welfare	

As per EPA requirements set forth in 40 CFR 79.52(d), ENVIRON limited the searches to encompass the last 30 years of literature. Article titles, authors, journal (or book) citations, and abstracts identified during these searches were used to select those studies (referred to as either –primary" or –review" sources) that provided the relevant health and welfare effects associated with the 18 subject chemicals. Attachments A through R list the original output of the literature searches for the 18 chemicals.

ENVIRON reviewed each of the identified studies and develop concise summaries of the relevant information for each chemical. The summaries are organized by chemical and by topics critical to the development of a comprehensive health and welfare effects analysis and will include: chemical emissions/releases, transport and transformation, environmental quality, exposure, and health and ecological effects (both qualitative and quantitative characterizations). As part of the review, in limited instances, ENVIRON provided comments, as necessary, that included the ability of the authors of the studies to draw specific conclusions given the conduct of the study and interpretation of the results. Tables 1 through 18 that appear after the references (Section 21) summarize the protocols and results of all cited studies for each of the 18 chemicals of interest.

4 1,2,3-Trimethylbenzene

4.1 Health Effects

In acute and subacute studies, low-level inhalation exposures to 1,2,3,-trimethylbenzene (TMB) in animals induced toxic effects (Korsak et al. 1997; Siawomir et al. 2001; Lutz et al. 2010). Laboratory animals exposed via inhalation to high quantities (> 500 ppm) of TMB showed respiratory irritative effects (Korsak et al. 1997). Korsak et al. (2000) examined the effects of subchronic inhalation of TMB in rats; the authors reported pulmonary lesions as well as an increased number of goblet cells and interstitial lung parenchyma infiltration. Neurobehavioral disturbances and behavioral sensitivity were observed in rats exposed to TMB isomers at 100 ppm, and in some cases these effects were detectable several weeks after termination of exposure (Gralewicz et al. 2001; Siawomir et al. 2001; Lutz et al. 2010). Several studies found evidence of central nervous system effects (Wiaderna et al. 1998; Tomas et al. 1999). One such study found that low-level inhalation exposure to TMB isomers led to long-lasting disturbances in CNS functions (Wiaderna et al. 1998). Tomas et al. (1999) found a relationship between changes in spontaneous EEG and blood concentrations of TMB isomers (Tomas et al.

1999). Hemimellitene, the 1,2,3-TMB isomer, was found to have the highest potential for inducing CNS effects.

One study found mutagenic effects of 1,2,3-TMB on *S. typhimurium* cells. The authors found limited evidence for the genotoxic activity of 1,2,3-TMB (Janik-Spiechowicz et al. 1998). In occupationally exposed human subjects, there was an increased incidence of hearing and vestibular disorders (Sulkowski et al. 2007). To obtain toxicokinetic data on absorption and elimination of TMB and its metabolites, biological monitoring was conducted among eight human subjects, and retention of TMB isomers ranged from 68-71 % in the lungs (Kostrzewski et al. 1997). The authors proposed a biological exposure limit (BEL) for TMB, with the current maximum allowable concentration value of 100 mg/m^3 (Polish standard) baseline value.

4.2 Welfare Effects

For welfare effects, there was limited information on lethality in aquatic organisms. One study reported the effect of TMB on microbial culture from a diesel fuel-contaminated aquifer; the culture was able to grow on TMB isomers under nitrogen oxide conditions, except 1,2,3-TMB (Haner et al. 1997).

4.3 Overall Summary

Overall, there is some evidence of toxic effects of TMB in laboratory animals; however, there is insufficient evidence of these effects in humans. There was limited information available on the welfare effects of TMB.

5 1,3-Diethylbenzene

5.1 Health Effects

One health effects study in rats administered a mixture of diethylbenzene and individual isomers orally reported no changes in neurological effects including motor and sensory conduction velocities or changes in amplitude of the sensory action potential of the tail nerve of rats exposed to 1,3-diethylbenzene. However, the authors concluded that the results indicated 1,2-diethylbenzene (not 1,3-diethylbenzene) was the isomer responsible for neurotoxicity.

5.2 Welfare Effects

One study evaluated the attractiveness of 79 compounds, including 1,3-diethylbenzene, to fruit flies. The authors reported that 1,3-diethylbenzene was an effective attractant to female fruit flies (Casana-Giner et al. 2001).

Sampling studies indicated that 1,3-diethylbenzene was contained within vehicle exhaust gas (Zielinska and Gung 1994; Caplain et al. 2006).

5.3 Overall Summary

Oral administration of 1,3-diethylbenzene did not appear to be neurotoxic in rats. Overall, there was insufficient evidence in animals and no evidence in humans of potential toxic effects of 1,3-diethylbenzene.

There was limited information available on the welfare effects of 1,3-diethylbenzene.

6 1-Methyl-1-ethyl-cyclopentane

6.1 Health Effects

There are no health effects studies of 1-methyl-1-ethyl-cyclopentane.

6.2 Welfare Effects

There are no welfare studies of 1-methyl-1-ethyl-cyclopentane.

6.3 Overall Summary

There were no available/relevant studies, so an assessment of the health and welfare effects of 1-methyl-1-ethyl-cyclopentane was not possible.

7 2,2-Dimethylhexane

7.1 Health Effects

There are no health effects studies of 2,2-dimethylhexane.

7.2 Welfare Effects

There are no relevant welfare effects studies of 2,2-dimethylhexane.

7.3 Overall Summary

There were no available/relevant studies, so an assessment of the health or welfare effects of 2,2-dimethylhexane was not possible.

8 2,4,4-Trimethyl-2-pentene

8.1 Health Effects

There are no health effects studies on 2,4,4,-trimethyl-2-pentene in animals or humans.

8.2 Welfare Effects

There were no welfare effects studies on 2,4,4,-trimethyl-2-pentene.

8.3 Overall Summary

For 2,4,4,-trimethyl-2-pentene, there were no available/relevant studies, so an assessment of the health and welfare effects of 2,4,4,-trimethyl-2-pentene was not possible.

9 2-Butyne

9.1 Health Effects

Three studies in rats were conducted to investigate the potential effects of 2-butyne. The acute effects observed among rats orally exposed to high amounts of 2-butyne included growth depression, increase in liver weight, an increase in cholesterol levels, decreased red cell counts, diarrhea, congestion of internal organs, perivascular edema, bronchopneumonia, hyperemia, liver necrosis, and nephrosis (Jedrychowski et al. 1992a,b; Komsta et al. 1989). Jedrychowski et al. (1992a) concluded that 2-butyne was a moderately toxic substance and that in acute

poisonings it was hepatotoxic and nephrotoxic. Tyce (1997) also reported 2-butyne as having its primary affect on the liver and kidney. 2-Butyne had no apparent skin sensitizing potential in animals based on dermal tests on rats, rabbits and guinea pigs (Jedrychowski et al. 1992a).

The only available human evidence included two case reports of human contact sensitization (Tyce 1997).

Genotoxicity studies for both the induction of gene mutations in *Salmonella typhimurium* and chromosome aberrations in hamster cells were negative (Tyce 1997).

9.2 Welfare Effects
There are no relevant welfare effects studies on 2-butyne.

9.3 Overall Summary
Oral administration of 2-butyne appeared to be a moderate toxicant in rats, primarily involving damage to liver and kidneys. Even though no carcinogenicity data were found, 2-butyne did not appear to be genotoxic. Overall, there was limited evidence in animals that 2-butyne was toxic. No inhalation studies were available and there was insufficient evidence in humans of the potential toxic effects of 2-butyne.

There were no relevant welfare effects studies on 2-butyne so an assessment was not possible.

10 2-Methylhexane

10.1 Health Effects
There are no relevant health effects studies of 2-methylhexane.

10.2 Welfare Effects
There are no relevant welfare effects studies of 2-methylhexane.

10.3 Overall Summary
There were no relevant health or welfare effects studies of 2-methylhexane specifically, so an assessment was not possible.

11 3,4-Dimethyl-1-pentene

11.1 Health Effects
There are no health effects studies on 3,4,-dimethyl-1-pentene in animals or humans.

11.2 Welfare Effects
The only welfare effects study was an analysis of volatile organic compounds (VOCs) in four different areas in Northeast China.

11.3 Overall Summary
For 3,4,-dimethyl-1-pentene, there were no available/relevant studies, so an assessment of the health and welfare effects of 3,4,-dimethyl-1-pentene was not possible.

12 3-Methyl-1-pentene

12.1 Health Effects

There are no health effects studies on 3-methyl-1-pentene in animals or humans.

12.2 Welfare Effects

There were no applicable welfare effects studies on 3-methyl-1-pentene.

12.3 Overall Summary

For 3-methyl-1-pentene, there were no available/relevant studies, so an assessment of the health and welfare effects of 3-methyl-1-pentene was not possible.

13 cis-1,2-Dimethylcyclohexane

13.1 Health Effects

There were no relevant health effects studies on cis-1,2-dimethylcyclohexane.

13.2 Welfare Effects

One ecotoxicity study of larval rainbow trout reported 100% mortality at 7.3 mg/L and no mortality at 5.0 mg/L (Edsall 1991). The author also reported that the LC50 (lethal concentration for 50% of the test population) of cis-1,2-dimethylcyclohexane was 7.1 ± 0.7 mg/L and was more toxic than the PAH compound 1,3-dimethylnapthalene (LC50 = 1.7 ± 0.2 mg/L). Only an approximate LC50 could be determined because mortality was not dose-dependent.

13.3 Overall Summary

There were no relevant health effects studies of cis-1,2-dimethylcyclohexane specifically, so an assessment was not possible. The single welfare effects study of cis-1,2-dimethylcyclohexane indicated that mortality in larval rainbow trout was not dose-dependent and that its toxicity was lower than the PAH compound 1,3-dimethylnapthalene.

14 cis-2-Octene

14.1 Health Effects

There are no health effects studies on cis-2-octene in animals or humans.

14.2 Welfare Effects

There were no welfare effects studies on cis-2-octene.

14.3 Overall Summary

For cis-2-octene, there were no available/relevant studies, so an assessment of the health and welfare effects of cis-2-octene was not possible.

15 Ethanol

15.1 Health Effects

A variety of non-cancer outcomes have been investigated in rats exposed to ethanol via inhalation. Neurobehavioral effects were observed in rats that failed unconditioned reflex tests when exposed to 8,000 ppm ethanol (Mullin and Krivanek 1982). Gohsh et al. (1991b) reported a decrease in the reinforcement rate at concentrations as low as 102 ppm. Massad et al. (1985, 1986) reported that ethanol-fueled engine exhaust was significantly less toxic, in terms of both acute and chronic toxicity, in rats and mice than gasoline-fueled engine exhaust. Another study of chronically exposed rats indicated that ethanol exposure did not produce a significant degree of oxidative stress in the rat lung (Rikans and Gonzalez 1990). Chu et al. (2005) reported that rats exposed to ethanol at 6,130 ppm, gasoline at 500 ppm, or a mixture of 85% ethanol and 15% gasoline at 6,130 and 500 ppm respectively, produced mild, reversible biochemical, hematological and histological effects. Ghosh et al. (1991a) found that ethanol inhaled at low doses could cause arousal action (waking) in sleeping rats. The authors noted that ethanol inhaled at all concentrations investigated (100, 400, 800 and 1,600 ppm) did not have any effect on EEG power spectrum. Guppy and Littleton (1999) reported that the hearts of ethanol-exposed rats were significantly protected against myocardial infarction.

Most reproductive studies of rats suggested that inhalation exposure to ethanol was not a developmental toxin. Nelson et al. (1985a) reported no significant increase in malformations of offspring following maternal exposure to ethanol in pregnant rats at concentrations ranging from 10,000 to 20,000 ppm. Nelson et al. (1985b) also reported no reduction in weight gain or reduced weight in offspring through 3 weeks of age. No significant difference from controls with respect to neuromotor coordination, activity levels, or learning ability and no reports of male infertility among the exposed were reported at concentrations ranging from 10,000 to 16,000 ppm (Nelson et al. 1985b). Offspring from paternally- or maternally-exposed animals performed as well as controls in tests of neuromotor coordination, activity levels, or learning ability (Nelson et al. 1988). Liopo et al. (1996), however, reported that low exposure to ethanol was toxic to rat progeny.

An inhalation and *in vitro* study of mice found a dose-dependent suppression of tumor growth when ethanol was added to culture medium for Lewis lung carcinoma. When injected, Lewis lung carcinoma was sequestered and following inhalation exposure to ethanol, a marked reduction of the pulmonary tumor growth was observed (Batkin and Tabrah 1990). Another carcinogenicity study of rats found that chronic inhalation of ethanol was not found to be carcinogenic (Cheever et al. 1990).

Some genotoxicity/mutagenicity studies of ethanol indicated that it may be genotoxic (Topping et al. 1982; Hayes 1985; Lofti et al. 1990; Blasiak et al. 2000; Kayani and Parry 2010). Other researchers suggested that it is ethanol‘s metabolite, acetaldehyde, and not ethanol itself, that is mutagenic (Obe et al. 1986; Cortes et al. 1986). Philips and Jenkinson (2001) concluded that some degree of genotoxicity in humans may result from ethanol drinking, but this is not considered relevant to any conceivable exposure obtainable either by inhalation or dermal exposure in the workplace. Several investigators also found that ethanol was not genotoxic

(Brusick et al. 1982; Kvelland 1983; Bariliak and Slu 1983; Gichner and Veleminsky 1987). Song et al. (2007) reported that E-10 and E-15 diesel fuel emissions as a whole showed lower genotoxic potency than E-0, E-5, or E2-0 diesel exhaust.

An inhalation study of humans found that exposure to ethanol vapor at the UK occupational exposure limit (1,900 mg/m^3) did not produce a significant blood alcohol concentration (Campbell and Wilson 1986). Another acute inhalation study found that healthy males exposed to ethanol concentrations that could be encountered during refueling and ranging from 250 to 1,000 ppm exhibited no significant changes in neuromotor function (Nadeau et al. 2003).

The author of a review of the potential developmental toxicity of ethanol in an occupational setting concluded that there is no evidence that industrial exposure to ethanol is a developmental hazard. The author explained that though developmental toxicity may result from drinking alcohol (of which a threshold level has yet to be defined), this was not considered relevant to the low blood alcohol concentrations resulting from any conceivable inhalation or dermal exposure to ethanol in the workplace (Irvine 2003). The authors of another review concluded that there appears to be little cause to suppose the UK occupational exposure limit of 1,000 ppm is associated with any appreciable increase in risk of cancer (Bevan et al. 2009).

15.2 Welfare Effects

An ecotoxicity study of freshwater snails found that ethanol reduced their ability to reproduce at a concentration of 198 mg/L. No reduction in fertility was observed at a concentration of 19.8 mg/L. Hatching retardation was also not observed at concentrations below 19.8 mg/L (Oliveira-Filho et al. 2009). Another study of fish found that ethanol exposure resulted in a decrease in body weight followed by a significant inhibition of total ATPase activities (Bhanu and Philip 2011). A third study on fish found that hatchability was not affected following exposure to 1% ethanol. The 1% ethanol group, however, suffered 89% mortality during 108 to 120 hour-post-fertilization. No developmental defects at the 0.01 and 0.1% concentrations were observed, but significantly higher deformity rates occurred in the 1% ethanol exposure group. Some behavioral effects, including hyperactivity, were observed in all ethanol concentration groups (Chen et al. 2011).

15.3 Overall Summary

Overall, the evidence from the health effects studies suggested that exposure to high concentrations of ethanol via inhalation may result in some mild reversible, neurobehavioral, biochemical, hematological and histological effects. Overall, exposure to ethanol via inhalation was at most, mildly toxic, and exposure to gasoline exhaust has been reported to be significantly more toxic than ethanol exhaust. Exposure to optimal concentrations of ethanol has actually been found to be beneficial to the heart in rats and did not cause oxidative stress in rat lungs. Genotoxicity data suggested that ethanol may be mildly genotoxic, and/or that it is ethanol‘s metabolite, acetaldehyde, that may be genotoxic. Carcinogenicity data suggested that exposure to ethanol via inhalation was not carcinogenic and that it may actually suppress tumor growth. Ethanol exposure via inhalation at concentrations experienced in the workplace is not expected to be carcinogenic in humans or result in any noticeable acute effects.

The evidence from the welfare effects studies indicated that at certain concentrations, ethanol reduced fertility, effected development, and resulted in behavioral effects in aquatic organisms.

16 Isopropylcyclopentane

16.1 Health Effects

There are no health effects studies on isopropylcyclopentane in animals or humans.

16.2 Welfare Effects

There are no applicable welfare effects studies on isopropylcyclopentane.

16.3 Overall Summary

There were no available/relevant studies, so an assessment of the health and welfare effects of isopropylcyclopentane was not possible.

17 Methacrolein

17.1 Health Effects

In an acute mouse inhalation study, methacrolein induced irritation of the upper respiratory tract and a concentration-dependent decrease in the respiratory rate. The extrapolated threshold for the respiratory depressing effect, RDO, was 1.3 ppm. The authors concluded that the main effect of methacrolein was sensory irritation (Larsen and Nielsen 2000).

Genotoxicity/mutagenicity effects were examined and methacrolein induced little increase over background expression of the SOS-regulated umu operon (Benamira et al. 1992).

Blinking frequency was monitored in ten human subjects exposed locally for twenty minutes in the non-dominant eye while viewing an educational film. Mean blinking frequency increased significantly among exposed subjects compared to participants exposed to clean air (Nøjgaard et al. 2005). The authors found the lowest observed effect level was 286 ppb.

17.2 Welfare Effects

Uptake rates by tree saplings of methacrolein and croton aldehydes were found to be higher than those of methyl vinyl ketone and methyl ethyl ketone (MEK). In particular, the rate of MEK uptake for *Q. myrsinaefolia* was exceptionally low. The loss rate by tree uptake was the highest for both methacrolein and MEK, suggesting that tree uptake provided a significant sink (Akira et al. 2010).

17.3 Overall Summary

Overall, limited evidence showed that methacrolein could elicit respiratory effects in animals. There was only very limited evidence of toxicity in humans. There was limited information available on the welfare effects of methacrolein – a single study that showed trees served as a significant sink for methacrolein.

18 Methanol

18.1 Health Effects

Laboratory studies of animals (rats, mice, and monkeys), conducted by exposing animals principally via the inhalation route of exposure, indicated that methanol intoxication resulted in delayed visual toxicity (Eells 1991; Teo 1991; Bolon et al. 1993; Lee et al. 1994). In general, studies of rats exposed to methanol via inhalation exhibited mild biochemical and histopathological effects (Reed et al. 2006; Poon et al. 1994), though rats exposed to higher concentrations of methanol still exhibited visual toxicity seen in other animals. Even so, Poon and colleagues (1995), who investigated the inhalation effects of both gasoline and methanol on rats, reported that gasoline was largely responsible for the adverse effects observed in the study. They also reported that there were no apparent interactive effects observed between methanol and gasoline. Though mice and monkeys were shown to be more sensitive to the inhalation effects of methanol, one study reported no consistent treatment-related effects for organ or body weights or for histopathologic or opthalmoscopic examinations of monkeys exposed to ethanol via inhalation (Andrews et al. 1987). The authors reported that the results from this study support the ACGIH TLV of 200 ppm. Several reproductive studies of mice exposed to high concentrations of methanol via inhalation indicated that it was a developmental toxin in a concentration-dependent manner (Bolon et al. 1993; Rogers et al. 1993 and 1997). The authors of one of these studies reported a NOAEL for developmental toxicity of 1,000 ppm (Rogers et al. 1993).

No increased neoplasms were observed in rats and mice chronically exposed to air levels of methanol up to 1,000 ppm (Cruzan 2009).

Genotoxicity/mutagenicity studies of methanol indicated that it was not genotoxic. In fact, several studies of the potential genotoxicity and cytotoxicity of gasoline and methanol exhaust reported strong evidence that gasoline exhaust was genotoxic while methanol exhaust was not (Llano et al. 2005; Zhang et al. 2005; Zhang et al. 2007). The author of a recent review of the carcinogenic potential of methanol concluded that genotoxicity studies did not suggest carcinogenic activity and that methanol is not likely to be carcinogenic in humans (Cruzan 2009).

Evidence from human observational studies and case reports also indicated that methanol poisoning can result in visual symptoms or delayed ocular damage, and acute renal toxicity in humans (Smith 1983; Teo 1991; Downieet al. 1992; Frenia and Schauben 1993; Verhelst et al. 2004; Wallace and Green 2009). Case reports of highly exposed humans suggested that methanol poisoning can lead to nausea, altered mental status, lethargy, or death. The authors of one case report, however, noted that even among humans highly exposed to methanol via inhalation (inhalant abusers), the risk of developing methanol-induced complications such as visual dysfunction was still low (Berbarta et al. 2006). The authors of a review of methanol toxicity described a study of human volunteers exposed to 200 ppm methanol for 6 hours. Blood formate (the metabolite of methanol believed to be the primary toxicant in humans) concentrations were not significantly increased above endogenous concentrations. The authors concluded that human populations may not be at added risk of neurotoxic effects resulting from exposure to methanol at these low concentrations (Medinsky and Dorman 1995). The authors of a cytotoxicity study of human lung cancer cells exposed to gasoline and methanol exhaust

demonstrated cytotoxic effects for gasoline exhaust but not for methanol exhaust (Zhanci et al. 2007).

18.2 Welfare Effects

An ecotoxicity study of rainbow trout, fathead minnow and bluegill fish revealed the lowest LC50 (lethal concentration for 50% of the test population) for methanol occurred in bluegill fish exposed for 96 hours. The LC50 was 15,400 mg/L with a median effect concentration (EC50) of 12,700 mg/L (Poirer et al. 1986). The authors of another ecotoxicity study of blue mussels reported a similar LC50 of 15,900 mg/L (Helmstetter et al. 1996), while a third study of fish and aquatic invertebrates reported a NOAEL of 23,750 mg/L for a freshwater aquatic ecosystem (Kavirai et al. 2004).

In summary, welfare effects studies indicated that methanol may be toxic in aquatic organisms but only at high concentrations – i.e., in the range of 12,700 mg/L – 23,750 mg/L.

18.3 Overall Summary

Overall, the evidence from toxicity studies and case reports suggested that methanol is acutely toxic in animals and humans exposed to high concentrations via inhalation and ingestion; however, genotoxicity and cytotoxicity studies suggested that methanol is not genotoxic and likely does not pose a carcinogenic risk to humans. These studies also suggested that the genotoxicity and cytotoxicity of gasoline is much more pronounced than methanol.
The evidence from the welfare effects studies indicated that methanol exhibited low ecotoxicity in aquatic species.

19 Styrene

19.1 Health Effects

Several acute and subchronic studies evaluated liver toxicity of styrene on laboratory animals via inhalation and oral exposures (Morgan et al. 1993a, 1993b, 1993c; 1997; Susan et al. 1997; Mahler et al. 1999; De Piceis et al. 2003). These studies found necrosis, hepatotoxicity and mortality in exposed rats and mice. In addition, increased levels of liver enzymes such as serum alanine aminotransferase and sorbitol dehydrogenase were observed in moribund animals (Morgan et al. 1993a). Hepatic GSH levels decreased in a dose-dependent manner (Morgan et al. 1993a, 1993b).

Significant dose-related increases in sister-chromatid exchange (SCE) frequencies were observed in cells (Conner et al. 1982; Kligerman et al. 1992, 1993). At concentrations above 387 ppm, a concentration-dependent increase in SCE frequencies was observed in all three types of cells in hepatectomized mice and in both the alveolar macrophages and bone marrow cells in non-hepatectomized mice (Conner et al. 1981). In addition, SCE frequency increases were found in lymphocytes from the spleen, peripheral blood as well as in cells from the lung (Kligerman et al. 1992). Nonetheless, styrene did not induce SCE in rats exposed via inhalation (150 to 1,000 ppm) and there were no increased chromosomal aberrations (Preston etal. 1993). The authors claimed these results were significant for prediction responses in people occupationally exposed to styrene. Kligerman et al. (1992) found no statistically significant dose-related increases in the frequency of chromosome aberrations in the cultured splenocytes

or lung cells, and no significant increases in micronucleus frequencies were observed in binucleated splenocytes or normochromatic erythrocytes in peripheral blood smears.

Other studies considered other noncancer effects of styrene such as hearing loss, respiratory, and pulmonary effects (Pryor et al. 1987; Rebert et al. 1993; Coccini et al. 1997; Boogaard et al. 2000). Hearing loss was assessed by behavioral and electrophysiologic methods in rats exposed via inhalation. The authors found that styrene demonstrated ototoxic potential compared to toluene and tricholoroethylene (Pryor et al. 1987; Rebert et al. 1993). In acute and subacute studies, pulmonary effects were seen in laboratory animals exposed to styrene vapors, and these effects were dose-dependent (Elovaara et al. 1990; Coccini et al, 1997; Cruzan et al. 1997). Additionally, GSH depletion accompanied by inhibition of cytochrome P450-dependent oxidative drug metabolism were the earliest biochemical lesions manifested in exposed lungs (Elovaara et al. 1990). Cruzan et al. (1997) found nasal tract effects in rats exposed via inhalation, with a NOEL (no observed effect level) of 200 ppm; however, no NOEL was found in mice with similar exposures.

Genotoxicity/mutagenic evaluation of styrene and its metabolites showed that styrene had a slight effect on induction of the *E. coli* SOS system, with a LOEL (lowest observed effect level) of 100 µg/ml (Glosnicka et al. 1986). At 200 µg/ml, there was evidence of alkaline phosphatase production inhibition compared with the control group in relation to β-galactosidase secretion. Carcinogenicity was induced in rats exposed via inhalation, gavage, intraperitoneal and subcutaneous injections and in mice exposed orally (Conti et al. 1988; Dogra et al. 1989; Cruzan et al. 2001). Styrene caused an increase in total (benign and malignant) and malignant mammary tumors (Conti et al. 1988). Styrene induced an increase in the number of lung tumors seen spontaneously in CD-1 mice (Cruzan et al. 2001).

Bronchiolo-alveolar tumors were observed in mice exposed chronically to 160 ppm styrene, whereas no tumors were seen in rats up to concentrations of 1,000 ppm (Boogaard et al. 2000). Furthermore, the authors evaluated the carcinogenicity of styrene by quantification of DNA adducts formed in the liver. Their results indicated that DNA adduct formation did not play a role in styrene tumorigenicity in chronically exposed mice (Boogaard et al. 2000). On the other hand, following chronic exposures, there was no evidence that styrene exposure caused treatment-related increases of any tumor type in males or females or in the number of tumor-bearing rats in the exposed groups compared to controls (Cruzan et al. 1998). Even so, in female rats, there were treatment-related decreases in pituitary adenomas and mammary adenocarcinomas. Based on an overall evaluation of eight oncogenicity studies, there is clear evidence that styrene does not induce cancer in rats (Cruzan et al. 1998). Engelhardt et al (2003) found no evidence of clastogenicity (a form of chromosome damage) in mice exposed to styrene.

There were several studies that examined the effects of styrene exposure in humans; however, the majority of these studies were conducted in occupationally-exposed subjects. Sassine et al. (1996) found progressive deterioration of wellbeing associated with neurotoxic exposure to styrene. Neurobehavioral toxicity was examined via psychometric tests in occupational exposed subjects (Jegaden et al. 1993). All tests conducted on exposed subjects were significantly ―less good" than control. However, the results were better in the evening than in

the morning; the authors claimed that this result proved a lack of acute intoxication at the end of the day. In conclusion, the results suggested the existence of minor but significant organic mental disorders in the subjects exposed to a mean dose of 30 ppm in this study. In a different cohort of exposed workers, there was an increased prevalence of headaches, dizziness, light headedness, fatigue, irritability, and memory loss with increasing blood styrene (Checkoway et al. 1992).

Evidence from mutagenicity/genotoxicity studies in humans revealed that styrene exhibited mild genotoxic activity (Karakava et al. 1997; Cakbarti et al. 1993; Laffon et al. 2002). Using human blood lymphocytes, Cakbarti et al (1993) found a dose-related increase in SCE frequency and cell length (up to 1000 µM). Average estimated values for styrene exposure were slightly below the threshold limit value (TLV) of 20 ppm recommended by the ACGIH, and significant increases for SCE and MN (micronucleus) frequencies were observed among exposed individuals (Laffon et al. 2002). Nevertheless, the mutagenic potential of styrene was examined in cultured lymphocytes of occupationally-exposed workers and the authors found no cytogenic effects as a result of styrene exposure (Watanabe et al. 1981). Likewise, no genotoxic effects related to exposure were detected by DNA adducts or DNA single strand breaks and SCE (Holz et al. 1995). The only effect related to exposure was an increase in kinetochore positive micronuclei in peripheral lymphocytes; the frequency of total micronuclei in peripheral lymphocytes did not change. The authors concluded that low occupational exposure to styrene, benzene, and ethylbenzene did not induce alterations of genotoxicological variables (Holz et al. 1995).

Styrene absorption and metabolism was monitored in human volunteers exposed via inhalation and dermal exposures (Wieczorek and Piotrowski 1985; Wieczorek 1985). The average retention of styrene vapors in the respiratory tract was 71 %. The yield of styrene metabolism measured within 24 hours was 39% and 17% for mandelic acid and phenylglyoxylic acid, respectively. When volunteers were exposed to styrene dermally, there were increases in levels of mandelic and phenylglyoxylic acids in urine.

19.2 Welfare Effects

An acute ecotoxicity study on fathead minnows, daphnids, amphipods, and freshwater green algae found styrene to be moderately toxic to these aquatic organisms (Cushman et al. 1997). The LC50 for fathead minnows and amphipods was 10 mg/liter and 9.5 mg/liter, respectively. The EC50 for daphnids and green algae was 4.7 mg/liter and 0.72 mg/liter, respectively. In the same study, styrene was found to be slightly toxic to earthworms. The authors concluded that there was no indication of a concern for chronic toxicity based on these studies since styrene's potential impact on aquatic and soil environments is significantly mitigated by its volatility and biodegradability.

The toxicity of styrene dimers and trimers to *Ceriodaphnia dubia* bred in polystyrene cups was evaluated (Tatarazako et al. 2002). Styrene dimers and trimers were eluted from the cups by hexane and methanol which led to a 25% reduction in *C.dubia* fertility. The authors concluded that styrene has the potential to impair crustacean populations in the aquatic environment.

19.3 Overall Summary

Overall, the evidence from health effects studies suggested that styrene was toxic in laboratory animals. There was some evidence that styrene was neurotoxic in humans; however, it is prudent to note that this effect was observed in occupationally-exposed individuals (exposed to high concentrations in air). Also, there was evidence that styrene was mutagenic/genotoxic in animals and mildly toxic in humans. There was some suggestive evidence of styrene carcinogenicity in animals; however, there was no evidence of this in humans.

The evidence from the welfare effects studies indicated the styrene was moderately toxic to aquatic organisms.

20 trans-1,3-Dimethylcyclohexane

20.1 Health Effects

There are no health effects studies of trans-1,3-dimethylcyclohexane.

20.2 Welfare Effects

There are no relevant welfare effects studies on trans-1,3-dimethylcyclohexane.

20.3 Overall Summary

There were no health effects studies of trans-1,3-dimethylcyclohexane so an assessment of health effects was not possible. Likewise, there were no relevant welfare effects studies on trans-1,3-dimethylcyclohexane so an assessment of welfare effects was not possible.

21 trans-1,4-Dimethylcyclohexane

21.1 Health Effects

There are no health effects studies on trans-1,4-dimethylcyclohexane in animals or humans.

21.2 Welfare Effects

There are no applicable welfare effects studies on trans-1,4-dimethylcyclohexane.

21.3 Overall Summary

For trans-1,4-dimethylcyclohexane, there were no available/relevant studies, so an assessment of the health and welfare effects of trans-1,4-dimethylcyclohexane was not possible.

22 Summary and Conclusions

An increase in ethanol content in gasoline from 10 percent (E-10) to 15 percent (E-15) requires the registration of ethanol as a fuel additive at increasing concentrations, and pursuant to Section 79.52(d), any new registration application must be accompanied by health impact information. The information in this report was developed in support of this increase.

Testing was conducted to identify the types and concentrations of emissions products of E-15 and a literature search was performed for 18 emission compounds found in both unleaded certification fuel (E-0) and E-15. Commercial databases were searched to identify the relevant health and welfare effects literature for ethanol and the 17 additional subject chemicals, and the databases selected encompassed available relevant information from industry, government, and public sources.

Each of the relevant studies identified in the literature searches was reviewed and concise study summaries were developed. For 10 of the 18 chemicals – 1-methyl-1-ethylcyclopentane, 2,2-dimethylhexane, 2,4,4-trimethyl-2-pentene, 2-methylhexane, 3,4-dimethyl-1-pentene, 3-methyl-1-pentene, cis-2-octene, isopropylcyclopentane, trans-1,3-dimethylcyclohexane, and trans-1,4-dimethylcyclohexane – there were no health or welfare effects studies found in the literature, so an assessment of the health and welfare effects of these chemicals was not possible. These chemicals, however, are all short carbon chain substituted alkanes and alkenes that most likely produce relatively mild neurological effects (central nervous system depression) at high concentrations in air, similar to the non-substituted alkanes such as propane, butane, pentane, etc.

Likewise, cis-1,3-dimethylcyclohexane, for which no health effects studies were found, is expected to be a mild central nervous system depressant at high concentrations. For the related short carbon chain alkyne 2-butyne, there was limited evidence in animals that it was toxic when administered via the oral route, and insufficient evidence that it was toxic in humans.

For the substituted benzenes – 1,2,3-trimethylbenzene (TMB) and 1,3-diethylbenzene – there was some evidence of toxic effects of TMB in laboratory animals but insufficient evidence of these effects in humans. Oral administration of 1,3-diethylbenzene did not appear to be neurotoxic in rats and there was no evidence in humans of potential toxic effects. There was limited information available on the welfare effects of TMB and 1,3-diethylbenzene.

Studies showed that exposure to high concentrations of ethanol via inhalation may result in some mild reversible, neurobehavioral, biochemical, hematological and histological effects. Studies also suggested that ethanol may be mildly genotoxic, and/or that it is ethanol‘s metabolite, acetaldehyde, that may be genotoxic. Carcinogenicity data suggested that exposure to ethanol via inhalation was not carcinogenic. Ethanol exposure via inhalation at concentrations experienced in the workplace is not expected to be carcinogenic in humans or result in any noticeable acute effects. The evidence from the welfare effects studies indicated that at certain concentrations, ethanol reduced fertility, effected development, and resulted in behavioral effects in aquatic organisms.

Studies showed that methacrolein could elicit respiratory effects in animals. There was only very limited evidence of toxicity in humans. There was limited information available on the welfare effects of methacrolein – a single study that showed trees served as a significant sink for methacrolein.

Evidence from toxicity studies and case reports suggested that methanol is acutely toxic in animals and humans exposed to high concentrations via inhalation and ingestion; however, genotoxicity and cytotoxicity studies indicated that methanol is not genotoxic and likely does not pose a carcinogenic risk to humans. Evidence from the welfare effects studies indicated that methanol exhibited low ecotoxicity in aquatic species.

Health effects studies suggested that styrene was toxic in laboratory animals. There was some evidence that styrene was neurotoxic in humans; however, this was observed in occupationally-exposed individuals who were exposed to high concentrations of styrene in air. Also, there was evidence that styrene was mutagenic/genotoxic in animals and mildly toxic in humans. There was some suggestive evidence of styrene carcinogenicity in animals; however, there was no evidence of this in humans. The evidence from the welfare effects studies indicated the styrene was moderately toxic to aquatic organisms.

It can be concluded from the available studies on 7 of the 18 emission compounds found in both unleaded certification fuel (E-0) and E-15 – i.e., 2-butyne, 1,2,3-trimethylbenzene, 1,3-diethylbenzene, ethanol, methacrolein, methanol, ands styrene – that sufficient health and welfare effects data exist such that further characterization of these effects through, for example, Alternative Tier 2 testing, is not warranted. For the remaining 11 emission compounds for which limited or no health or welfare effects data were found – i.e., 1-methyl-1-ethylcyclopentane, 2,2-dimethylhexane, 2,4,4-trimethyl-2-pentene, 2-methylhexane, 3,4-dimethyl-1-pentene, 3-methyl-1-pentene, cis-2-octene, isopropylcyclopentane, trans-1,3-dimethylcyclohexane, trans-1,4-dimethylcyclohexane, and cis-1,4-dimethylcyclohexane – further health and welfare effects testing likewise is not required since these chemicals are all short carbon chain substituted alkanes and alkenes that most likely display similar effects to the non-substituted alkanes and alkynes for which ample data exist.

23 References

1,2,3-Trimethylbenzene

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Tables – Health and Welfare Effects Summaries

Table 1 – 1,2,3-Trimethylbenzene

Health Effects Studies

Citation	Study methods	Results	Authors' conclusions/comments
Kimura K Negate T Hara K, Kaqeura M 1988. Gasoline and kerosene components in blood – a forensic analysis. Hum Toxicol. Jul;7(4):299-305.	Using two simultaneous procedures, head space and solvent extraction methods were used to analyze small amounts of fuel components in laboratory animals exposed to gasoline vapors.	Aliphatic hydrocarbons with carbon numbers of 5 to 8 and aromatics such as benzene, toluene and xylenes were detected in laboratory animals, following exposure to gasoline vapor, using the head space method. Aliphatic hydrocarbons with carbon numbers over 9 as well as the aromatics with carbon number 9 group including cumene, mesitylene, pseudocumene and 1,2,3-trimethylbenzene were determined by the solvent extraction method following exposure to kerosene vapor. The lower limits of detection were 0.01 µg and 50 pg in gasoline and kerosene components, respectively.	
Korsak Z, Rydzynski K. 1996. Neurotoxic effects of acute and subchronic inhalation exposure to trimethylbenzene isomers (pseudocumene, mesitylene, hemimellitene) in rats. Int J Occup Med Environ Health.9(4):341-9.	An acute inhalation study in rats exposed to pseudocumene, mesitylene, hemimellitene (1,2,3-trimethylbenzene), 250 to 2,500 ppm for 4 hrs, and a subchronic study with exposure at 25, 100, and 250 ppm.	Rotarod performance EC50 for pseudocumene, mesitylene, hemimellitene was 954, 963, 768 ppm. Pain sensitivity EC50 for pseudocumene, mesitylene, hemimellitene was 1,115, 1,212, and 848 ppm. Acute and subcronic exposures led to concentration-dependent disturbances in rotarod performance behavior and ↓ in pain sensitivity.	Two weeks after cessation of exposure to pseudocumene or hemimellitene no recovery in rotarod performance behavior was observed.
Korsak Z, Rvdzviiski K, Jaffe J.1997 Respiratory irritative effects	Pseudocumene, mesitylene, and	Respiratory rate	Study results showed the respiratory irritative effects of hemimellitene

Table 1 – 1,2,3-Trimethylbenzene

Citation	Study methods	Results	Authors' conclusions/comments
of trimethylbenzenes: an experimental animal study. Int J Occup Med Environ Health, 10(3):303-11.	hemimellitene acute exposure in male Balb/C mice, and pseudocumene repeated 90-day exposure in male Wistar rats	In mice RD50s for pseudocumene, mesitylene, and hemimellitene were 78, 519, 541 ppm. In rats, ↑ in total cell macrophages, polymorphonuclear leukocytes and lymphocytes compared to controls and ↑ in total protein lactate dehydrogenase (LDH) and acid phosphatase activity in bronchoalveolar lavage (BAL) in all exposed groups.	(1,2,3-trimethylbenzene). A TLV of at least 10 ppm should be considered for occupational exposures to trimethlybenzene isomers.
Wiaderna D, Gralewicz S, Tomas T. 1998. Behavioural changes following a four-week inhalation exposure to hemimellitene (1,2,3-trimethylbenzene) in rats. Int J Occup Med Environ Health. 11(4):319-34.	Report of a prior study rats exposed to pseudocumene via inhalation to 100 or 250 ppm 6h/day, 5 days/wk for 4 weeks, as well as a report of hemimellitene (HM) inhalation exposure to 0, 25, 100, 250 ppm 6h/day, 5 days/wk for 4 weeks.	For the latency of the paw-lick response to heat, the effect of 100 ppm HM was more pronounced than that of 250 ppm. For the test performed day 14 and 61 after last exposure, radial-maze performance and open-field activity showed no significant effect at all exposures tested. At 250 ppm and control groups, no behavioral changes were observed. At 25 or 100 ppm group impaired learning of the passive avoidance, i.e. refraining from performance of a punished response (stepping off an elevated platform) occurred. At 100 ppm acquisition of the two-way active avoidance in the shuttle-box was slower and the footshock-induced increase in latency of the paw-lick response to heat persisted longer than in the unexposed animals.	The results suggested that a low-level inhalation exposure to HM, just like low-level exposure to PS, may lead to long-lasting disturbances in the CNS functions. The nonlinear concentration-effect relationship observed in the case of both chemicals requires clarification in further studies.
Kostrzewski P, Wiaderna-Brycht A, Czerski B. 1997. Biological monitoring of experimental human exposure to trimethylbenzene. Sci Total Environ. Jun 20;199(1-2):73-81.	Pseudocumene, mesitylene, hemimellitene inhataion exposure (5 to 150 mg/m^3 air for 8 hours) in 8 human subjects	Pulmonary ventilation ranged from 0.56 to 1.0 m^3/h. Retention of (pseudocumene, mesitylene, hemimellitene) in the lungs was 68%, 67% and 71%. The elimination of TMB from capillary blood occurred in accordance with the open three-compartment model. Urinary excretion of	Study aim was to obtain toxicokinetic data on absorption and elimination of TMB and its metabolites. The relationship between the levels of TMB or DMBA in biological material and TMB air concentration or absorbed dose were determined. The biological

Table 1 – 1,2,3-Trimethylbenzene

Citation	Study methods	Results	Authors' conclusions/comments
	with no history of exposure to trimethylbenzene (TMB) isomers (20-39 years).	dimethylbenzoic acids (DMBA) proceeded according to the open two-compartment model. Based on the toxicokinetic data, a simulation model of accretion and excretion of DMBA in urine during a 14-day period was developed. The highest rates of metabolite excretion and the highest quantities of DMBA in urine during 24-h intervals were observed on day 5 of exposure.	exposure limit (BEL) for TMB has been proposed, with the current maximum allowable concentration (MAC) value of 100 mg/m^3 (Polish standard) baseline value.
Janik-Spiechowicz E Wyszvnska K, Dziubattowska E. 1998 Genotoxicity evaluation of trimethylbenzenes. Mutat Res. Feb 13;412(3):299-305.	Pseudocumene, mesitylene, hemimellitene were used in *Salmonella typhimurium* TA97a, TA98, TA100 and TA102 strains in the presence and absence of rat liver S9 metabolic activation. Also, bone marrow cells of Balb/c mice treated with 1,2,3-TMB (730, 1,470, 2,200 mg/kg), 1,2,4-TMB (900, 1,800, 2,700 mg/kg), and 1,3,5-TMB (1,800, 2,700 mg/kg).	Only the isomer of benzene with the methyl-group at position 1, 2, 3 was found to have mutagenic effect on *S. typhimurium* cells. ↑ in bacterial reversions was observed in four conventional strains used in this study, but most clearly in TA97a. *Salmonella* tester strains were observed without enzymatic activation, and mutagenic responses of 1,2,3-TMB were as follows: • No influence on the frequency of micronucleated polychromatic erythrocytes • Cytogenetic potential of increasing the SCE level in these cells was observed • Significant responses in SCE induction, compared with the level of those changes in corresponding solvent-administered controls.	Data provided limited evidence for the genotoxic activity of 1,2,3-TMB and inadequate evidence for genotoxic activity of 1,2,4-TMB and 1,3,5-TMB.
Wiaderna D.; Gralewicz S.; Tomas T. 1998. A comparison of the long-	Trimethylbenzenes, pseudocumene, m-	Neurobehavioral impairments in rats	At 100 ppm, exposure to TMBs may result on neurobehavioral

Table 1 – 1,2,3-Trimethylbenzene

Citation	Study methods	Results	Authors' conclusions/comments
term behavioral effect after 28 days inhalation exposure to dimethylbenzene (M-xylene) and isomers of trimethylbenzene (pseudocumene, hemimellitene, mesitylene) in rat. Toxicology. Letters, Volume 95, Supplement 1, July, pp. 69-69(1).	xylene were used in rats exposed via inhalation to 100 ppm, 6 hours/day, 5d/wk for 28d. Measurements included short-term memory in radial maze, 14 days after exposure, and long -term memory (passive and active avoidance), 60 to 70 days after exposure.	No differences in radial maze testing occurred in exposed rats. In the pseudocumene group, passive avoidance learning impairment occurred. There also occurred a significant effect of exposure on active avoidance acquisition – theTMB group required significantly more acquisition trials to reach avoidance criterion than control and xylene rats	disturbances detectable several weeks after termination of exposure.
Yuji Tsujimoto, Tsutomu Noda, Mitsuru Shimizu, Hiroshi Moriwaki and Masanobu Tanaka 1999. Synthesis of regioisomeric dimethylbenzyl mercapturic acids anticipated from the metabolism of 1,2,3-trimethylbenzene. Chemosphere, Volume 38, Issue 9, April 1999, Pages 2065-2070.		The synthesis of two regioisomeric mercapturic acids, N-acetyl-S-(2,3-dimethylbenzyl)-L-cysteine and N-acetyl-S-(2,6-dimethylbenzyl)-L-cysteine, was undertaken to investigate the operation of mercapturic acid pathway in the metabolism of 1,2,3-trimethylbenzene.	
Yuji Tsujimoto, Tsutomu Noda, Mitsuru Shimizu, Hiroshi Moriwaki and Masanobu Tanaka 1999. Identification of the dimethylbenzyl mercapturic acid in urine of rats treated with 1,2,3-trimethylbenzene. Chemosphere, Volume 39, Issue 5, August Pages 725-730.	Mercapturic acid identification in rat urine after intraperitoneal administration of 1,2,3-trimethylbenzene.	Of the two regioisomeric mercapturic acids; i.e., N-acetyl-S-(2,3-dimethylbenzyl)-L-cysteine and N-acetyl-S-(2,6-dimethyl-benzyfi-L-cysteine, only the former was isolated by preparative HPLC and identified, by comparison with an authentic specimen. The excretion rate of the mercapturate was estimated to be ~5% of dose, not a substantial metabolic route.	

Table 1 – 1,2,3-Trimethylbenzene

Citation	Study methods	Results	Authors' conclusions/comments
Tomas T, Wiaderna D, Swiercz R. 1999 Neurotoxicity assessment of selected organic solvents based on spontaneous and evoked cortical and hippocampal activity in rats. Int J Occup Med Environ Health,12(1):73-84.	Toluene, mesitylene, hemimellitene, and pseudocumene were used in acute intraperitoneal injections in rats (doses not provided).	With all solvents, there occurred significant quantitative differences between hippocampal and cortical EEG.	A relationship was found between the changes in spontaneous EEG and blood concentration of the solvents. Hemimellitene, with the lowest recorded blood leve,l was found to have the highest potential for inducing the CNS effects
Tomas T, Swiercz R, Wiaderna D, Gralewicz S. 1999. Effects of acute exposure to aromatic hydrocarbons C 9 on locomotor activity in rats. Trimethylbenzene isomers. Int J Occup Med Environ Health. 12(4):331-43.	Toluene, mesitylene, hemimellitene & pseudocumene were used in rats exposed orally to 0.008, 0.016, and 0.032 mol/kg in olive oil.	For toluene (.008 mol/kg), biphasic changes in the animal locomotor activity were reported. For TMB (all doses), there occurred slightly increased animal locomotor activity, but the magnitude of changes did not indicate their stimulating effect. No time-effect relationship was observed after administration. Mean blood concentrations were dose-related. The highest blood concentrations were observed after toluene administration.	After administration of TMB isomers, no time-effect relationship was observed. TMB isomers slightly increased locomotor activity.
Tomas T, Lutz P, Wiaderna D. 2000. Changes in electrocortical arousal following acute trimethylbenzene administration in rats. Int J Occup Med Environ Health. 13(1):67-78.	Toluene, mesitylene, hemimellitene & pseudocumene were used in WAG/Rij rats exposed intragastrically via gavage to 0.002, 0.008, and 0.032 mol/kg in olive oil.	Electrocortical arousal monitored via high voltage spindles (HVS) activity sensitive to arousal level was measured, and in all solvents there occurred ab inhibition of the HVS activity that was dose-related. The effect produced by TMB isomers was in each case less pronounced than that of toluene.	TMB produced electrocortical arousal in exposed rats.
Korsak Z, Stetkiewicz J, Maicherek	Inhalation exposure	Overall, no changes in body weight gain or	There were dose-related responses in

Table 1 – 1,2,3-Trimethylbenzene

Citation	Study methods	Results	Authors' conclusions/comments
W, Stetkiewicz I, Jaite J Rvdzynski K. 2000. Subchronic inhalation toxicity of 1,2,3-trimethylbenzene (hemimellitene) in rats. Int J Occup Med Environ Health.13(3):223-32.	in rats to hemimellitene at 123, 492 and 1,230 mg/m^3, 6 hr/day, 5 days/wk for 3 months.	food consumption were reported, as was low systemic toxicity. At 1,230 mg/m^3, the following effects were observed: • ↑in relative liver weight was observed in male rats • a slight ↑ in sorbitol dehydrogenase activity • ↑ in alkaline phosphatase activity was found in females only • some disturbances in haematological parameters: ↓ decrease in red blood cells, slight ↑ in white blood cells • segmented neutrophils and lymphocytes • pulmonary lesions as well as an increased number of goblet cells and interstitial lung parenchyma infiltration in male and female rats.	rats exposed to TMB via inhalation.
Siawomir Gralewicz and Dorota Wiaderna 2001. Behavioral Effects Following Subacute Inhalation Exposure to m-Xylene or Trimethylbenzene in the Rat: A Comparative Study. NeuroToxicology, Volume 22, Issue 1, Pages 79-89.	m-Xylene mesitylene, hemimellitene, and pseudocumene were used in rats (10/group) exposed via inhalation to 100 ppm for 6 hrs/ day, 5 days per week for 4 weeks.	No difference in radial maze performance was observed between exposed and control groups. Acquisition, but not retention, of the two-way active avoidance response was significantly impaired in all solvent-exposed groups. In control, xylene, mesitylene, and pseudocumene-exposed animals, there occurred ↑ spontaneous locomotor activity in the open field, impaired passive avoidance learning, and significantly longer paw-lick latencies 24 h after footshock.	For behavioral parameters, the xylene group did not differ significantly from the TMB isomers. Results showed that a short-term exposure to any of the TMB isomers or m-xylene at concentration as low as 100 ppm may induce persistent behavioral alterations in the rat.
Sulkowski WJ, Kowalska S, Matvia W, Guzek W, Wesotowski W, Szimczak W, Kostrzewski P. 2002.	Ethylbenzene, xylene pseudocumene,	Symptoms of vestibular dysfunction, as well as the decreased duration, amplitude and slow phase angular velocity of induced	The findings closely corresponded with the rate of the total exposure to

Table 1 – 1,2,3-Trimethylbenzene

Citation	Study methods	Results	Authors' conclusions/comments
Effects of occupational exposure to a mixture of solvents on the inner ear: a field study. Int J Occup Med Environ Health. 15(3):247-56.	mesitylene and hemimellitene exposure in 61 workers (controls: were 40-age-matched non-exposed subjects).	nystagmus occurred in subjects exposed and controls (47.5% vs. 5%). Sensorineural high frequency hearing loss occurred in exposed and controls (42% vs. 5%), as well as reduced amplitudes of transiently evoked and distortion-product otoacoustic emissions.	the solvent mixture.
Baines CJ, McKeown-Evssen GE Riley N Cole DE, Marshall L Loescher B Jazmaji V. 2004 Case-control study of multiple chemical sensitivity, comparing haematology, biochemistry, vitamins and serum volatile organic compound measures. Occup Med (Lond). Sep;54(6):408-18. Epub 2004 Sep 3.	A study involving exposure to VOCs, including 1,2,3-trimethyl benzene and 1,3,5-TMB, in females (30-64 yrs) – 223 multiple chemical sensitivity cases and 194 controls.	Odds ratio showed MCS was negatively associated with lymphocyte counts and plasma homocysteine. MCS was positively associated with mean cell hemoglobin concentrations, alanine aminotransferase and serum vitamin B6 and not associated with the thyroid stimulating hormone, folate or serum vitamin B12. Cases had significantly lower means of detectable serum levels of 1,2,3-trimethylbenzene. 1,3,5-TMB.	Authors reported that findings were inconsistent with proposals that MCS is associated with vitamin deficiency or thyroid dysfunction, but the association of lower lymphocyte counts with an increased likelihood of MCS was consistent with theories of immune dysfunction in MCS.
Yoko Hieda, Yoshio Tsujino and Haruo Takeshita 2005. Skin analysis to determine causative agent in dermal exposure to petroleum products. Journal of Chromatography B, Volume 823, Issue 1, 25 August 2005, Pages 53-59.	Gasoline, kerosene, light oil, o-xylene, saturated aliphatic hydrocarbons, naphthalene, pseudocumene, mesitylene and hemimellitene dermal exposure in male rats (6/group), as well as case reports (73 year-old woman with erythema over abdomen by	Extraction recoveries for TMBs were 88 – 91%. In rats, % AHCs of skin samples were slightly greater than standard products, while those of blood samples were different in kerosene and oil. TMB concentration in Case 1 in skin and blood were 1.70 and 0.02 µg/g. High aliphatics were detected in skin samples with a similar pattern and components for kerosene. In Case 2, skin and blood concentrations were 1.67, 0.24 µg/g, and aliphatics were detected with a similar pattern for kerosene in skin samples.	In Case 1, patient was diagnosed with kerosene dermatitis. Based on evaluation, patient committed suicide by self-burning using kerosene.

Table 1 – 1,2,3-Trimethylbenzene

Citation	Study methods	Results	Authors' conclusions/comments
	dermal exposure to chemical and a 37 year old woman dead at fire scene).		
Jeremy Ratela and Erwan Engel 2009. Determination of benzenic and halogenated volatile organic compounds in animal-derived food products by one-dimensional and comprehensive two- dimensional gas chromatography-mass spectrometry. Journal of Chromatography A, Volume 1216, Issue 45,6 November, Pages 7889-7898.	Occurrence and levels of the 52 BHVOCs in animal products were studied. One muscle and three fat tissues were analyzed by GC-Quad/MS in 16 lambs.	46 BHVOCs were found in the three fat tissues and 29 in all four tissues, confirming that VOCs are widely disseminated in the body. Twenty-six BHVOCs were quantified in fat tissues, and risk for consumer health was assessed for six of these compounds regulated by the US Environmental Protection Agency (EPA). The BHVOC content was found to be consistent with previous reports and was below the maximum contaminant levels set by the EPA. In the second part of the study, the performance of GCxGC-TOF/MS for comprehensively detecting BHVOCs and showing their entryways in animal-derived food chains was assessed. Meat, milk and oysters were analysed by GC-Quad/MS and GCxGC-TOF/MS. For all these products, at least a 7-fold increase in the contaminants detected was achieved with the GCxGC-TOF/MS technique. The results showed that the production surroundings, through animal feeding or geographical location, were key determinants of BHVOC composition in the animal products.	
Piotr Lutz, Stawomir Gralewicz, Dorota Wiaderna, Radoslaw Swierczi, Zofia Grzelinska, Wanda Majcherek. 2010 Contrasting effects of 4-week inhalation	Pseudocumene (PS) and hemimellitene (HM) exposure in male Wistar rats via	Behavioral sensitivity to the psychostimulant amphetamine (AMPH) was measured. In the hemimellitene group, there occurred an increased sensitivity, as augmenting was significantly more evident compared to	The concentration-effect relationship was nonlinear; 100 ppm was the most effective. The results confirmed that low-level inhalation exposure to trimethylbenzene isomers may induce

Table 1 – 1,2,3-Trimethylbenzene

Citation	Study methods	Results	Authors' conclusions/comments
exposure to pseudocumene or hemimellitene on sensitivity to amphetamine and propensity to amphetamine sensitization in the rat. International Journal of Occupational Medicine and Environmental Health, Volume 23, Number 1	inhalation to 0, 25, 100 or 250 ppm 6 h/day, 5 days a week for 4 weeks.	controls. In the pseudocumene group, no sensitivity was observed, as augmenting was significantly less evident compared to controls. In controls, there occurred an augmented behavioral response to AMPH.	behavioural sensitisation and/or increase the susceptibility of the animals to develop this state upon repeated psychostimulant treatment. The authors showed, however, that HM and PS differed markedly in their ability to induce such alterations.

Table 1 – 1,2,3-Trimethylbenzene

Welfare Effects Studies

Citation	Study methods	Results	Authors' conclusions/comments
G.R.B. Webster, K.J. Friesen, L.P. Sarna and D.C.G. Muir. 1985. Environmental fate modeling of chlorodioxins: Determination of physical constants. Chemosphere, Volume 14, Issues 6-7, Pages 609-622.	Environmental fate modeling of polychlorinated dibenzo-P -dioxins (PCDDs).	Environmental fate modeling of polychlorinated dibenzo-P -dioxins (PCDDs) requires knowledge of a number of fundamental physical parameters of these compounds , viz., octanol-water partition coefficient (Kow), solubility in water (Sw), vapor pressure (p), and Henry's constant (H). Classical methodology for determination of these parameters is often not suitable for use with compounds as hydrophobic as PCDDs. Methods have been developed or refined to enable these properties to be measured.	This study does not appear to mention 1,2,3-TMB.
Butts, C. P., Eberson, L., Hartshorn, M. P., Robinson, W. T., Timmerman-Vaughan, D. J. and Young, D.A.W. 1996. Photochemical Nitration by Tetranitromethane. Part XXVI. Adduct Formation in the Photochemical Reaction of 1,2,3-Trimethylbenzene: the Formation of „Double' Adducts Including Nitronic Esters. Acta Chemica Scandinavica. 1996: 50: 29-47.		The photolysis of the charge-transfer complex of 1,2,3-trimethylbenzene and tetranitromethane gives a complex mixture of products, most of which arise by initial attack of trinitromethanide ion on the unsubstituted ring positions at C4(C6) and C5 of the radical cation of 1,2,3-trimethylbenzene. The products 7-19 are adducts resulting directly or indirectly from the addition of the elements of tetranitromethane to 1,2,3-trimethylbenzene, and the trinitromethyl aromatic compounds 22-25 are formed by eliminations from intermediate adducts. Six adducts are simple „single' adducts, nitro-trinitromethyl adducts 7, 8, 10-12, while nitro cycloadduct 9 is formed by cycloaddition of nitro-trinitromethyl adduct 8.	

Table 1 – 1,2,3-Trimethylbenzene

Citation	Study methods	Results	Authors' conclusions/comments
		The remaining addition products are "double" adducts, formed by secondary addition reactions initiated by attack of nitrogen dioxide on the buta-1,3-diene system of „single' adducts, and include trinitro-trinitromethyl compounds 13 and 15, the hydroxy-dinitro-trinitromethyl compound 14, and a group of four nitronic esters 16-19 formed by nitro-denitrocyclization of initially formed hydroxy-trinitromethyl and nitro-trinitromethyl 'single' adducts. Minor amounts of other products are formed including two nitrodienones 21 and 22, and the rearrangement product, 4,5,6-trimethyl-2-nitrophenol (28), and the 2,3,4-trimethyl- and 3,4,5 trimethylnitrobenzenes 26 and 27. The modes of formation of the above products are discussed, and X-ray crystal structure determinations are reported for compounds.	
A Haner, P Hohener and J Zeyer 1997. Degradation of Trimethylbenzene Isomers by an Enrichment Culture under N2O-Reducing Conditions. Appl. Environ. Microbiol., Mar, 1171-1174, Vol 63, No. 3.	1,3,5-TMB, 1,2,4-TMB, 1,2,3- TMB in a microbial culture enriched from a diesel fuel-contaminated aquifer.	Cultures were able to grow on 1,3,5-TMB and 1,2,4-TMB under N2O-reducing conditions, but it did not degrade 1,2,3-TMB. The oxidation of 1,3,5-TMB to CO2 was coupled to the production of biomass and the reduction of N2O (to avoid toxic effects caused by NO2 accumulation during growth with NO3 as the electron acceptor). In addition to 1,3,5-TMB and 1,2,4-TMB, the culture degraded toluene, m-xylene, p-xylene, 3-ethyltoluene, and 4-ethyltoluene.	
Heidi L. Bethe, Roger Atkinson, and Janet Arey 2000. Products of the Gas-Phase Reactions of OH	Products of the gas-phase reactions of the OH	Analyses showed that the ring-cleavage products 2,3-butanedione (from 1,2,3- and 1,2,4-trimethylbenzene) and 3-hexene-2,5-	

Table 1 – 1,2,3-Trimethylbenzene

Citation	Study methods	Results	Authors' conclusions/comments
Radicals with p-Xylene and 1,2,3- and 1,2,4-Trimethylbenzene: Effect of NO2 Concentration. J. Phys. Chem. A, 2000, 104 (39), pp 8922-8929.	radical with p-xylene and 1,2,3- and 1,2,4-TMB have been measured by GC in the presence of varying concentrations of NO2.	dione (from p-xylene and 1,2 ,4-trimethylbenzene) exhibited a dependence of their formation yields on the NO2 concentration, with higher yields from the reactions of the OH-aromatic adducts with O2 than from their reactions with NO2. Data also showed that these ring-cleavage products were primary products of the OH-aromatic adduct reactions. Formation yields extrapolated to zero NO2 concentration should be applicable to ambient atmospheric conditions (provided that there is sufficient NO that peroxy radicals react dominantly with NO), and are from p-xylene, p-tolualdehyde, 0.0706 ± 0.0042 (independent of NO2 concentration), 2,5-dimethylphenol, 0.138 ± 0.016 (independent of NO2 concentration), and 3-hexene-2,5-dione, 0.32 (extrapolated); from 1,2,3-trimethylbenzene, 2,3-butanedione, 0.52 (extrapolated); and from 1,2,4-trimethylbenzene, 2,3-butanedione, 0.10 (extrapolated) and 3-hexene-2, 5-dione, 0.31 (extrapolated).	
Vayenas DV, Michalopoulou E, Constantinides GN, Pavlou, Payatakes AC 2002. Visualization experiments of biodegradation in porous media and calculation of the biodegradation rate. Advances in Water Resources Volume 25, Issue 2, February, Pages 203-219.	Toluene, phenol, o-cresol, naphthalene and 1,2,3-trimethlybenzene were used in visualization experiments made under flow velocities and organic loadings to study the	Biodegradation efficiency decreased as the aqueous concentration of NAPL (representing creosote) at the inlet increased and /or as velocity increased. Biofilm thickness decreased as the distance from the inlet increased and/or the pore diameter decreased.	A quasi-steady-state theoretical model of biodegradation was used to calculate the values of the mesoscopic biochemical rates and to predict the profile of NAPL concentration in the porous medium and the thickness of biofilm in pores. The agreement between experimental data and model predictions was satisfactory.

Table 1 – 1,2,3-Trimethylbenzene

Citation	Study methods	Results	Authors' conclusions/comments
	morphology and thickness of the biofilm as a function of pore size and the distance from the entrance and the efficiency of biodegradation.		
S. Lavarias, F. Garcia, R.J. Pollero and H. Heras 2007. Effect of the water- soluble fraction of petroleum on microsomal lipid metabolism of Macrobrachium borellii (Arthropoda: Crustacea) Aquatic Toxicology, Volume 82, Issue 4, 31Pages 265-271.	The bioavailability and toxicity of leachable constituents in *Daphnia* of four solid and three liquid resins were evaluated by analyzing water-accommodated fractions prepared with each resin, using solid phase microextraction (SPME) techniques.	Liquid resins exhibited concentrations of bioavailable constituents that were sufficiently elevated to cause acute toxicity to the aquatic organism *Daphnia magna*. All solid resins exhibited lower bioavailable concentrations of leachable constituents that were unlikely to pose an aquatic toxicity concern.	Since observed toxicity of both resin types was generally consistent with bioavailable concentrations determined using SPME fiber measurements, it was concluded that this approach provided a convenient *in vitro* screening tool that can help reduce the use of animal testing in environmental hazard assessment of complex hydrocarbon-based substances.
R.W. Woods, D.J. Letinskia, E.J. Febbo, C.L. Dzamba, M.J. Connelly and T.F. Parkerton 2007. Assessing the aquatic hazard of commercial hydrocarbon resins. Ecotoxicology and Environmental Safety Volume 66, Issue 2, Pages 159-168.	The effect of the water-soluble fraction of crude oil (WSF) on lipid metabolism was studied at critical metabolic points, namely fatty acid activation, enzymes	After exposure, microsomal palmitoyl-CoA synthetase (ACS) showed a two-fold increase in adult midgut gland. Embryo's ACS activity was also affected, the increment being correlated with the developing stage. Endoplasmic reticulum acylglycerol synthesis was also increased by WSF exposure in adults and stage 5 embryos, but not at earlier stages of	Hydrocarbons elicited membrane fluidity alterations in *in vitro* experiments at concentrations that could be found in the environment after an oil spill.

Table 1 – 1,2,3-Trimethylbenzene

Citation	Study methods	Results	Authors' conclusions/comments
	of triacylglycerol and phospholipid synthesis, and membrane (lipid packing) properties in the freshwater prawn *Macrobrachium Borellii* (adults and embryos at different stages of development were exposed to a sublethal concentration of WSF for 7 days).	development. Triacylglycerol synthesis was particularly increased (18.5%) in adult midgut gland. The microsomal membrane properties were studied by fluorescent steady-state anisotropy, using the rotational behavior of the fluorescent probe 1,6-diphenyl-1,3,5-hexatriene (DPH). Microsomes from midgut gland of WSF-exposed prawn showed no differences in fluidity. Nevertheless, microsomes incubated with WSF *in vitro* increased their fluidity in a temperature- and WSF concentration- dependent fashion. Both, aliphatic and aromatic hydrocarbons individually tested elicited an increase in membrane fluidity at 10 mg/liter, but at 4 mg/liter only C10-C16 aliphatics did. *In vivo* results indicated that WSF increased the activity of microsomal enzymes that are critical in lipid metabolism, though this change was not due to direct alterations in membrane fluidity, suggesting a synthesis induction, or an enzyme- regulatory mechanism.	

Table 2 – 1,3-Diethylbenzene

Health Effects Studies

Citation	Study methods	Results	Authors' conclusions/comments
Molhave L. 1982. Indoor air pollution due to organic gases and vapours of solvents in building materials. Environment International, Volume 8, Issues 1-6, 117-127.	The emissions of organic gases and vapors of solvent type from 42 commonly used building materials were measured under atmospheric conditions. A mathematical model was established for the indoor air concentrations of pollutants originating from building materials. The model was tested on three model rooms constructed from the materials investigated.	An average of 22 compounds was found in the air around each building material, and the total concentration of gases and vapors was from 0.01 to 1,410 mg/m^3. The calculated total air concentrations of gases and vapors in the three rooms ranged from 1.6 to 23.6 mg/ m^3, and the number of compounds in the air from 23 to 32. These concentrations and number of compounds did not differ from those found in actual rooms similar in size and construction to the model rooms. The mean concentration of 1,3-diethylbenzene was 20.15 ppb; range was 13.03 to 30.48 ppb.	The authors concluded that the possibility of negative health effects cannot be neglected, especially not for the more sensitive minority of the general population.
Gacmaire F, Marienac B, de Ceaurriz J. 1990. Diethylbenzene-induced sensorimotor neuropathy in rats. J Appl Toxicol. Apr;10(2):105-12.	A study in rats given 500 mg/kg orally once daily, 5 days/week for 8 weeks.	No changes were observed in motor and sensory conduction velocities or for the amplitude of the sensory action potential of the tail nerve.	The authors concluded that 1,2-DEB is the isomer responsible for neurotoxicity.
Mehlman MA. 1992. Dangerous and cancer- causing properties of products and chemicals in the oil	The health effects of motor fuels are		Author's conclusions were as follows • Significant increases in tumors of

Table 2 – 1,3-Diethylbenzene

Citation	Study methods	Results	Authors' conclusions/comments
refining and petrochemical industry: VIII. Health effects of motor fuels: Carcinogenicity of gasoline—Scientific update. Research, Volume 59, Issue 1, October 238-249.	reviewed.		kidney, liver, and other tissues and organs following exposure to gasoline provide sufficient evidence of carcinogenicity. • Sufficient evidence for the carcinogenicity of alkyl benzenes, very significant components of gasoline, has also been established. • Human epidemiologic studies show important increases in cancers of the kidney, stomach, brain, pancreas, prostate, lung, and skin as well as hematopoietic and lymphatic leukemias as a result of exposure to gasoline, its components, and its vapors. No specific mention of 1,3-diethylbenzne in this review.
Srivastava A, Joseph AE, Patil S, More A, Dixit RC, Prakash M. 2005. Air toxics in ambient air of Delhi. Atmospheric Environment, Volume 39, Issue 1, January 59-71.	The study estimated target VOCs at 15 locations in residential, industrial, commercial, traffic intersections and petrol refueling stations in Delhi, India. Ambient air was absorbed on absorbent tubes,	The authors reported that the levels of VOCs were high and stressed the need for a regular monitoring program for VOCs in the urban environment.	Not certain that 1,3-diethylbenzene was one of the chemicals measured in the study.

Table 2 – 1,3-Diethylbenzene

Citation	Study methods	Results	Authors' conclusions/comments
	thermally desorbed and analyzed on GC-MS.		

Table 2 – 1,3-Diethylbenzene

Welfare Effects Studies

Citation	Study methods	Results	Authors' conclusions/comments
Zielinska B and Fung KK. 1994. The composition and concentration of hydrocarbons in the range of C2 to C18 emitted from motor vehicles. Science of The Total Environment, Volumes 146-147, 23 May, 281-288.	Sampling was carried out in the Caldecott Tunnel, located in the San Francisco area. Three daily samples were collected, using stainless steel canisters and Tenax-TA solid adsorbent cartridges, over 2 days in June 1991. Sample were analyzed using high resolution gas chromatographic separation and Fourier transform infrared/mass spectrometric detection or flame ionization detection of individual hydrocarbons.	Mean ppbC of 1,4- (or 1,3)-diethylbenzene was 20.15 (13.03 to 30.48). Mean % was 0.84% (0.61 to 1.14).	1,3-Diethylbenzene was found in the Caldecott Tunnel.
Kaplan IR, Galperin Y, Lu S-T, Lee R-P. 1997. Forensic Environmental Geochemistry: differentiation of fuel- types, their sources and release time. Organic Geochemistry, Volume 27, Issues 5-6, 15 November, 289, 299, 301-317.	Discussion of forensic environmental geochemistry, and the identification of escaped hydrocarbon products.	None presented in abstract.	

Table 2 – 1,3-Diethylbenzene

Citation	Study methods	Results	Authors' conclusions/comments
Casana-Giner V, Gandia-Balaquer A, Hernànder-Alamos MM, Menqod-Puerta C, Garrido-Vivas A, Primo-Millo J, Primo-Yufera E. 2001. of 79 compounds and mixtures to wild Ceratitis capitata (Diptera: Tephritidae) in field trials. J Econ Entomol. Aug; 94(4): 898-904.	79 compounds were tested for attractiveness to fruit flies.	1,3-diethylbenzene was an effective attractant for females.	
Yoshida T and Matsunaga I. 2006. A case study on identification of airborne organic compounds and time courses of their concentrations in the cabin of a new car for private use. Environment International Volume 32, Issue 1, January 58-79.	Sampling of compounds of interior air of a new car were identified, and examined for over 3 years after delivery.	High concentrations of n-nonane (458 pg/m^3 on the day following delivery), n-decane (1,301 pg/m^3), n-undecane (1,616 pg/m3), n-dodecane (716 pg/m^3), n-tridecane (320 pg/m^3), 1-hexadecene (768 pg/m^3), ethylbenzene (361 pg/m^3), xylene (4,003 pg/m^3) and 2,2'-azobis (isobuty ronitrile) (429 pg/m^3) were detected, and the sum of the concentrations determined for all compounds excluding formaldehyde The total VOC concentration (TVOC) was approximately 14 mg/m^3 on the day after the delivery. The concentrations of most compounds decreased with time, but increased with a rise of the interior temperature.	Not certain that 1,3-diethylbenzene was one of the chemicals measured in the study.
Caplain I, Cazier F, Nouali H, Mercier A, Dechaux J-C, Nollet V, Joumard R, Andre J-M, Vidon R. 2006. Emissions of unregulated pollutants from European gasoline and diesel passenger cars. Atmospheric Environment, Volume 40, Issue 31, October	Emissions of unregulated compounds were measured on new tech. passenger cars. Cars were tested on a chassis dynamometer with constant volume	1,3-diethylbenzene was identified by GC/MS in the exhaust gas.	

Table 2 – 1,3-Diethylbenzene

Citation	Study methods	Results	Authors' conclusions/comments
5954-5966.	sampling for about 100 different volatile organic compounds in the C2-C6 and C7-C16 range and carbonyl compounds. The sampling of these compounds was made using sorbent tubes followed by analysis with liquid and gas phase's chromatography.		
Prince RC and Walters CC. 2007. 11 - Biodegradation of oil hydrocarbons and its implications for source identification. Oil Spill Environmental Forensics Fingerprinting and Source Identification 349-379.	Discussion of hydrocarbons and biodegradation of fuel or crude oil.	Results not presented in abstract.	
Fingas M. 2011. Chapter 23 - An Overview of In-Situ Burning. Oil Spill Science and Technology 737-903.	Discusses *in situ* burning of oil spills. Emissions of burning are covered in the chapter.	Provides equations for predicting concentrations of emissions for the various groups and for more than 150 compounds, as well as tables of results.	Not certain that 1,3-diethylbenzene was specifically mentioned.
Guo H, Cheng HR, Ling ZH, Louiec PKK, Ayokod GA. 2011. Which emission sources are responsible for the volatile organic compounds in the atmosphere of Pearl River Delta? Journal of Hazardous Materials Volume 188,	Field measurement study of VOCs carried out at an inland Pearl River Delta site and Hong Kong urban site.	The major sources identified in the region were vehicular emissions, solvent use and biomass burning, whereas extra sources found in inland PRD included liquefied petroleum gas and gasoline evaporation. In Hong Kong, the vehicular emissions made the most significant contribution to	Not certain that 1,3-diethylbenzene was one of the chemicals measured in the study.

Table 2 – 1,3-Diethylbenzene

Citation	Study methods	Results	Authors' conclusions/comments
Issues 1-3, 15 April 116-124.		ambient VOCs (48 ± 4%), followed by solvent use (43 ± 2%) and biomass burning (9 ± 2%). In inland PRD, the largest contributor to ambient VOCs was solvent use (46 ± 1%), and vehicular emissions contributed 26 ± 1% to ambient VOCs.	

Table 3 – 1-Methyl-1-ethyl-cyclopentane

Welfare Effects Studies

Citation	Study methods	Results	Authors' conclusions/comments
Kumar P, Kuchhal RK, Kumar B, Chandolaa HC, Dixit L, Gupta PL. 1987. Characterization and compositional study of lighter fractions from coal-derived liquids. Fuel, Volume 66, Issue 8, August 1036-1045.	Synfuel fractions boiling in the range IBP (initial boiling point)-150 °C and 150-250 °C were characterized employing high resolution capillary gas chromatography and mass spectrometric techniques independently.	More than 180 compounds including 60 olefins have been identified and quantified in the IBP-150 °C fraction only.	Uncertain whether 1-methyl-1-ethyl-cyclopentane is specifically identified.

Table 4 – 2,2-Dimethylhexane

Welfare Effects Studies

Citation	Study methods	Results	Authors' conclusions/comments
Corapcioglu MY and Hossein MA. 1990. Theoretical modeling of biodegradation and biotransformation of hydrocarbons in subsurface environments. Journal of Theoretical Biology, Volume 142, Issue 4, 22 February 503-516.	An investigation of the biodegradation and biotransformation, and transport of hydrocarbons in groundwater.	None presented in abstract.	Uncertain whether 2,2-dimethylhexane is specifically discussed.
Solano-Serena F, Marchal R, Casaregola S, Vasnier C, Lebeault J-M, Vandecasteele J-P. 2000. A Mycobacterium Strain with Extended Capacities for Degradation of Gasoline Hydrocarbons. Applied and Environmental Microbiology, Vol. 66, No. 6, June, 2392-2399.	*Mycobacterium* sp. strain IFP 2173, the first isolate with the capacity to use 2,2,4-trimethylpentane as a sole carbon and energy source was identified by 165 ribosomal DNA analysis.	*Mycobacterium* sp. strain IFP 2173 used an unusually wide spectrum of hydrocarbons as growth substrates, including n-alkanes and multimethyl-substituted isoalkanes with chains ranging from 5 to 16 carbon atoms long, as well as substituted monoaromatic hydrocarbons. It also attacked ethers, such as methyl C.-butyl ether. During growth on gasoline, it degraded 86% of the substrate. The results indicated that strain IFP 2173 was capable of degrading 3- methyl groups, possibly by a carboxylation and deacetylation mechanism.	Uncertain whether 2,2-dimethylhexane is specifically discussed.
Wang D, Fuentes JD, Travers D, Dann T, Connolly T. 2005. Non-methane hydrocarbons and carbonyls in the Lower Fraser Valley during PACIFIC 2001. Atmospheric Environment Volume 39, Issue 29, September, 5261-5272.	Sampling study of hydrocarbon and carbonyl loadings in ambient air at urban and rural sites in BC, Canada.	Results indicated that in the urban atmosphere (3-h) non-methane hydrocarbon levels exceeded 20 ppbv. Rural sites had lower levels of non-methane hydrocarbons. The dominant anthropogenic hydrocarbon species were propane, butane, 2-methylbutane and toluene. The most common biogenic hydrocarbons included isoprene, a-pinene,	Uncertain whether 2,2-dimethylhexane is specifically discussed.

Table 4 – 2,2-Dimethylhexane

Citation	Study methods	Results	Authors' conclusions/comments
		13- pinene and limonene. On average, biogenic hydrocarbons represented approximately 23% of the total hydrocarbon reactivity. Formaldehyde, acetaldehyde, and acetone were the dominant carbonyl compounds found. Three-hour averaged carbonyls levels reached 10 ppbv (3-h) and resulted from both direct emissions and hydrocarbon photooxidation.	
McGillen MR, Percivala CJ, Raventos-Duran T, Sanchez-Reyna G, Shallcross DE. 2006. Can topological indices be used to predict gas-phase rate coefficients of importance to tropospheric chemistry? Free radical abstraction reactions of alkanes. Atmospheric Environment, Volume 40, Issue 14, May 2488-2500.	Investigation of hydrocarbons ranging from C2 to C10 using topological indices to provide a reliable and accessible method of estimating rate coefficients for these species.	Rate coefficients of free radical abstraction reactions of NMHCs were correlated with the Randid and Balaban topological indices for radical moieties OH, CI and NO3. The Balaban index did not correlate for branched alkanes except for NO3. Where only unbranched alkanes were considered, the Balaban index proved most reliable, demonstrating a clear linear relationship.	Uncertain whether 2,2-dimethylhexane is specifically discussed.
Fuentes MJ, Font R, Gomez-Rico MF, Martin-Gullon I. 2007. Pyrolysis and combustion of waste lubricant oil from diesel cars: Decomposition and pollutants. Journal of Analytical and Applied Pyrolysis, Volume 79, Issues 1-2, May 215-226.	Pyrolysis and combustion of waste lubricant oil were studied to its potential value as a fuel, carried out in a tubular reactor at 500 and 850 °C.	The semivolatile PAH compounds (naphthalene, phenanthrene, anthracene, etc.) were detected as much in pyrolysis as in combustion (850 °C), but benzo(a)pyrene was only found in pyrolysis at 850 °C. On the other hand, the levels of PCDD/Fs and dioxin-like PCBs were estimated after a fuel-rich combustion process at around 113 pg WHO-TEQ g-1 burnt oil (corresponding to 80 pg I-TEQ g-1) and 3 pg WHO-TEQ g-1 burnt oil, respectively. The PCDD/Fs concentration in the raw sample was estimated at around	Uncertain whether 2,2-dimethylhexane is specifically discussed.

Table 4 – 2,2-Dimethylhexane

Citation	Study methods	Results	Authors' conclusions/comments
		7-16 pg WHO-TEQ g-1 used oil (or 6-13 pg I-TEQ g-1) and PCBs were not detected.	
Cho J, Zein MM, Suidan MT, Venosa AD. 2007. Biodegradability of alkylates as a sole carbon source in the presence of ethanol or BTEX. Chemosphere, Volume 68, Issue 2, June 266-273.	The biodegradability of alkylate compounds in serum bottles was investigated in the presence and absence of ethanol or benzene, toluene, ethylbenzene, and p-xylene (BTEX).	Compounds degraded more slowly in the presence of BTEX than in their absence because BTEX inhibited the microbial utilization of alkylates. However, in the presence of ethanol, their slower biodegradation was not related to inhibition by the ethanol. Throughout the experiments alkylates, ethanol, and BTEX concentrations did not change in the sterile controls.	Uncertain whether 2,2-dimethylhexane is specifically discussed.

Table 5 – 2,4,4-Trimethyl-2-pentene

Health Effects Studies

Citation	Study methods	Results	Authors' conclusions/comments
Christine V. Hampton," William R. Pierson, 1. Michael Harvey, William S. Updegrove, andHydrocarbon Gases Emitted from Vehicles on the Road. 1. A Qualitative Gas chromatography/ Mass Spectrometry Survey Richard S. Marano 1982. Environ. Sci. Technol. 16, 287-298	A study measuring components of exhaust gas.	2,4,4,-trimethyl-2-pentene was identified as an exhaust gas was but not identified at Allegheny Tunnel in 1979 (in the present study)	
Edward W. Kaiser,' Walter 0. Siegi, Davld F. Cotton, and Richard W. Anderson 1993. Effect of Fuel Structure on Emissions from a Spark-Ignited Engine. 3.Olefinic Fuels. Environ. Sci, Technol., 27, 1440-1447	A single-cylinder, production-type engine has been run at four operating conditions on four olefinic fuels (ethylene,1-butene, 1-hexene, and diisobutylene) and two blends (n-hexane with toluene and 20% diisobutylene with a fully blended gasoline).	The diisobutylene contained 76.9 % 2,4,4-trimethyl-1-pentene, 18.4% 2,4,4-trimethyl-2-pentene, 2 % isomeric octanes, and 2.7 % unidentified impurities. Total HC emissions from the olefinic fuels increase with the molecular weight of the fuel (e.g., from 320 ppm C1 for ethylene to 1,420 ppm C1 for diisobutylene during lean operation). The HC emission for each olefin is lower, and the NO, emission is higher than that of the corresponding alkane. 1,3-Butadiene is significant for the straight-chain terminal olefins, 1-butene and 1-hexene, but is much less important for the highly branched olefin, diisobutylene. For the diisobutylene-gasoline blend, the mole fractions of products unique to diisobutylene combustion can be predicted to within 10% based on data from diisobutylene, gasoline, and the concentration of diisobutylene in the blend. Thus, the exhaust emissions are approximately additive. For the hexane-toluene blend, no appreciable formation of alkyl-	

Table 5 – 2,4,4-Trimethyl-2-pentene

Citation	Study methods	Results	Authors' conclusions/comments
		substituted toluenes was observed.	

Table 6 – 2-Butyne

Health Effects Studies

Citation	Study methods	Results	Authors' conclusions/comments
d'Amboise M, Mathieu D, Piron DL. 1988. A chemical study of 2-butyne-1,4-diol. Talenta. Oct;35(10):763-8.	Report of chemical properties of 2-butyne-1,4-diol.	Brownish technical grade 2-butyne-1,4-diol contains the monomer, the dimer and some trimer. Pure monomeric 2-butyne-1,4-diol is a white solid obtained by evaporation of the technical grade product. The monomer is slowly transformed into timer and possibly into a trimer when dissolved in water.	Study of 2-butyne-1,4-diol; not 2-butyne.
Komsta E, Secours VE, Chu I, Valli VE, Morris R, Harrison J, Baranowski E, Villeneuve DC. 1989. Short-Term Toxicity of Nine Industrial Chemicals. Bulletin of Environmental Contamination and Toxicology, Vol. 43, No. 1, 87-94.	An oral study in rats treated with 1, 10, and 100 mg/kg for 14 days.	2-Butyne-1,4-diol demonstrated toxic properties. A significant growth depression was observed in male rats dosed with 100 mg/kg bw and an increase in liver weight for males and females occurred at this dosing. Males and females experienced an increase in serum cholesterol levels at 100 mg/kg bw as well as decreased red cell counts, hemoglobin content, and hematocrit among females.	Study of 2-butyne-1,4-diol; not 2-butyne. The authors concluded that additional studies should be conducted to determine potential long-term effects.
Rappaport SM. 1991. Assessment of long-term exposures to toxic substances in air. Ann Occup Hyg. Feb;35(1):61-121.	Commentary on exposure assessment of toxic substances in air.	Sampling strategy must allow the distribution of individual mean exposures across the population at risk. Little effort should be devoted to evaluation of short-term peak exposures.	No specific mention of 2-butyne in the study.
Jedrychowski RA, Czajkowska T, Stetkiewicz J, Stetkiewicz I. 1992. Acute Toxicity of 2-Butyne-1,4-diol in Laboratory Animals. Journal of Applied Toxicology, Vol. 12, No. 2, 113-115.	An oral study in rats treated with 10% aqueous 2-butyne at 100, 150, 180, 200, or 250 mg/kg for 14 days.	The median lethal dose (LD50) was 132 mg/kg in males and 176 mg/kg in females. Gross pathologies included diarrhea and congestion of internal organs while histopathological change included perivascular edema, bronchopneumonia, hyperemia, liver necrosis, and nephrosis.	Study of 2-butyne-1,4-diol; not 2-butyne. The authors concluded that 2-butyne-1,4-diol was a moderately toxic substance. In acute poisonings it was hepatotoxic and nephrotoxic, but it had no apparent skin sensitizing potential.

Table 6 – 2-Butyne

Citation	Study methods	Results	Authors' conclusions/comments
		Slight lymphocytic infiltrations and mitotic cells were seen after 14 days.	
	Dermal exposure to 100% or 40% aqueous 2-butyne (applied to clipped sides or dorsum) in rats. Dermal exposure in White Vienna Rabbits (irritation test) to 100%, 40% and 20% 2-butyne at 1, 24, 48 and 72 hours after application, as well as a short- term dermal irritation tested using 40% 2-butyne painted behind ears for 10 consecutive days. Dermal exposure in Guinea Pigs to 2% 2-butyne injected intradermally and 20% 2-Butyne applied topically.	The 40% solution of 2-butyne killed 8 of 16 rats within 48 hours. Diffuse necrosis of liver and kidneys was seen. By 14 days, most prominent feature was extensive vacuolation of liver parenchyma. Dermal irritation and allergic contact dermatitis tests were negative. Solid application was not lethal. It was to be a slight irritant. No allergic contact dermatitis was observed.	
	Occular exposure in New Zealand rabbits to 100 mg in conjunctival sac of	Marked lacrimation and slightly closed lids were observed in all rabbits after 1 hr of application. After 24 and 48 hrs. Minimal conjunctival erythema was evident.	

Table 6 – 2-Butyne

Citation	Study methods	Results	Authors' conclusions/comments
	one eye; other eye was control. Scored after 1, 24, 48, 72 hrs, and after 7 days.		
Jedrychowski RA, Czajkowska T, Stetkiewicz J, Stetkiewicz I. 1992. Subacute Oral Toxicity of 2-Butyne-1,4-diol in Rats. Journal of Applied Toxicology, Vol. 12, No. 2, 117-122.	Oral exposure in rats to single doses of 1, 10, and 50 mg/kg for 28 days.	In the high dose group, 3 of 8 males and 3 of 8 females died, congested internal organs, pulmonary edema, and necrosis of kidneys and liver were observed. Weight gain was less than controls (males), with no difference in females. Hematological differences in females included a decrease in erythrocyte (RBC) count, hematocrit value, and hemoglobin concentration. Increased reticulocyte and leukocyte counts occurred In both sexes. Significant increases in sorbitol-dehydrogenase in both sexes were observed, as well as a higher total protein concentration in females, and elevated glucose in males. In the medium dose group, a diminished ROC count in females was reported. Hepatic lesions were seen on microscopic examination of medium and high dose animals. Splenic changes in animals from these two groups reflected disturbances in peripheral blood.	Study of 2-butyne-1,4-diol; not 2-butyne. The authors concluded that prolonged exposure to 2-butyne can cause serious health problems.
Tyce R. 1997. Toxicology of 2-Butyne-1,4-diol [110-65-6], Review of Literature. Integrated Laboratory Systems, Research Triangle Park, North Carolina. January 14.	Review.	This review included two case reports of human contact sensitization. One involved a female cleaner and the other involved a male worker in an electroplating dept. of a factory who occasionally prepared aqueous solutions. Author reported that 2-butyne-1,4-diol appears to be activated to a toxic metabolite by liver alcohol dehydrogenase	Study of 2-butyne-1,4-diol; not 2-butyne. There does not appear to be any human carcinogenicity data for this chemical. Genotoxicity data were negative.

Table 6 – 2-Butyne

Citation	Study methods	Results	Authors' conclusions/comments
		and that it induced mortality in a dose-dependent manner in rats. Seven-day acute oral LD50s for mice, rats, rabbits, and guinea pigs were between 100 and 200 mg/kg bw. The 2-hour inhalation LCLo for mice and rats was approx. 50 mg/kg bw. Treatment acutely or subchronically resulted in irritation and/or systemic toxicity, primarily involving damage to the liver and kidneys, and depending on route, to the skin and lungs. It also induced hypothermia in rats. The only reproductive study – a study whereby it was administered by gavage at 653 mol/kg to male quail – did not show a sufficient decrease in fertility or testis weight to be classified as an avian chemosterilant. No carcinogenicity data were found. Genotoxicity data were negative for both the induction of gene mutations in *Salmonella typhimurium* and chromosome aberrations in hamster cells.	
Hakkola MA and Saarinen LH. 2000. Customer exposure to gasoline vapors during refueling at service stations. Appl Occup Environ Hyg. Sep;15(9):677-80.	Exposure study of gasoline vapors at Stage I (vapor recovery only during recovery from road tanker) and Stage II stations (additional vapor recovery during refueling). Exposure of 20 customers measured from breathing zone	Geometric mean concentrations of hydrocarbons (C3-C11) at Stage I was 85 mg/m^3 (range 2.5-531 mg/m^3); at Stage II was 18 mg/m^3 (range < 0.2-129 mg/m^3). [Note: butyne is a C3 hydrocarbon.] The differences in exposure were statistically significant ($p < 0.05$).	The exposure level of customers at the Stage II station during refueling was around 20-25 percent of the exposure at the Stage I service station when conditions were equal and no other confounding factors such as leaks or spills were present.

Table 6 – 2-Butyne

Citation	Study methods	Results	Authors' conclusions/comments
	during 95-octane refueling.		
Hewett P. 2001. Misinterpretation and misuse of exposure limits. Appl Occup Environ Hyg. Feb;16(2):251-6.	Commentary on how the failure to distinguish between the complementary processes of risk assessment and exposure (risk) management leads to misinterpretation and misuse of OELs.	The authors noted the following points: • OELs are occasionally improperly applied, resulting in a reduction of the expected level of protection. • OELs are frequently misinterpreted as upper limits to exposures averaged over weeks, months or even years, rather than a single shift.	This study does not appear to be specific for 2-butyne. The author suggested that OEL-setting organizations consider harmonizing definitions and statistical interpretations for both existing and new OELs.
Jeon H-B, Lee Y, Qiao C, Huang H, Lawrence M. 2003. Inhibition of bovine plasma amine oxidase by 1,4-diamino-2-butenes and -2-butynes. Bioorganic & Medicinal Chemistry Volume 11, Issue 21, 15 October 4631-4641.		2-Butyne was reported to be a potent inactivator of bovine plasma amine oxidase.	The authors noted that this finding was suggestive of unexpected similarities between plasma amine oxidase and the diamine oxidases and implies that it may be unwise to attempt to develop selective inhibitors of diamine oxidase using a diamine construct.
Stouten H, Bouwman C, Wardenbach P. 2008. Reassessment of occupational exposure limits. Am J Ind Med, Jun;51(6):407-18.	Criteria documents for 161 compounds prepared by the Health Council of the Netherlands to reassess the toxicological hazards and health based OELs.	The toxicological database met the committee's criteria for a health-based OEL for only about 40% of the compounds.	This report does not appear to be specific for 2-butyne. The author concluded that many older maximum allowable concentration (MAC) values were either too high or not scientifically supported and therefore not health-based.
Nielsen GD, Ovrebø S. 2008. Background, approaches and	Review discussing approaches and		This review does not appear to be specific for 2-butyne.

Table 6 – 2-Butyne

Citation	Study methods	Results	Authors' conclusions/comments
recent trends for setting health-based occupational exposure limits: a minireview. Regal Toxicol Pharmacol, Aug;51(3):253-60, Epub Apr 14.	trends to setting OELs.		
Kupczewska-Dobecka M and Swiercz R. 2009. Setting limit values for chemical substances in the workplace: DNEL(INH) setting according to REACH principles following the example of 2-butyne-1,4-diol. Med Pr. 60(5):347-57.	Discussion of setting Derived No Effect Level (DNEL) for 2-butyne. Data from two inhalation studies in rats and three oral studies have been used to calculate the DNEL.	The estimated DNEL values show significant differences and fall within the range of 0.02 to 0.33 mg/m^3, depending on the chosen experiment and critical effect. The authors noted that a 30-day inhalation study best reflects the penetration of xenobiotics in humans. This study was used to set a MAC value of 0.25 mg/m^3 and it is close to the local DNEL of 0.10 mg/m^3.	The authors concludes that the DNEL will tend to be lower than any corresponding health-based OEL and indicated that the OEL does not provide the appropriate level of protection required by REACH. This is due to differences in methodologies for calculating DNELs and OELs.

Table 6 – 2-Butyne

Welfare Effects Studies

Citation	Study methods	Results	Authors' conclusions/comments
Frêbort I, Sebela M, Svendsen I, Hirota S, Endo M, Yamauchi O, Bellelli A, Lemr K, Pec P. 2000. Molecular mode of interaction of plant amine oxidase with the mechanism-based inhibitor 2-butyne-1,4-diamine. European Journal of Biochemistry, Vol 267; Issue 5; 1423-1433, March.	Chemical study of the interaction between plant amine oxidase and 2-butyne. Peptides containing the 2-butyne binding site were obtained by proteolysis of inactivated enzyme, isolated by HPLC and analyzed by amino acid sequencing and MS.	Inhibition of the amine oxidase is caused mainly by the highly reactive 2-butyne product, 4-amino-2-butynal, binding to a nucleophilic residue at the entrance to the substrate channel.	As other 2-butyne labeled peptides were also found and no free 2-butyne product was detected by MS after complete inhibition of the enzyme, it is likely that the 2-butyne product binds also to other solvent-exposed nucleophilic residues on the enzyme surface.
Sebela M, Frebort I, Petrivalsky M, Pec P. 2002. Copper/topa quinine-containing amine oxidases — Recent research developments. Studies in Natural Products Chemistry Volume 26, Part 7, 1259-1299 Bioactive Natural Products.	Report that discusses information on the general properties of the enzymes and their physiological functions of amine oxidases that contain copper/topa quinone cofactor.	In plants, enzymes reduce the concentration of toxic amines produced during exposure to stress conditions, provide hydrogen peroxide for wall stiffening and lignifications and precursor compounds for biosynthesis of some alkaloids.	This report does not appear to mention 2-butyne.
Fahr A and Laufer AH. 2005. UV-absorption spectra of the radical transients generated from the 193-nm photolysis of allene, propyne, and 2-butyne. J Phys Chem A. Mar 24;109(11):2534.9.	A study that examined the 193-nm photochemistry of 2-butyne and the UV spectral region between 220 and 350 nm of transient species generated from the photolysis of 2-butyne. Time-	Photolysis of 2-butyne in the 240 and 320 nm region indicated an instant rise followed by an additional slower absorption rise. The methyl (CH3) and propynyl (CH3C[triple bond]C) radicals are likely to be among the photodissociation products of 2-butyne.	

Table 6 – 2-Butyne

Citation	Study methods	Results	Authors' conclusions/comments
	resolved UV-absorption spectroscopy was used for detection of transient absorption. Gas chromatographic/ mass spectroscopic (GC/MS) analysis of the photolyzed samples were employed for identification of the final photodissociation products.		
Becerra R, Cannady JP, Dormer G, Walsh R. 2009. The gas-phase reaction between silylene and 2-butyne: kinetics, isotope studies, pressure dependence studies and quantum chemical calculations. Pill's Chem Chem, Phys. Jul 14;11(26):5381-44. Epub Apr 17.	Time-resolved kinetic studies of the reactions of silylene, SiH(2), and dideutero-silylene, SiD(2), generated by laser flash photolysis of phenylsilane and phenylsilane-d(3), respectively, have been carried out to obtain rate coefficients for their bimolecular reactions with 2-butyne.	Calculations indicated the probable involvement of several other intermediates, and possible products. RRKM calculations were in reasonable agreement with the pressure dependences at an enthalpy value for 2,3-dimethylsilirene fairly close to that suggested by the *ab initio* calculations. The experimental isotope effects deviated significantly from those predicted by RRKM theory. The differences can be explained by an isotopic scrambling mechanism, involving H-D exchange between the hydrogens of the methyl groups and the D-atoms in the ring in 2,3-dimethylsilirene-1,1-d(2). A detailed mechanism involving several intermediate species, which is consistent with the G3 energy surface, is proposed to account for this.	
Kaiser RI, Zhang F, Gu X, Kislov VV, Mebei AM. 2009. Reaction	The reactions of the phenyl radical (C6H5)	Both reactions involved indirect scattering dynamics and were initiated by additions of	

Table 6 – 2-Butyne

Citation	Study methods	Results	Authors' conclusions/comments
dynamics of the phenyl radical (C6H5) with 1-butyne (HCCC2H5) and 2-butyne (CH3CCCH3). Chemical Physics Letters, Volume 461, Issues 1-3, 19 October 46-53.	with 1-butyne (HCCC2H5) and 2-butyne (CH3CCCI-13) were studied in a crossed molecular beam machine to shed light on the formation of C10H10 isomers in combustion flames.	the phenyl radical with its radical center to the acetylenic carbon atoms. The reaction intermediates fragmented through the ejection of hydrogen atoms yielded 1-phenyl-3-methylallene and 1- phenyl-1-butyne (both from 1-butyne) as well as 1-phenyl-1-methylallene (from 2-butyne).	
Lalli PM, Corilo YE, Abdelnur PV, Ebertin MN, Laali KK. 2010. Intrinsic acidity and electrophilicity of gaseous propargyl/allenyl carbocations. Org Biomol Chem, Jun 7;8(11):2580-5, Epub Mar 29.	The ion/molecule chemistry of 2-butyne-1-ol (CH(3)-CH(+)-C[triple bond]C-H, 3a) was studied via pentaquadrupole mass spectrometry.	With pyridine, proton transfer was observed as the sole reaction channel for the isomeric 2-butyne. Proton transfer was also observed as a dominant process in reactions with ethanol for 2-butyne. No adduct formation was observed. Limited reactivity was exhibited by 2-butyne in a cycloaddition reaction with isoprene.	

Table 7- 2-Methylhexane

Health Effects Studies

Citation	Study methods	Results	Authors' conclusions/comments
Krotoszynski BK and O'Neill HJ. 1982. Involuntary bioaccumulation of environmental pollutants in nonsmoking heterogeneous human population. Journal of Environmental Science and Health, Part A: Toxic/Hazardous Substances and Environmental Engineering, Volume 17, Issue 6, 855 – 883.	Biomarker study: bioaccumulations of environmental pollutants were identified and evaluated using a non-invasive expired air technique.	2-Methylhexane was detected in expired air.	2-Methylhexane may accumulate in humans.
Klopman G. 1985. Predicting toxicity through a computer automated structure evaluation program. Environ Health Perspect. September; 61: 269-274.	Computer automated structure evaluation program has been extended to perform automatic quantitative structure-activity relationships.	The authors reported that agreement with experimental data is satisfactory.	Not certain if 2-methylhexane was included in this study.
Kleindienst TE, Smith DF, Hudgens EE, Snow RF, Perry E, Claxton LD, Bufalini JJ, Black FM, Cupitt LT. 1992. The photo-oxidation of automobile emissions: measurements of the transformation products and their mutagenic activity. Atmospheric Environment, Part A. General Topics, Volume 26, Issue 16, November 3039-3053.	Genotoxicity study: dilute mixtures of automobile emissions (50% exhaust and 50% surrogate evaporative emissions) were irradiated in a 22.7 m^3 smog chamber and tested for mutagenic activity by using a variant of the Ames test.	The gas-phase data for TA100 and TA98 showed increased activity for the irradiated emissions when compared to the nonirradiated mixture, which exhibited negligible activity with respect to the control values. The particulate phase for both the irradiated and nonirradiated mixtures showed negligible activity when results were compared to the control values for both strains. 2-Methylhexane was measured in the automobile exhaust: 0.0308 ppmC of 3.75 ppmC auto exhaust.	2-Methylhexane did not appear to be genotoxic.

Table 7- 2-Methylhexane

Citation	Study methods	Results	Authors' conclusions/comments
Laurent C, Lakhanisky T, Jadot P, Joris I, Ottogali M, Planard C, Bazzoni D, Foidart JM, Ros Y. 1993. Increased sister chromatid exchange frequencies observed in a cohort of inhabitants of a village located at the boundary of an industrial dumping ground: phase I. Cancer Epidemiol Biomarkers Prev July, 2; 355.	Biomarker and genotoxicity study: biomarkers were measured in a group of people living near a dumping ground for industrial waste. The sister chromatid exchange assay was also performed.	A significant increase in sister chromatid exchange (SCE) frequency was detected among the inhabitants of the village compared to that of the control group, especially among the children living in Mellery as compared to the matched control children. The concentration of 2-methylhexane measured from the superficial bed of soil of the dumping ground was 27 mg/m^3.	Though SCE frequency was increased in the exposed group; however, 2-methylhexane was one of many chemicals measured at the dumping ground.
Basak SC and Grunwald GD. 1994. Molecular Similarity and Risk Assessment: Analog Selection and Property Estimation Using Graph Invariants. SAR and QSAR in Environmental Research, Volume 2, Issue 4, 289 – 307.	Four molecular similarity measures have been used to select the nearest neighbor of chemicals in two data sets of 139 hydrocarbons and 15 nitrosamines, respectively.	The results showed that for these data sets, all four methods give reasonable estimates of the properties studied.	Not certain if 2-methylhexane was included in this study.
Gamble JF, Pearlman ED, Nicolich MJ. 1996. A nested case-control study of kidney cancer among refinery/petrochemical workers. Environ Health Perspect. June; 104(6): 642-650.	Nested case-control study: to evaluate whether a nearly two-fold excess of kidney cancer among workers at a refinery/petrochemical plant was associated with cumulative exposure to C2-05 saturated, C2-05 unsaturated, C6-C10 aliphatic saturated, C6-C10 aliphatic	Categorical analysis showed increased odds ratios only in the second (low) and fourth (high) quartiles compared to the first quartile reference group of lowest exposed workers, and a three-quarter-fold increased odds ratio for > 32 years' tenure compared to the < 25-year reference group.	2-Methylhexane was one of many chemicals in petroleum production streams.

Table 7- 2-Methylhexane

Citation	Study methods	Results	Authors' conclusions/comments
	unsaturated, and C6-C10 aromatic process streams.		
Johnson R, Macina OT, Graham C, Rosenkranz HS, Cass GR, Karol MH. 1997. Prioritizing testing of organic compounds detected as gas phase air pollutants: structure-activity study for human contact allergens. Environ Health Perspect. September; 105(9): 986-992.	Air pollutants were evaluated using computer programs to determine the potential to act as contact allergens.	CASE and MULTICASE predicted the activity of 238 compounds found in the atmosphere for their ability to act as contact allergens. The analysis found that 21 of 238 compounds were predicted to be active contact allergens (probability >0.5), with potencies ranging from mild to very strong. The compounds came from chemical classes that included chlorinated aromatics and chlorinated hydrocarbons, N-containing compounds, phenols, alkenes, and an S-containing compound.	Not certain if 2-methylhexane was included in this study.
Daughtrey W, Newton P, Rhoden R, Kirwin C, Haddock L, Duffy J, Keenan T, Richter W, Nicolich M. 1999. Chronic inhalation carcinogenicity study of commercial hexane solvent in F-344 rats and B6C3F1 mice. Toxicol. Sci. 48 (1): 21-29.	Study in which rats and mice were exposed via inhalation to 0, 900, 3,000, and 9,000 ppm 6 h/day, 5 days/week for 2 years.	There were no significant differences in survivorship between control and hexane-exposed groups, and clinical observations were generally unremarkable. Small, but statistically significant decreases in body weight gain were seen in rats of both sexes in the mid- and high-exposure groups and in high-exposure female mice. The only noteworthy histopathological finding in rats was epithelial cell hyperplasia in the nasoturbinates and larynx of exposed groups. This response was judged to be indicative of upper respiratory tract tissue irritation. No significant differences in tumor incidence between control and hexane-exposed rats were found. In mice, uterine tissue from the high-exposure females exhibited a significant decrease in the severity of cystic endometrial	Commercial hexane does not include 2-methylhexane. The authors concluded that chronic exposure to commercial hexane solvent at concentrations up to 9,000 ppm was not carcinogenic to F-344 rats or to male B6C3F1 mice, but did result in an increased incidence of liver tumors in female mice.

Table 7- 2-Methylhexane

Citation	Study methods	Results	Authors' conclusions/comments
		hyperplasia compared to controls. An increase in the combined incidence of hepatocellular adenomas and carcinomas was observed in high-exposure female mice. The incidence of liver tumors was not increased in the mid- or low-exposure female mice or in male mice exposed to hexane.	
Karelson M, Sild S, Maran U. 2000. Non-Linear QSAR Treatment of Genotoxicity. Molecular Simulation, Volume 24, Issue 4 & 6, 229-242.	The mutagenic toxicity of heteroaromatic and aromatic amines, measured by the Ames test, was correlated with the molecular descriptors calculated from the molecular structures using quantum-chemical methods. The quantitative models obtained were compared with the results of the linear QSAR treatment.	Results not presented in the abstract.	The authors explained that the descriptors appearing in the models revealed the importance of mutagenic interactions of heteroaromatic amines via hydrogen bonding, of effects induced by the solvent, and of the size of compound. Not certain if 2-methylhexane was included in this study.
Novak BJ, Blake DR, Meinardi S, Rowland FS, Pontello A, Cooper DM, Galassetti PR. 2007. Exhaled methyl nitrate as a noninvasive marker of hyperglycemia in type 1 diabetes. Proc Natl Acad Sci U S A. October 2; 104(40): 15613-15618.	Exhaled VOC profiles were evaluated in children with type 1 diabetes mellitus to investigate the effect of differing metabolic characteristics.	Exhaled methyl nitrate strongly correlated specifically with the acute, spontaneous hyperglycemia of T1 DM children. Among the ~100 measured exhaled gases, the kinetic profile of exhaled methyl nitrate, commonly present in room air in the range of 5-10 parts per trillion, was most strongly statistically correlated with that of plasma	Not certain if 2-methylhexane was included in this study.

Table 7- 2-Methylhexane

Citation	Study methods	Results	Authors' conclusions/comments
		glucose (P = 0.003-0.001).	
Chang CT and Chen BY. 2008. Toxicity assessment of volatile organic compounds and polycyclic aromatic hydrocarbons in motorcycle exhaust. Journal of Hazardous Materials, Volume 153. Issue 3, 30 May 1262-1269.	Toxicity study of various pollutants from motorcycle exhaust was evaluated using *E. coli*.	Motorcycle exhaust was significantly more toxic than BTEX due to the highly toxic VOCs generated from incomplete combustion. Overall toxicity evaluation showed that the toxicity, indicated as EC50, was approximately as follows: PAHs > two-stroke engines > four- stroke engines > BTEX. 2-Methylhexane makes up 1.39% by volume of gasoline.	Exhaust gases contain many chemicals in addition to 2-methylhexane (and at far greater concentrations).
Price K and Krishnan K. 2011. An integrated QSAR-PBPK modeling approach for predicting the inhalation toxicokinetics of mixtures of volatile organic chemicals in the rat. SAR and QSAR in Environmental Research, Volume 22, Issue 1 & 2, 107-128.	QSAR-PBPK modeling study to predict the inhalation toxicokinetics of chemicals in mixtures.	QSAR-estimated values of Vmax varied compared with experimental results by a factor of three for 43 out of 53 studied volatile organic compounds (VOCs). Km values were within a factor of three compared with experimental values for 43 out of 53 VOCs.	The authors reported that the study indicated the potential usefulness of the QSAR-PBPK modeling approach to provide first-cut evaluations of the kinetics of chemicals in mixtures of increasing complexity, on the basis of chemical structure.

Table 7- 2-Methylhexane

Welfare Effects Studies

Citation	Study methods	Results	Authors' conclusions/comments
Henry RC, Spiegelman CH, Collins JF, Park E. 1997. Reported emissions of organic gases are not consistent with observations. Proc Natl Acad Sci U S A. June 24; 94(13): 6596-6599.	Comparison study of self-reported emissions of organic gases in Houston to measurements at a receptor site near the Houston ship channel (a major petrochem. complex).	The study demonstrate severe inconsistencies between reported emissions and major sources as derived from the data using a multivariate receptor model. The composition and the location of the sources as deduced from the data were not consistent with the reported industrial emissions.	The authors conclude that their research provides strong empirical evidence that regulatory agencies and photochemical models are making predictions based on inaccurate industrial emissions. Not certain if 2-methylhexane was included in this study.
Solano-Serena F, Marchal R, Casaregola S, Vasnier C, Lebeault J-M, Vandecasteele J-P. 2000. A Mycobacterium Strain with Extended Capacities for Degradation of Gasoline Hydrocarbons. Appl Environ Microbiol. June; 66(6): 2392-2399.	Strain IFP 2173 *Mycobacterium* was selected and its ability to degrade gasoline hydrocarbons was investigated.	Strain IFP 2173 *Mycobacterium* was capable of degrading 3-methyl groups, possibly by a carboxylation and deacetylation mechanism. Evidence that it attacked the quaternary carbon atom structure by an as-yet-undefined mechanism during growth on 2,2,4-trimethylpentane and 2,2-dimethylpentane was also obtained.	
Srivastava A, Joseph AE, Patil S, More A, Dixit RC, Prakash M. 2005. Air toxics in ambient air of Delhi. Atmospheric Environment, Volume 39, Issue 1, January 59-71.	Sampling study: estimation of target VOCs at 15 locations in five categories: residential, industrial, commercial, traffic intersections and petrol refueling stations in Delhi, India.	The results showed that levels of VOCs are high and the authors stressed the need for a regular monitoring program of VOCs in urban environment.	Not certain if 2-methylhexane was included in this study.
Davis ME, Blicharz AP, Hart JE, Laden F, Garshick E, Smith TJ.	Sampling study: measurements of	Occupational exposure to VOCs and aldehydes varied significantly across the	Not certain if 2-methylhexane was included in this study.

Table 7- 2-Methylhexane

Citation	Study methods	Results	Authors' conclusions/comments
2007. Occupational Exposure to Volatile Organic Compounds and Aldehydes in the U.S. Trucking Industry. Environ Sci Technol. October 15; 41(20): 7152-7158.	VOCs and aldehydes at 15 different U.S. trucking terminals and in city truck drivers were conducted.	different sampling locations within each terminal, with significantly higher exposures noted in the work environments over background levels ($p < 0.01$).	
Cheng HR, Guo H, Saunders SM, Lam SHM, Jiang F, Wang XM, Simpson IJ, Blake DR, Louie PKK, Wang TJ. 2010. Assessing photochemical ozone formation in the Pearl River Delta with a photochemical trajectory. Atmospheric Environment, Volume 44, Issue 34, November 4199-4208.	Photochemical trajectory model, coupled with the Master Chemical Mechanism describing the degradation of 139 VOCs in the troposphere, was developed and used to simulate the formation of photochemical pollutants during photochemical pollution episodes.	The simulated diurnal variations and Mmxing ratios of ozone were in good agreement with observed data ($R2 = 0.80$, $P < 0.05$), indicating that the photochemical trajectory model – an integration of boundary layer trajectories, precursor emissions and chemical processing – provided a reasonable description of ozone formation in the Pearl River Delta (PRD) region. Alkanes and oxygenated organic compounds had relatively low reactivity, while alkenes and aromatics presented high reactivity. Analysis of the emission inventory found that the sum of 60 of the 139 VOC species accounted for 92% of the total POCP-weighted emission. Mobile sources were the largest contributor to regional O3 formation (40%).	
Fuselli S, De Felice M, Morlino R, Turrio-Baldassarri L. 2010. A Three Year Study on 14 VOCs at One Site in Rome: Levels, Seasonal Variations, Indoor/Outdoor Ratio and Temporal Trends. Int J Environ Res Public Health. October; 7(10): 3792-3803.	Sampling study: twelve hydrocarbons and two organochlorine compounds were monitored both outdoors and indoors for three years at one site in Rome.	Results showed that 118 out of 168 indoor seasonal mean values were higher than the corresponding outdoor concentrations. The most relevant source of outdoor hydrocarbons was automotive exhaust emissions.	

Table 8 – 3,4-Dimethyl-1-pentene

Welfare Effects Studies

Citation	Study methods	Results	Authors' conclusions/comments
Chunming Liu, Zili Xu, Yaoguo Du and Haich en Guo 2000. Analyses of volatile organic compounds concentrations and variation trends in the air of Changchun, the northeast of China. Atmospheric Environment, Volume 34, Issue 26,2 August 2000, Pages 4459-4466	Analysis of volatile organic compounds (VOCs) at 4 different zones in Northeast China.	VOCs levels by the roadside (the center zone of the main street) and in the downtown area were higher than those in other three areas, with the lowest concentration observed at a rural site. Seasonal variation of VOCs was noted at all sampling sites – maximum in the winter and minimum in the spring	The diurnal variation of the total mean VOC concentrations showed two peaks related with traffic density for the A, B and D sites, and a single peak for the industrial area.

Table 9 – 3-Methyl-1-pentene

Health Effects Studies

Citation	Study methods	Results	Authors' conclusions/comments
Khadikar, Padmakar V.; Diudea, Mircea V.; Singh, Jyoti; John, Peter E.; Shrivastva, Anjalee; Singh, Shalini; Karmarkar, Sneha; Lakhwani, Meenakshi; Thakur, Purnima 2006. Use of PI Index in Computer-Aided Designing of Bioactive Compounds. Current Bioactive Compounds, Volume 2, Number 1, March, pp. 19-56(38)	Review.	In this review the authors critically examined index of organic compounds acting as drugs and discussed its applications in Computer-Aided Designing of bioactive compounds with special reference to designing of carbonic anhydrase inhibitors, lipophilicity, toxicity, tadpole narcosis, bio-concentration factor, diuretic activity and carcinogenic activity of aromatic hydrocarbons and heterocycles.	Not certain that 3-methyl-1-pentene was specifically included in this review.
Sihua Lu, Ying Liu, Min Shao and Shan Huang 2004. Chemical speciation and anthropogenic sources of ambient volatile organic compounds (VOCs) during summer in Beijing. Frontiers of Environmental Science & Engineering in China, 2007, Volume 1, Number 2, 147-152	Quantification of VOCs, alkanes (C3 to C12), alkenes (C3 to C11), aromatics (C6 to C12), and halogenated hydrocarbons using a chemical mass balance (CMB) model.	The results showed that urban VOCs are predominant from mobile source emissions, which contribute more than 50% of the VOCs (in mass concentrations) to ambient air at most sites. Other important sources are gasoline evaporation, painting, and solvents. The exception is at the Tongzhou site where vehicle exhaust, painting, and solvents had about equal contribution, around 35%, of the ambient VOC concentration	Not certain that 3-methyl-1-pentene was specifically included in this study.
Hongzhi R. Zhang, Eric G. Eddings and Adel F. Sarofim 2008. Pollutant Emissions from Gasoline Combustion. 1. Dependence on Fuel Structural Functionalities. Environ. Sci. Technol., 2008, 42 (15), pp 5615-5621	A study of the formation of air pollutants and soot precursors from aliphatic and aromatic fractions of gasoline fuels, techniques.	The mechanism yielded very good predictions of species concentrations in premixed flames of n-heptane, isooctane, benzene, cyclohexane, olefins, oxygenates, and gasoline using a 23-component surrogate formulation. The 1,3-butadiene emission comes mainly from minor fuel fractions of olefins and cyclohexane. The benzene formation potential of gasoline components shows the following trends as	3-Methyl-1-pentene was not directly measured in this study.

Table 9 – 3-Methyl-1-pentene

Citation	Study methods	Results	Authors' conclusions/comments
		functions of (i) chemical class: n-paraffins < isoparaffins < olefins < naphthalenes < alkylbenzenes < cycloparaffins toluene; (ii) carbon number: n-butane < n-pentane n-hexane; and (iii) branching: n-hexane < isohexane < 2,2,4-trimethylpentane < 2,2,3,3-tetramethylbutane. In contrast, fuel structure is not the main factor in determining acetylene formation.	

Table 9 – 3-Methyl-1-pentene

Welfare Effects Studies

Citation	Study methods	Results	Authors' conclusions/comments
Grosjeana, Daniel 1992. Formic acid and acetic acid: Emissions, atmospheric formation and dry deposition at two southern California locations. Atmospheric Environment, Part A. General Topics, Volume 26, Issue 18, December 1992, Pages 3279-3286	Formation rates and removal rates by dry deposition were estimated for formic and acetic acid	Data for eight unreactive tracers, direct emission rates during the fall 1987 are estimated to be 5.6 and 12.8 metric tons d-1 (d-1 = per day) for C1 and C2, respectively, at a coastal source-dominated site. These emissions rates increased to 9.6 (C1) and 20.4 (C2) metric tons d-1 during the summer. *In situ* formation in the atmosphere via the ozone- olefin reaction is an important source for both acids. This reaction produced an estimated 25.0 and 10.1 metric tons d-1 of C1 and C2, respectively, during the day and 34.5 (C1) and 4.3 (C2) metric tons d-1 at night. More acetic acid than formic acid was emitted by direct sources, with C2/C1 emission rate ratios of 2.1-2.3. The reverse was true of *in situ* formation, with C1/C2 production rate ratios of 2.5 (day) and 8.0 (night). Dry deposition removal rates depended on season (fall > summer) and location (inland > coastal) and were 22-52 metric tons d-1 for C1, and 32-83 metric tons d-1 for C2. Source (emissions + *in situ* formation) and sink (dry deposition) terms were of the same magnitude in all six cases studied and balance each other well in three of these cases. Uncertainties in emission, *in situ* production and removal rates reflected uncertainties in olefin and unreactive tracer emission rates, yields of organic acids from the Criegee biradical (oz one-olefin reaction), and dry deposition velocity,	Formic acid and acetic acid which are the most abundant acids in southern California air and together account for much of the airborne acidity and are the leading contributors to acid dry deposition.

Table 9 – 3-Methyl-1-pentene

Citation	Study methods	Results	Authors' conclusions/comments
		respectively.	
Hope A. Johnson, Dale A. Pelletier, and Alfred M. Spormann 2001. Isolation and Characterization of Anaerobic Ethylbenzene Dehydrogenase, a Novel Mo-Fe-S Enzyme. J Bacterial. August; 183(15): 4536-4542	A study to isolate purified ethylbenzene dehydrogenase.	Ethylbenzene dehydrogenase was purified to apparent homogeneity and showed that the enzyme is a heterotrimer. Purified ethylbenzene dehydrogenase contained approximately 0.5 mol of molybdenum, 16 mol of iron, and 15 mol of acid-labile sulfur per mol of holoenzyme, as well as a molydopterin cofactor. In addition to ethylbenzene, purified ethylbenzene dehydrogenase was found to oxidize 4-fluoro-ethylbenzene and the nonaromatic hydrocarbons 3-methyl-2-pentene and ethylidenecyclohexane. Sequencing of the encoding genes revealed that ebdA encodes the a subunit, a 974-amino-acid polypeptide containing a molybdopterin-binding domain. The ebdB gene encodes the 6 subunit, a 352-amino-acid polypeptide with several 4Fe-4S binding domains. The ebdC gene encodes the y subunit, a 214-amino-acid polypeptide that is a potential membrane anchor subunit.	Sequence analysis and biochemical data suggested that ethylbenzene dehydrogenase is a novel member of the dimethyl sulfoxide reductase family of molybdopterin-containing enzymes
Roger C. Prince and Clifford C. Walters 2007. 11 - Biodegradation of oil hydrocarbons and its implications for source identification. Oil Spill Environmental Forensics Fingerprinting and Source Identification 2007, Pages 349-379	Review.	Hydrocarbons have been part of the biosphere from its inception, produced initially by prebiotic processes, and subsequently both by living organisms and during the generation of fossil fuels. As highly reduced forms of carbon, hydrocarbons provide a rich source of energy and carbon to those organisms, typically microorganisms, which are able to consume them. Almost all hydrocarbons are readily degraded under appropriate	

Table 9 – 3-Methyl-1-pentene

Citation	Study methods	Results	Authors' conclusions/comments
		conditions. There is a clear preference for the catabolism of some molecules before others; hence, the composition of a fuel or crude oil changes as biodegradation proceeds.	
Zhang, Zhuo-Min; Wu, Wen-Wei; Li, Gong-Ke 2009. Study of the Alarming Volatile Characteristics of Tessaratoma papillose Using SPME-GC-MS. Journal of Chromatoarashic Science, Volume 47, Number 4, April 2009, pp. 291-296(6)	A study on the volatile composition of the stinkbug using a headspace microextraction sampling method	The number of volatiles identified was 16 and 22 before and after stinkbug irritation, respectively. Long-chain alkanes, alkenes, and alcohols consisted of the main volatile compositions of *Tessaratoma papillose*. When stinkbugs were disturbed, the typical unsaturated volatiles were released, especially including a series of tridecane derivatives. In comparison with the volatile compounds of lichi leaf and flower (plants the stinkbug eats), it could be seen that most stinkbug alarming volatiles were synthesized by the insects themselves, and that they do not originate from their food. However, temperature and light did not affect the alarming volatile characteristics. The variety of the stinkbug alarming volatile characteristics before and after irritation was specified by common model strategy. Tridecane, [E]-2-hexenal, dodecane, [E]-2-hexen-1-ol acetate, and 2,3-dimethyl-1-pentene contributed most to the various alarming volatile characteristics before and after irritation, which might be the potential alarming volatiles.	

Table 10 – cis-1,2-Dimethylcyclohexane

Health Effects Studies

Citation	Study methods	Results	Authors' conclusions/comments
Karelson M, Sild S, Maran U. 2000. Non-Linear QSAR Treatment of Genotoxicity. Molecular Simulation, Volume 24, Issue 4 & 6, 229-242.	The mutagenic toxicity of heteroaromatic and aromatic amines, measured by the Ames test, was correlated with the molecular descriptors calculated from the molecular structures using quantum-chemical methods. The quantitative models obtained were compared with the results of the linear QSAR treatment.	No results presented in the abstract.	Results not available. The authors explained that the descriptors appearing in the models revealed the importance of mutagenic interactions of heteroaromatic amines via hydrogen bonding, of effects induced by the solvent, and of the size of compound.
Yoshida T, Matsunaga I, Tomioka K, Kumagai S. 2006. Interior Air Pollution in Automotive Cabins by Volatile Organic Compounds Diffusing from Interior Materials: I. Survey of 101 Types of Japanese Domestically Produced Cars for Private Use. Indoor and Built Environment, Vol. 15(5): 425-444.	The types and concentrations of organic compounds in the interior air of 101 different types of Japanese domestically-produced private-use cars were examined.	A total of 275 organic compounds, including many aliphatic hydrocarbons and aromatic hydrocarbons, were identified, and 242 of them could be quantified for each cabin. The sum of the concentrations of 241 compounds excluding formaldehyde was approximately 600 g/m^3 as a median, ranging from 136 to 3968 g/m^3 for the tested cars. The findings demonstrated that the air in the cabin of these cars was contaminated by high concentrations of a large variety of organic compound diffusing from the interior	

Table 10 – cis-1,2-Dimethylcyclohexane

Citation	Study methods	Results	Authors' conclusions/comments
		materials.	
Yoshida T, Matsunaga I, Tomioka K, Kumagai S. 2006. Interior Air Pollution in Automotive Cabins by Volatile Organic Compounds Diffusing from Interior Materials: II. Influence of Manufacturer, Specifications and Usage Status on Air Pollution, and Estimation of Air Pollution Levels in Initial Phases of Delivery as a New Car. Indoor and Built Environment, Vol. 15(5): 445-462.	The influence of the manufacturer, specifications and usage status of these cars on the interior air pollution was evaluated by covariance analysis.	The findings showed greater air pollution in the cabins of luxury cars, with leather seats or leather steering wheels, or high-end catalogue prices. Differences in the specifications contributed more markedly to interior air pollution than differences in manufacturers. Also, usage status, such as everyday ventilation affected the long- term interior air quality. The sum values of interior concentrations of 154 compounds, for which there were time-course data, were estimated to be approximately 1,700 $\mu g/m^3$ as a median (max. 11,000 $\mu g/m^3$) at 1 month from delivery (interior temperature. 32 C; interior humidity, 45%).	

Table 10 – cis-1,2-Dimethylcyclohexane

Welfare Effects Studies

Citation	Study methods	Results	Authors' conclusions/comments
Edsall CC. 1991. Acute toxicities to larval rainbow trout of representative compounds detected in Great Lakes fish. Bull. Environ. Contam. Toxicol. 46:173-176.	Hazard assessment: determination of the occurrence and abundance of the compounds in Great Lakes fish, and the determination of acute toxicities of representative compounds of 19 chemical classes.	Mortality was 100% at 7.3 mg/L and there was no mortality at 5.0 mg/L.	cis-1,2~ dimethylcyclohexane (LC50 = 7.1 ± 0.7) was less toxic than the PAH compound 1,3-dimethylnaphthalene (LC50 = 1.7 ± 0.2).
Devillers J and Pham-Deleque M-H. 2002. Honey Bees: Estimating the Environmental Impact of Chemicals. London and New York: CRC Press.	Discussion of ecotox tests with honey bees.	Laboratory tests are described in the book.	This is a book. Would need to get book to determine whether cis-1,2-dimethylcyclohexane is discussed.

Table 11- cis-2-Octene

Health Effects Studies

Citation	Study methods	Results	Authors' conclusions/comments
Christine V. Hampton, William R. Pierson, T. Michael Harvey, William S. Updegrove, and Richard S. Marano 1982. Hydrocarbon Gases Emitted from Vehicles on the Road. 1. A Qualitative Gas Chromatography/Mass Spectrometry Survey. Environ. Sci. Technol, 16, 287-298.	Gas-phase hydrocarbons ≥C5 from motor vehicles in highway operations were surveyed in the Allegheny Mountain Tunnel of the Pennsylvania Turnpike in 1979.	Over 300 compounds were either completely or partially identified. In the various tunnel samples the same peaks were always present, with relative intensities varying according to the traffic composition.	
Daniel Grosjean 1992. In situ organic aerosol formation during a smog episode: Estimated production and chemical functionality. Atmospheric Environment, Part A. General Topics, Volume 26, Issue 6, April, Pages 953-963	Amount and chemical identities of the organic aerosol formed *in situ* in the atmosphere during a smog episode.	The estimated amounts produced during a typical smog episode of carbonyls, aliphatic nitrates, aliphatic carboxylic acids, phenols and nitro aromatics were 670, 240, 1,360, 3,120 and 3,120 kg/day, respectively.	Similarities and differences between calculated organic aerosol composition and experimental observations are discussed in terms of organic aerosol formation pathways and sampling artifact for the following functional groups: aliphatic carboxylic acids, carbonyls, aliphatic nitrates, phenols, aromatic acids, nitro aromatics, amides and esters. No specific mention of cis-2-octene in this study.
C.R. Calkins and J.M. Hodgen 2007. A fresh look at meat flavor. Meat Science, Volume 77, Issue 1, September 2007, Pages 63-80	Review.	New research reveals important relationships in flavor among multiple muscles within a single animal carcass. This animal effect includes the presence of off- flavors. Diets high in polyunsaturated fatty acids may be contributing to the appearance of off-flavors in beef. Compounds associated with liver-like off-flavor notes in beef have been identified in	No specific mention of cis-2-octene in this study.

Table 11- cis-2-Octene

Citation	Study methods	Results	Authors' conclusions/comments
		raw tissue.	

Table 12 - Ethanol

Health Effects Studies

Citation	Study methods	Results	Authors' conclusions/comments
Sabourault D, Bauche F, Giudicelli Y, Nordmann J, Nordmann R. 1981. Alpha- and beta-adrenergic receptors in rat myocardium membranes; rater prolonged ethanol inhalation. Cellular and Molecular Life Sciences, Volume 37, Number 3, 227-229.	Rats exposed via inhalation for 3 weeks (continuous exposure).	The maximal number and affinity of the α- and β-receptors of rat heart were unchanged.	The authors concluded that adrenergic receptor disturbances were not involved in the mechanism of chronic ethanol-induced triglyceride deposition in the heart.
Morgan ET, Devine M, Skett P. 1981. Changes in the rat hepatic mixed function oxidase system associated with chronic ethanol vapor inhalation. Biochemical Pharmacology, Volume 30, Issue 6, 15 March 595-600.	Inhalation exposure in rats.	An increase in aniline hydroxylase activity as a result of ethanol treatment was attributed to an increase in a form of cytochrome P-450 with a high specific activity toward aniline. Since the ethanol effect on aniline hydroxylation had disappeared 24 hr after treatment was discontinued, a high rate of turnover of this enzyme was deduced. Dimethylsulfoxide (56 mM) produced a reverse type I spectral change in ethanol-induced, but not in control, microsomes.	
Zuskin E, Bouhuys A, Sarid M. 1981. Lung function changes by ethanol inhalation. Clin Allergy. May;11(3):243-8.	Acute inhalation exposure in humans volunteers	There was a significant decrease in flow rates on partial expiratory flow-volume (PEFV) curves up to 90 min after ethanol inhalation. FEV1 values did not change significantly, either after ethanol or saline aerosol. Pretreatment with atropine did not prevent the acute reductions in flow rates in comparison with the reductions without atropine. Pretreatment with disodium cromoglycate (DSCG) considerably diminished the acute reductions of flow	The authors concluded that ethanol in some persons may act, at least partly, through releasing mediators with bronchoconstrictive action.

Table 12 - Ethanol

Citation	Study methods	Results	Authors' conclusions/comments
		rates caused by ethanol inhalation.	
Radike MJ, Stemmer KL, Bingham E. 1981. Effect of ethanol on vinyl chloride carcinogenesis. Environ Health Perspect. Oct;41:59-62.	Chronic ingestion toxicity study in rats with vinyl chloride (VC) and ethanol. Ingestion of ethanol began 4 weeks prior to inhalation of VC and continued for life or 2.5 years from the first VC exposure.	Ethanol potentiated the carcinogenic response to vinyl chloride (VC) in the liver and produced an excess of neoplasms in animals receiving ethanol alone. Inhalation of VC induced angiosarcoma of the liver in 23% of the exposed animals; ethanol in addition to VC inhalation increased the incidence to 50%. Concomitant administration of VC and ethanol also produced an excess of hepatocellular carcinoma and lymphosarcoma. Ethanol with or without VC had a strong tumorigenic effect on the endocrine system.	The authors concluded that ethanol with or without VC had a strong tumorigenic effect on the endocrine system. These results indicated that ethanol is a co-carcinogen in relation to the carcinogen VC.
Seitz HK, Garro AJ, Lieber CS. 1981. Sex dependent effect of chronic ethanol consumption in rats on hepatic microsome mediated mutagenicity of benzo[a]pyrene. Cancer Letters, Volume 13, Issue 2, July, 97-102	Chronic oral exposure in rats	Treatment resulted in a 42% increase in AHH activity ($p < 0.01$), as measured in isolated microsomes, and also resulted in a significantly enhanced capacity ($P < 0.01$) of these microsomes to activate B[a]P to a mutagen detectable in the Ames bacterial mutagenesis assay. Hepatic microsomes from male rats on did not exhibit any significant differences, either in AHH activity or in their capacity to activate B[a]P to a mutagen after chronic ethanol feeding.	
Mullin LS and Krivanek ND. 1982. Comparison of unconditioned reflex and conditioned avoidance tests in rats exposed by inhalation to carbon monoxide, 1,1,1-trichloroethane, toluene or ethanol. Neurotoxicology. Jul:3(1):126-37.	Inhalation exposure in rats to 0, 4,000, 8,000, 16,000 or 32,000 ppm for 4 hrs. Rats were tested for behavioral changes	In unconditioned reflex testing the presence or absence of specific unconditioned reflexes (such as corneal, placing, grasping and righting reflexes) and simple behavior patterns including locomotor activity and coordination were observed. The conditioned reflex task consisted of shock	The authors concluded that for both methods, the concentrations at which changes were detected in rats were two- to ten-fold higher than those reported for human effects.

Table 12 - Ethanol

Citation	Study methods	Results	Authors' conclusions/comments
	at 1/2, 1, 2 and 4 hours during exposure and 18 hours after exposure.	avoidance by lever press following simultaneous light and sound stimuli. Rats began to fail unconditioned reflex tests and decrements in conditioned avoidance at 8,000 ppm ethanol.	
Brusick DJ, Jaoannath DR, Matheson D. 1982. The activity of 2-(2',4'-diaminophenoxy)ethanol in 3 genetic toxicity bioassays. Mutat Res. Dec;102(4) 361-72.	Mouse dermal exposure to 2-(2',4'-Ddaminophenoxy)-ethanol evaluated for genetic activity *in vivo*. Dose levels ranged from 15 to 1,500 mg/kg bw.	The results of these studies were considered to be negative although urine analysis and spot-test data showed non-significant dose-related increases.	Ethanol was not found to be genotoxic.
Topping DL, Weller RA, Nader CJ, Calvert GD, Iliman RJ. 1982. Adaptive effects of dietary ethanol in the pig: changes in plasma high-density lipoproteins and fecal steroid excretion and mutagenicity. Am J Clin Nutr. Aug;36(2):245-50.	Pigsexposed to ethanol as a substitute for sucrose.	Ethanol treatment raised plasma total cholesterol, an increase that was solely due to a rise in high-density lipoproteins. Plasma triacylglycerols and apo-B concentrations were unchanged and although apo-A1 rose with ethanol, this was not statistically significant. Ethanol did not alter total fecal steroids but both bile acids and the ratio of bile acids/neutral sterols were increased. In fecal extracts from these animals, mutagenic activity in the Ames bacterial test was also raised.	
Ribiere C, Sinaceur J, Nordmann J, Nordmann R. 1983. Liver superoxide dismutases and catalase during ethanol inhalation and withdrawal. Pharmacol Biochem Behay. 1983;18 Suppl 1:263-6.	Rats exposed during a 4-day inhalation period.	Manganese superoxide dismutase (Mn-SOD) studied during ethanol vapor inhalation showed no changes during the inhalation period (4 days) and a transient increase 12 hours after ethanol withdrawal. A significant decrease in cytosolic Cu-Zn-SOD was found at the end of the inhalation	

Table 12 - Ethanol

Citation	Study methods	Results	Authors' conclusions/comments
		period and was sustained during 48 hours following ethanol withdrawal. Cytosolic catalase was reduced at the end of the ethanol inhalation period.	
Rice SA, Dooley JR, Mazze RI. 1983. Metabolism by rat hepatic microsomes of fluorinated ether anesthetics following ethanol consumption. Anesthesiology. Mar,58(3) 237-41.	Oral exposure in rats to 16 % ethanol *ad libitum* for 14 days.	Fourteen days of *ad libitum* consumption of 16% ethanol resulted in maximal defluorination rates of anesthetics. No overt signs of ethanol toxicity were observed. Ethanol-treated rats with a normal caloric intake had significantly increased microsomal defluorination rates per mg protein compared with pair-fed control rats as follows: methoxyflurane, 190% of control; enflurane, 298% of control; and sevoflurane, 301% of control. Ethanol-treated animals with 50% of normal caloric intake showed similar elevations in microsomal defluorination rates when compared with pair-fed controls.	
Goldstein DB and Zaechelein R. 1983. Time course of functional tolerance produced in mice by inhalation of ethanol. J Pharmacol Exp Thor. Oct;227(1):150-3.	Mice exposure via inhalation for 1 to 9 days.	Tolerance, tested at 6 hr after withdrawal, was measurable after only 1 day of ethanol treatment and increased further in experiments of 3 and 6 days duration, but did not continue to increment during 9 days of alcohol inhalation. The tolerance disappeared rapidly after withdrawal; it was maximal at the earliest test, 2 hr after withdrawal, but decayed progressively and was no longer appreciable 30 hr after withdrawal.	The authors concluded that the method was suitable for accurate measurement of ethanol sensitivity, even when residual alcohol remains from the chronic treatment.
Rouach H, Clement M, Orfanelli MT, Janvier B, Nordmann J,	Rats exposed to ethanol via	The level of hepatic lipid peroxide started to increase significantly after the first day of	Hepatic peroxide levels and the mitochondrial sensitivity to

Table 12 - Ethanol

Citation	Study methods	Results	Authors' conclusions/comments
Nordmann R. 1983. Hepatic lipid peroxidation and mitochondria! susceptibility to peroxidative attacks during ethanol inhalation and withdrawal. Biochim Biophys Ada. Oct 11;753(3):439-44.	inhalation at concentrations ranging from 15-22 mg/l for 4-days.	ethanol inhalation, whereas the *in vitro* mitochondrial sensitivity to peroxidation induced by ADP X Fe3+ in the presence of an O2-generating system was still unaltered after a 2-day inhalation period. Both the hepatic peroxide level and the mitochondrial sensitivity to peroxidation were significantly enhanced at the end of the 4-day inhalation period. Such an enhancement was still apparent 24 h after withdrawal, a time at which no more ethanol was present in the blood. Lipid peroxidation returned to normal values only 48 h after withdrawal.	peroxidation were significantly enhanced after ethanol inhalation. Microsomes were less affected than mitochondria by the ethanol treatment.
Kvelland I. 1983. The mutagenic effect in bacteriophage T4D of a hair dye, 1,4 diaminoanthraquinone, and of two solvents, dimethylsulfoxide and ethanol. Hereditas 99(2).209-13.	Mutagenicity study – induction of rapid lysis mutants in bacteriophage T4D.	Ethanol had no mutagenic effect at doses ranging from 47.6-130.4 microliters/ml.	Ethanol was not found to be mutagenic.
Clark CR, Dutcher JS, McClellan RO, Naman TM, Seizinger DE. 1983. Influence of ethanol and methanol gasoline blends on the mutagenicity of particulate exhaust extracts. Arch Environ Contam Toxicol. May;12(3):311-7.	Mutagenicity study in *Salmonella typhimurium*.	Dichloromethane extracts of the exhaust particles from all tests were mutagenic in *Salmonella typhimurium* strains TA 100 and TA 98. The extracts were less mutagenic in the nitroreductase deficient strains TA 98NR and TA 98DNPR suggesting that nitro-substituted polycyclic aromatic hydrocarbons may be responsible for part of the mutagenicity. In all the alcohol blended fuel tests, the mass of particle-associated organics emitted from the exhaust was lower than that observed during the control tests using gasoline alone. Thus, in most cases, estimates of the emission of mutagenic combustion products from the	Alcohol blended fuel produced lower mutagenic combustion products than methanol blended fuels.

Table 12 - Ethanol

Citation	Study methods	Results	Authors' conclusions/comments
		exhaust were lower in the alcohol blend tests.	
Bariliak IR, Kozachuk Slu. 1983. [Embryotoxic and mutagenic activity of ethanol and acetaldehyde in intra-amniotic exposure]. [Article in Russian]. Tsitol Genet. Sep-Oct;17(5):57-60.	Rats exposed to ethanol via intra-amniotic administration.	The administration of acetaldehyde on the 13th day of pregnancy resulted in a considerable embryolethal effect, appearance of developmental abnormalities in rat embryos and chromosomal aberrations in fetal cells.	Ethanol was found to have no teratogenic or mutagenic activity.
Nelson BK, Brightwell WS, Setzer JV, O'Donohue TL. 1984. Reproductive toxicity of the industrial solvent 2-ethoxyethanol in rats and interactive effects of ethanol. Environ Health Perspect. Aug;57:255-9.	Ethoxyethanol and ethanol exposure via inhalation and oral routes in rats.	Ingestion of ethanol with concomitant inhalation of ethoxyethanol vapors early in pregnancy appeared to reduce the number of both behavioral and neurochemical deviations found for ethoxyethanol. In contrast, the concomitant exposure to ethanol and ethoxyethanol later in gestation potentiated the behavioral and neurochemical effects of ethoxyethanol.	
Rouach H, Clement M, Orfanelli MT, Janvier B, Nordmann R. 1984. Fatty acid composition of rat liver mitochondrial phospholipids during ethanol inhalation. Biochim Biophys Acta. Aug 15;795(1):125-9.	Rat exposed via inhalation to 15-22 mg/l ethanol for 4 days.	After a 2 -day inhalation period, the proportion of monoenoic acids in total phospholipids increased, whereas that of arachidonic acid decreased.	
Massad E, Saldiva CD, Cardoso LM, Da Silva R, Saldiva PH, Bohm GM. 1985. Acute toxicity of gasoline and ethanol automobile engine exhaust gases. Toxicol Lett. Aug; 26(2-3):187-92.	Acute toxicity study in rats exposed to ethanol-fueled and gasoline-fueled engine exhaust via inhalation.	The acute toxicity, in terms of the LC50, the gasoline-fueled engine exhaust was significantly higher than that of the ethanol-fuelled engine exhaust.	Ethanol-fueled engine exhaust was less toxic than gasoline-fueled engine exhaust.

Table 12 - Ethanol

Citation	Study methods	Results	Authors' conclusions/comments
Hobara N, Watanabe A, Kobayashi M, Nakatsukasa H, Nagashima H, Fukuda T, Araki Y. 1985. Tissue Distribution of Acetaldehyde in Rats following Acetaldehyde Inhalation and Intragastric Ethanol Administration. Bulletin of Environmental Contamination and Toxicology, Volume 35, Number 1, 393-396.	Rats exposed to acetaldehyde via inhalation and to ethanol via intragastric administration.	Standard curves of acetaldehyde concentrations were linear in the ranges of 0.4-2.8 nmol/ml for blood. Those of ethanol levels also were linear in the ranges of 1.5-189.8 micromol/ml for blood. Disappearance of acetaldehyde from blood following discontinuation of acetaldehyde inhalation was rapid. Its level was reduced to less than 2% of the initial value within 20 minutes.	The authors concluded that acetaldehyde exposure by inhalation serves as a viable model for studying acetaldehyde toxicity.
Lewis MJ. 1985. Inhalation of Ethanol Vapour: A Case Report and Experimental Test Involving the Spraying of Shellac Lacquer. Journal of the Forensic Science Society, Volume 25, Issue 1, January 5-9.	Case report of a worker occupationally exposed to ethanol via inhalation.	Under relatively arduous conditions, a blood alcohol level of 1.3 mg/100 ml was achieved.	The author concluded that continued exposure would not have led to a significant increase in that level.
Lewis MJ. 1985. A Theoretical Treatment for the Estimation of Blood Alcohol Concentration Arising from Inhalation of Ethanol Vapour. Journal of the Forensic Science Society Volume 25, Issue 1, January 11-22.	Theoretical model for the estimation of blood alcohol concentration in a subject exposed to ethanol vapor.	Available experimental data were tested against the model and found to be in good agreement.	
Nelson BK, Brightwell WS, MacKenzie DR, Khan A, Burg JR, Weigel WW, Goad PT. 1985. Teratological assessment of methanol and ethanol at high inhalation levels in rats. Fundam Apol Toxicol. Aug;5(4):727-36.	Rats exposed via inhalation to 20,000 ppm (20ET), 16,000 ppm (16ET), 10,000 ppm (10ET), and 0 ppm (ETCO) for 7 hr/day on Days 1-19	Dams in the 20ET group were narcotized by the end of exposure, and maternal weight gain and feed intake were decreased during the first week of exposure. The 16ET dams had slightly depressed weight gain (p less than 0.01) during the first week of exposure, but there were no significant effects on feed	There was no significant increase in malformations at any level of ethanol exposure.

Table 12 - Ethanol

Citation	Study methods	Results	Authors' conclusions/comments
	of gestation. ET = ethanol.	consumption. There was no definite increase in malformations at any level of ethanol, although the incidence in the 20ET group was of borderline significance.	
Nelson BK, Briohtwell WS, Burg JR. 1985. Comparison of behavioral teratogenic effects of ethanol and n-propanol administered by inhalation to rats. Neurobehav Toxicol Teratol. Nov-Dec;7(6):779-83.	Rats exposed via inhalation to 0, 16,000, or 10,000 ppm for 7 hours/day for six weeks or throughout gestation, respectively.	There occurred no reduction in weight gain or reduced weight in offspring through 3 weeks of age. One female and one male pup per litter were administered tests of neuromotor coordination (ascent on wire mesh screen, rotorod), activity levels (open field, running wheel), or learning ability (avoidance, operant conditioning), but no significant differences from controls were found with ethanol. There occurred no instances of male infertility in exposed rats.	
Hayes S. 1985. Ethanol-induced genotoxicity. Mutat Res. May-Jun;143(1-2):23-7.	Genotoxicity study.	Unrelated, nondenatured ethanol preparations, derived synthetically or by fermentation, were found to induce qualitatively similar concentration-dependent toxic and genotoxic effects as measured by the RK mutatest. In this system ethanol was found genotoxic above a threshold concentration of 18-19% (v/v) when RK+ selector cells were transiently exposed for 10 min before selection for RK- survivors at 42 ^{o}C.	Ethanol was found to be genotoxic in this test.
Nets JM, te Brömmelstroet BW, van Gemert PJ, Roelofs HM, Henderson PT. 1985. Influence of ethanol induction on the metabolic activation of genotoxic agents by isolated rat hepatocytes. Arch	Rats Oral exposure for 6 weeks. Activation of genotoxic compounds was studied in isolated	Benzidine (BZ), dimethylnitrosamine (DMN), diethylnitrosamine (DEN), isoniazid (INH) and cyclophosphamide (CP) were more effectively activated to products mutagenic towards *Salmonella typhimurium* by hepatocytes from ethanol-pretreated rats	The authors concluded that an alteration of the hepatic drug-metabolizing system may be responsible for the ethanol-induced increase in susceptibility to certain genotoxic compounds.

Table 12 - Ethanol

Citation	Study methods	Results	Authors' conclusions/comments
Toxicol. Sep;57(4):217-21.	rat hepatocytes.	than by hepatocytes from controls. The mutagenic potency of 2-aminofluorene (2-AF) and 2-acetylaminofluorene (2-AAF) was not influenced by ethanol pretreatment. Ethanol consumption was found to be associated with increased cytochrome P-450 content and enhanced N-acetylation in the isolated hepatocytes.	
Massad E, Saldiva PH, Saldiva CD, Caldeira MP, Cardoso LM, de Morals AM, Calheiros DF, da Silva R, Böhm GM. 1986. Toxicity of prolonged exposure to ethanol and gasoline autoengine exhaust gases. Environ Res. Aug; 40(2):479-86.	Rats and mice exposed via inhalation for 5 weeks.	The results demonstrated that the chronic toxicity of the gasoline-fueled engine is significantly higher than that of the ethanol engine.	Chronic exposure to ethanol exhaust was less toxic than exposure to exhaust from a gasoline engine.
Mattucci-Schiavone L and Ferko AP. 1986. An inhalation procedure to produce tolerance to the behavioral effects of ethanol. Physiology & Behavior, Volume 36, Issue 4, 643-646.	Exposure to ethanol in rats.	Ethanol in doses of 2.0 and 3.0 g/kg produced significant impairment of motor coordination with corresponding elevated blood ethanol levels. The rate of ethanol disappearance from the blood was 0.32 ± 0.03 mg/ml/hr. Functional tolerance to the effect of ethanol on motor coordination and sleep time was produced in rats by a 24 hr period of exposure to ethanol vapor (28 mg/liter of air) in a chamber. Animals tested 48 hr after the ethanol inhalation period showed less motor impairment from acute ethanol (3.0 g/kg, IP) and other animals exhibited a reduced sleep time from ethanol (4.0 g/kg, IP) when they were compared with controls. The rate of ethanol elimination from the blood was unchanged in ethanol vapor treated animals (0.30 ±	Acute ethanol administration and inhalation resulted in impaired motor coordination and reduced sleep time.

Table 12 - Ethanol

Citation	Study methods	Results	Authors' conclusions/comments
		0.01 mg/ml/hr) and air-treated animals (0.33±0.02 mg/ml/hr).	
Campbell L and Wilson HK. 1986. Blood Alcohol Concentrations Following the Inhalation of Ethanol Vapour Under Controlled Conditions. Journal of the Forensic Science Society Volume 26, Issue 2, March 129-135.	Human inhalation exposure.	Exposure to ethanol vapor at the UK occupational exposure limit (1,900 mg/m^3) dis not produce a significant blood alcohol concentration.	
Morris JB, Clay RJ, Cavanagh DG. 1986. Species differences in upper respiratory tract deposition of acetone and ethanol vapors. Fundam Appl Toxicol. Nov;7(4):671-80.	Rats and guinea pigs exposed via inhalation.	Deposition efficiencies were significantly dependent upon inspiratory flow rate. Despite the fact that the surface area of the upper respiratory tract (URT) of the guinea pig is greater than that of the rat, URT deposition of these vapors was as much as twice as efficient in the rat as in the guinea pig ($p < 0.0005$).	The authors concluded that there may be important anatomic and/or physiologic differences in the URT of the Hartley guinea pig and Fischer rat. Such differences may have to be considered when comparing the response(s) of these species to toxic gases or when extrapolating data obtained from these species to the human.
Barillo DJ, Rush BF Jr, Goode R, Lin RL, Freda A, Anderson EJ Jr. 1986. Is ethanol the unknown toxin in smoke inhalation injury? Am Surq. 1986 Dec;52(12):641-5.	Investigation of fire victims through examination of the fire scene, autopsy studies, and toxicologic analysis.	Ethanol was detected in 21/26 adults (80%) and 0/12 children (0%). 18/26 adult victims had ethanol levels above the statutory level of intoxication (10 mg%). Victims found in bed (no escape attempt) had a mean blood ethanol level of 268 mg%, compared with a mean level of 88 mg% in those victims found near an exit ($p = 0.006$).	The authors concluded that ethanol intoxication significantly impairs the ability to escape from fire and smoke and is a contributory factor in smoke-related mortality.
Obe G, Jonas R, Schmidt S. 1986. Metabolism of ethanol in vitro produces a compound which induces sister-chromatid	Genotoxicity study with human lymphocytes *in vitro*.	Ethanol (EtOH) in the presence of the EtOH-metabolizing enzyme, alcohol dehydrogenase (ADH), led to the induction of sister-chromatid exchanges (SCEs) in	The authors concluded that not ethanol itself, but its first metabolite acetaldehyde, was mutagenic.

Table 12 - Ethanol

Citation	Study methods	Results	Authors' conclusions/comments
exchanges in human peripheral lymphocytes in vitro: acetaldehyde not ethanol is mutagenic. Mutat Res. May;174(1).47-51.		human peripheral lymphocytes *in vitro*. Acetaldehyde (AA) induced SCEs, whose frequencies are lowered in the presence of the AA-metabolizing enzyme, aldehyde dehydrogenase (ALDH). EtOH in the presence of ADH produced more SCEs than EtOH in the presence of ADH and ALDH.	
Cortes F, Mateos S, Escalza P. 1986. Cytotoxic and genotoxic effects of ethanol and acetaldehyde in root-meristem cells of Allium cepa. Mutation Research/Genetic Toxicology, Volume 171, Issues 2-3, August-September, 139-143.	Genotoxicity study using root-tip cells of onion plants. Also, ethanol and acetaldehyde treatment and measurement of sister chromatid exchanges.	The results obtained showed that the chemicals differ in their action. For all the parameters analyzed, acetaldehyde treatments resulted in a stronger effect on meristematic cells.	The authors concluded that acetaldehyde is the actual mutagenic agent and, it can be stated that the mode of metabolism of ethanol in a given system is very important as regards its effectiveness in inducing chromosome damage.
de Vries DJ, Johnston GAR, Ward LC, Wilce PA, Shanley BC. 1987. Effects of chronic ethanol inhalation on the enhancement of benzodiazepine binding to mouse brain membranes by GABA. Neurochemistry International, Volume 10, Issue 2, 231-235.	Mouse inhalation exposure.	Chronic ethanol inhalation produced no change in the number or affinity of [3H] flunitrazepam binding sites on well-washed synaptic membranes prepared from male Quackenbush mice, but produced a significant decrease in the capacity of GABA to enhance [3H]flunitrazepam binding. Acute ethanol treatment or ethanol incubated *in vitro* with the brain membranes did not produce changes in any of the [3H]flunitrazepam binding parameters.	The authors concluded that chronic ethanol may affect the coupling of various sites on GABA-A receptor-ionophore complexes in brain.
Belykh AG, Strelkoy RB. 1987. [Influence of hypoxia on the effects of ethanol and acetaldehyde].	Mouse inhalation study.	During experiments on male SHK mice it was established that a preliminary normobaric normocapnic hypoxic	

Table 12 - Ethanol

Citation	Study methods	Results	Authors' conclusions/comments
[Article in Russian]. Farmakol Toksikol. Nov-Dec;50(6):89-91.		stimulation (30-minute inhalation of a gas mixture containing 10% of oxygen) increased the animal organism resistance to toxic effects of ethanol and its metabolite acetaldehyde.	
Gichner T, Veleminskỳ J. 1987. The organic solvents acetone, ethanol and dimethylformamide potentiate the mutagenic activity of N-methyl-N'-nitro-N-nitrosoguanidine, but have no effect on the mutagenic potential of N-methyl-N-nitrosourea. Mutat Res. Sep;192(1):31-5.	Plant (*Arabidopsis thaliana*) seeds exposed to N-methyl-N'-nitro-N-nitrosoguanidine (MNNG) in presence of 4-16% ethanol.	The frequency of recessive chlorophyll and embryonic lethals was markedly increased when exposure of the seeds to MNNG (3 h) was carried out in the presence 4-16% ethanol. The enhancement of MNNG mutagenicity was proportional to the concentrations of ethanol. In contrast, ethanol applied at the same conditions and doses did not influence the mutagenic activity of N-methyl-N-nitrosourea.	Ethanol without mutagens did not influence the spontaneous rate of mutations and revealed no or very weak toxic effects as measured by seed germination.
Nelson BK, Brightwell WS, MacKenzie-Taylor DR, Burg JR, Massari VJ. 1988. Neurochemical, but not behavioral, deviations in the offspring of rats following prenatal or paternal inhalation exposure to ethanol. Neurotoxicol Teratol. Jan-Feb;10(1):1 5-22.	Male and female rats exposed for 7 hours/day for six weeks or throughout gestation to 16,000, 10,000, or 0 ppm ethanol by inhalation and then mated with untreated rats.	Offspring from paternally or maternally exposed animals performed as well as controls on days 10-90 in tests of neuromotor coordination (ascent on a wire mesh screen, rotorod), activity levels (open field, modified-automated open field, and running wheel), and learning ability (avoidance conditioning and operant conditioning). Levels of acetylcholine, dopamine, substance P, and beta-endorphin were essentially unchanged in the offspring of rats exposed to ethanol. Complex, but significant changes in levels of norepinephrine occurred only in paternally exposed offspring. 5-Hydroxytryptamine levels were reduced in the cerebrum, and Met-enkephalin levels were increased in all brain regions of offspring from both maternally and	

Table 12 - Ethanol

Citation	Study methods	Results	Authors' conclusions/comments
		paternally exposed rats.	
Nakajima T, Okino T, Okuvama S, Kaneko T, Yonekura I, Sato A. 1988. Ethanol-induced enhancement of trichloroethylene metabolism and hepatotoxicity: difference from the effect of phenobarbital. Toxicol Appl Pharmacol. Jun 30;94(2):227-37.	Rats exposed via the oral route to 2 g in 80 ml liquid diet/day for 3 weeks were also exposed by inhalation to 500, 1,000, 2,000, 4,000, or 8,000 ppm trichloroethylene (TRI) for 2 or 8 hr.	Ethanol enhanced TRI metabolism as evidenced by accelerated disappearance of TRI from the blood and increased excretion of total trichloro compounds (TCE + TCA) in the urine. Ethanol markedly enhanced the metabolism particularly at TRI concentration of 2,000 ppm or lower, whereas phenobarbital enhanced it only at 4,000 ppm or higher. Ethanol potentiated TRI hepatotoxicity at a TRI concentration as low as 500 ppm.	The authors concluded that the toxicity in ethanol-treated rats was generally more marked in rats exposed to TRI for a longer period than in rats exposed to a higher concentration.
Seidel HJ, Bader R, Weber L, Barthel E. 1990. The influence of ethanol on the stem cell toxicity of benzene in mice. Toxicol Appl Pharmacol. Aug; 105(1):13-8.	Mice exposed via inhalation to 100, 300, and 900 ppm benzene vapor with and without ethanol in drinking water for 4 weeks, 6 hr/day, 5 days/week.	It was shown that the number of CFU-E per femur was depressed in a dose-dependent manner by benzene alone and also by ethanol combined with a given benzene concentration. CFU-E showed rapid regeneration after the end of the exposure, but not BFU-E and CFU-C. Prolongation of the ethanol exposure after withdrawal of benzene had only a marginal effect on progenitor cell regeneration.	Ethanol combined with benzene depressed progenitor cell regeneration in mice following inhalation.
Rikans LE and Gonzalez LP. 1990. Antioxidant Protection Systems of Rat Lung after Chronic Ethanol Inhalation. Alcoholism: Clinical and Experimental Research, Volume 14, Issue 6, 872-877.	Rats exposed via inhalation for 5 weeks.	Ethanol treatment increased the activities of catalase (117%) and Cu/Zn-superoxide dismutase (25%) in lung but not in liver. Although chronic ethanol inhalation lowered hepatic glutathione (19%) and hepatic vitamin E (30%), there was no increase in malondialdehyde content in either liver or lung of ethanol-exposed rats.	The authors concluded that long-term ethanol exposure did not produce a significant degree of oxidative stress in rat lung.
Batkin S and Tabrah FL. 1990.	Studies in mice –	A dose-dependent suppression of tumor	The authors concluded that the

Table 12 - Ethanol

Citation	Study methods	Results	Authors' conclusions/comments
Ethanol vapour modulation of Lewis lung carcinoma, a murine pulmonary tumour. J Cancer Res Clin Oncol. 116(2):187-9.	*in vitro* study whereby ethanol was added to the culture medium for Lewis lung carcinoma; also an *in vivo* study (injection); also an inhalation study with 0.4% ethanol vapor from an aerosol for 70 min daily for 17 days.	growth occurred. Sequestration of the Lewis lung carcinoma in the lungs followed tail-vein injection in C57BL/6 mice. Marked reduction of the pulmonary tumor growth occurred. Exposure to 0.1% ethanol vapor did not significantly affect the growth of Lewis lung carcinoma tumor.	optimum ethanol aerosol applications may thus be tumoricidal.
Cheever KL, Cholakist JM, El-Hawari AM, Kovatch RM, Weisburger EK. 1990. Ethylene dichloride: The influence of disulfiram or ethanol on oncogenicity, metabolism, and DNA covalent binding in rats. Fundamental and Applied Toxicology, Volume 14, Issue 2, February 243-261.	Rats exposed via inhalation to 50 ppm ethylene dichloride (EDC) for 7 hr/day, 5 days/week, for 2 years by inhalation. Additional rats were exposed to 50 ppm EDC either with 0.05% disulfiram in the diet or with 5% ethanol in the drinking water.	No significant increase in the number of any tumor type was observed in rats exposed to only EDC, disulfiram, or ethanol. No significant increase in the number of tumors was observed in rats exposed to inhaled EDC and ethanol in water. The urinary metabolite HPLC profile was qualitatively unchanged by long-term EDC, disulfiram, or ethanol treatment, either alone or in combination.	Ethanol was not found to be carcinogenic.
Lotfi CF, Brentani MM, Böhm GM. 1990. Assessment of the mutagenic potential of ethanol auto engine exhaust gases by the Salmonella typhimurium microsomal mutagenesis assay, using a direct exposure method.	Mutagenicity assay in *Salmonella typhimurium*.	This methodology was used to determine the mutagenic activity of gasoline, revealing mutagenic activity of base-pair substitution without any need for metabolic activation, indicating the presence of direct-action mutagens.	Experiments with ethanol suggest an indirect mutagenic activity of the oxidant type.

Table 12 - Ethanol

Citation	Study methods	Results	Authors' conclusions/comments
Environ Res. Aug;52(2):225-30.			
Ghosh TK, Copeland RL Jr, Pradhan SN. 1991. Effects of ethanol inhalation on EEG in rats. Pharmacol Biochem Behay. Feb;38(2):293-7.	Rats exposed via inhalation to 100, 400, 800 and 1,600 ppm for 2 hours on different days.	Ethanol was found to increase the duration of waking (W) with a decrease in duration of rapid eye movement (REM) sleep at 100 and 400 ppm. No effect was observed at 800 and 1,600 ppm on the stages of sleep-wake cycle or at 100-1,600 ppm on EEG power spectra from the somatosensory or visual cortices.	The authors concluded that ethanol administered by inhalation could produce arousal action at low doses, but did not have any effect on EEG power spectrum at the concentrations used.
Montpied P, Morrow AL, Karanian JW, Ginns El, Martin BM, Paul SM. 1991. Prolonged ethanol inhalation decreases gamma-aminobutyric acidA receptor alpha subunit mRNAs in the rat cerebral cortex. Mol Pharmacol. Feb;39(2):157-63.	Rats exposed via inhalation for 14 days.	A 40-50% reduction occurred in the level of gamma-aminobutyric acidA (GABAA) receptor alpha 1 subunit mRNAs in the cerebral cortex. The level of alpha 2 subunit mRNA was reduced by 29%. No effect of prolonged exposure on the level of alpha 3 subunit mRNA occurred. Ethanol exposure did not alter the steady state levels of cerebral cortical glutamic acid decarboxylase or beta-actin mRNAs. Moreover, no alterations in the levels of total RNA, poly(A)+ RNA, or rRNA were observed.	The authors concluded that these ethanol-induced reductions in GABAA receptor alpha subunit mRNAs may underlie alterations in GABAA receptor function or number observed following prolonged ethanol exposure in rats.
Ghosh TK, Copeland RL Jr, Alex PK, Pradhan SN. 1991. Behavioral effects of ethanol inhalation in rats. Pharmacol Biochem Behay. Apr;38(4):699-704.	Rats exposed via inhalation.	Behavioral effects were studied on two fixed-ratio (FR) liquid-reinforced schedules and a continuous reinforcement (CRF) schedule intracranial self-stimulation (SS). In the FR-24 schedule ethanol caused a decrease of reinforcement rate at 161 ppm and higher concentrations. In the FR-50 schedule, decreases of the rate were observed at 102 ppm and 203 ppm. In the SS behavior, ethanol produced a decrease	The authors concluded that ethanol inhalation could produce adequate blood concentrations so as to produce behavioral effects.

Table 12 - Ethanol

Citation	Study methods	Results	Authors' conclusions/comments
		in the rate of reinforcement at 603 ppm and higher concentrations. In rats of this schedule, blood ethanol concentrations were measured to be 393 micrograms/ml and 545 micrograms/ml after exposure to 600 ppm and 1,200 ppm of ethanol respectively. Acute tolerance to ethanol was observed in these experiments, particularly in the FR-24 schedule.	
Padilla S, Lyerly DL, Pope CN. 1992. Subacute ethanol consumption reverses p-xylene-induced decreases in axonal transport. Toxicology. Nov 1;75(2):159-67.	Oral (10% ethanol in drinking water) exposure to ethanol in rats with and without co-exposure to xylene. Two groups which were either exposed by inhalation to 1,600 ppm p-xylene for 6 h/day, 5 days/week for 8 exposure-days or were treated identically except that they were exposed to air while in the inhalation chambers.	The xylene exposure group showed a significant reduction in axonal transport of proteins and glycoproteins, whereas the ethanol exposure alone produced no significant reductions in the transport of either proteins or glycoproteins. In the animals receiving both ethanol and xylene, however, the ethanol treatment prevented the decreased transport characteristic of the xylene-only animals.	The authors conclude that the xylene-induced reduction in rapid axonal transport was reversed (or prevented) by subacute ethanol consumption.
Winston GW, Traynor CA, Shane BS, Halos AK. 1992. Modulation of the mutagenicity of three dinitropyrene isomers in vitro by rat-liver S9, cytosolic, and microsomal fractions following chronic ethanol ingestion. Mutat	Mutagenicity of three dinitropyrene (DNP) isomers *in vitro* by rat-liver S9, cytosolic, and microsomal fractions following	The mutagenicity of the DNP isomers toward *S. typhimurium* TA98 and TA100 was attenuated in the presence of post-mitochondrial supernatants (S9) from both ethanol-fed and pair-fed rats albeit, that from the ethanol-fed group was more efficient in lowering the mutagenicity. The	The authors concluded that ethanol feeding modulates both the augmented cytosolic activation of DNP to mutagens and the deactivation of the direct-acting mutagenicity of DNP by microsomes. In combination, as is the case with S9, the microsomal

Table 12 - Ethanol

Citation	Study methods	Results	Authors' conclusions/comments
Res. Jun 16;279(4):289-98.	chronic ethanol ingestion in rats.	cytosolic fraction from ethanol-fed rats enhanced the mutagenicity of all of the DNP isomers in TA100. The most notable enhancement was with 1,3-DNP in which a more than 4-fold enhancement was obtained. Cytosol from pair-fed rats enhanced only the mutagenicity of 1,3-DNP, this by 2.9-fold. Cytosolic NADPH-nitroreductase activity from ethanol-treated rats toward 1,6-, 1,8- and 1,3-DNP was increased 2.8-, 1.7- and 1.3-fold, respectively over pair-fed controls. Cytosolic NADH-nitroreductase from ethanol-fed rats was increased with 1,3-DNP (1.7-fold) and 1,8-DNP (1.4-fold) as substrates, but not with 1,6-DNP. Microsomes decreased the mutagenicity of DNP similarly to S9, i.e., fractions from ethanol-fed rats were more efficient than those of pair-fed rats in deactivating all the DNP isomers.	detoxifying activity outcompetes the cytosolic activation.
Creasia DA and Thurman JD. 1993. Comparative Acute Inhalation Toxicity of a Saline Suspension and an Ethanol Solution of T -2 Mycotoxin in Mice. Inhalation Toxicology, Vol. 5, No. 1 33-41.	Inhalation exposure in mice.	The LC50 for mice exposed for 10 min to an aerosol of a saline suspension of T-2 was 0.035 ± 0.02 mg T-2 per liter air, which was lower than the LC50 (0.380 ± 0.08 mg T-2 per liter air) for an aerosol of T-2 dissolved in ethanol. However, within about 15 min postexposure, most of the T-2 deposited in the respiratory tract was translocated from the respiratory tract regardless of whether the T-2 aerosol was from a saline suspension or ethanol solution.	A saline suspension of T-2 was more toxic than an aerosol of T-2 dissolved in ethanol.
Wang PY, Kaneko T, Tsukada H, Sato A. 1994. Dose and route	Rats treated with chloroform	Ethanol treatment had no influence on the intake of food and water, but increased	Ethanol increased chloroform metabolism, resulting in higher toxicity

Table 12 - Ethanol

Citation	Study methods	Results	Authors' conclusions/comments
dependency of metabolism and toxicity of chloroform in ethanol-treated rats. Arch Toxicol.69(1):18-23.	administered or by inhalation. Ethanol (2 g/kg) or water alone at 4 p.m. on the previous day were challenged with chloroform at 10 a.m. p.o (0.01, 0.2, or 0.4 g/kg), i.p. (0, 0.1, 0.2, or 0.4 g/kg), or inhalation (for 6 h each at 0, 50, 100, or 500 ppm).	chloroform metabolism *in vitro* about 1.5-fold with no significant influence on liver glutathione content. The treatment had a dose-dependent effect on the metabolism and toxicity of chloroform. Although ethanol treatment had no significant influence on the AUC at any dose by any route of administration, the toxicity of p.o.-administered chloroform was significantly higher in ethanol-treated rats than in control rats at a dose as low as 0.1 g/kg, whereas no significant difference was observed in toxicity between both groups of rats at such a low dose administered i.p.	of chloroform.
Sharkawi M, Granvil C, Faci A, Plaa GL. 1994. Pharmacodynamic and metabolic interactions between ethanol and two industrial solvents (methyl n- butyl ketone and methyl isobutyl ketone) and their principal metabolites in mice. Toxicology. (1-3);187-95.	Ethanol treated mice were given methyl n- butyl ketone (MnBK) and methyl isobutyl ketone (MiBK).	MnBK and MiBK prolong the duration of ethanol-induced loss of righting reflex (LRR) in mice. MnBK was significantly more effective (twice as effective) than its isomer with respect to enhancing ethanol-induced LRR. The mean elimination rate of ethanol was slower in groups pretreated with MnBK or 2-HOL as compared to control animals. Ethanol elimination in animals pretreated with MiBK, HMP, 4-MPOL, or 2,5-HD was similar to that in control animals.	The authors reported that these ketones are known to have some central depressant action on their own. This by itself could lead to prolongation of ethanol-induced LRR. However, MnBK, as well as one of its principal metabolites, 2-HOL, markedly reduced ethanol elimination. This could explain the observation that MnBK has a greater potentiating effect on ethanol-induced LRR that its isomer, MiBK, which does not affect ethanol elimination.
Burke DA, Wedd DJ, Herriott D, Bayliss MK, Spalding DJ, Wilcox P. 1994. Evaluation of pyrazole and ethanol induced S9 fraction in bacterial mutagenicity testing. Mutagenesis. Jan;9(1):23-9.	Mutagenicity (Ames) tests of known mutagens after pretreatment with ethanol (inducer of CYP2E1) and	Both pyrazole- and ethanol-induced S9 were superior to PB/beta-NF-S9 and uninduced-S9 for the activation of N-nitrosopyrrolidine, a known CYP2E1 substrate. However, there was no evidence of mutagenic activity with urethane, aniline,	The authors concluded that because these compounds have demonstrated genotoxicity *in vivo*, additional important metabolic pathways must be required which are not present in rat liver S9 fraction.

Table 12 - Ethanol

Citation	Study methods	Results	Authors' conclusions/comments
	pyrazole.	benzene, procarbazine or acetaminophen.	
Zerilli A, Lucas D, Amet Y, Beaude F, Volant A, Floch HH, Berthou F, Menez JF. 1995. Cytochrome P-450 2E1 in rat liver, kidney and lung microsomes after chronic administration of ethanol either orally or by inhalation. Alcohol Alcohol. May;30(3):357-65.	Oral exposure in rats to 10% ethanol solution for 4 weeks and inhalation exposure for 4 weeks.	Inhalation was clearly the most efficient way of inducing CYP2E1, probably due to the continuous and high alcohol exposure. Among the organs tested, lung appeared to be the tissue least sensitive to induction even after ethanol inhalation. After ethanol intoxication, immunostaining was increased in the centrilobular region of the liver, in the alveolar cells of the lung and in the proximal convoluted tube of the kidney. The CYP2E1 activities decreased to control values in the three tissues tested, within 24 h after cessation of intoxication.	Ethanol induced CYP2E1 activity, and activity decreased within 24 hours following cessation of intoxication.
Liopo AV, Omelyanchik MS, Chumakova OV. 1996. Effects of low-dose ethanol on rat progeny upon inhalation exposure during gestation. Bulletin of Experimental Biology and Medicine, Volume 121, Number 3, 244-246.	Exposure in rats via inhalation during gestation.	Inhalation exposure to ethanol was found to retard the development of the progeny. The consumption of ethanol by the progeny in the experimental group was 1.5 times higher than in the control.	The authors concluded that even trace amounts of ethanol in the atmosphere are toxic for the organism.
Sessink PJM, Vaes WHJ, van den Broek PHH, de Roos JHC, Noordhoek J, Bos RP. 1996. Influence of Aroclor 1254, phenobarbital, p-naphthoflavone, and ethanol pretreatment on the biotransformation of cyclophosphamide in male and female rats. Toxicology, Volume 112, Issue 2, 16 August 1996, Pages 141-150.	Rats	No influence on the biotransformation of cyclophosphamide (CP) with pretreatment with ethanol was found.	The authors concluded that the influence of the environmental factors, alcohol consumption and smoking, on the biotransformation of CP in man will be negligible.

Table 12 - Ethanol

Citation	Study methods	Results	Authors' conclusions/comments
Nielsen GD, Hansen LF, Nem BA, Poulsen OM. 1996. Indoor Air Guideline Levels for 2-ethoxyethanol, 2-(2-ethoxyethoxy)ethanol, 2-(2-butoxyethoxy)ethanol and 1-methoxy-2-propanol. Indoor Air, Volume 8, Issue S5, 37-54.	Discussion of proposed guidelines for indoor air levels of various substances.	Three guideline values have been proposed for each of the substances, i.e. one for evaluation of odor, one for sensory irritation and one value covering both the lung and the systemic effects. Genotoxic and carcinogenic effects have also been considered.	Uncertain if this discussion includes ethanol specifically.
Choy WN, Mandakas G, Paradisin W. 1996. Co-administration of ethanol transiently inhibits urethane genotoxicity as detected by a kinetic study of micronuclei induction in mice. Mutat Res. Apr 6;367(4):237-44.	Urethane or urethane with ethanol in mouse bone marrow and micronucleaus assays. Ethanol was administered via intraperitoneal injection (i.p.) at 2,500 mg/kg.	Polychromatic erythrocytes (PCE) from bone marrow were obtained at 24 and 48 h after injection and scored for micronuclei. Urethane induced an increase of micronucleated PCE (MN PCE) frequency from 0.19% in the control to 8.63% at 24 h, followed by a decrease to 6.98% at 48 h. When urethane was co-administered with ethanol, the MN PCE frequency was suppressed to 0.49% at 24 h, but markedly increased to 7.35% at 48 h. This delay of MN PCE occurrence indicated that ethanol inhibition was transient. Urethane alone induced a peak MN PCE frequency of 11.6% at 52 h. Urethane and ethanol induced a peak MN PCE frequency of 11.2% at 64 h, a delay of 12 h.	The authors concluded that ethanol delays but does not diminish urethane genotoxicity.
Wang PY, Kaneko T, Tsukada H, Nakano M, Sato A. 1997. Dose- and route-dependent alterations in metabolism and toxicity of chemical compounds in ethanol-treated rats: difference between highly (chloroform) and poorly (carbon tetrachloride) metabolized	Rats were treated with ethanol (containing ethanol at 2 g/rat/day) or a control (containing no ethanol) liquid diet for 3 weeks were challenged	Ethanol consumption, which increased the *in vitro* metabolism of both compounds six- to sevenfold, affected the metabolism and toxicity of CCl4 differently from those of CHCl3. Ethanol increased the metabolism and toxicity of CHCl3 at 500 ppm only, whereas it increased the metabolism and toxicity of CCl4 at either 50 or 500 ppm.	Ethanol increased the metabolism and toxicity of CHCl3 and CCl4.

Table 12 - Ethanol

Citation	Study methods	Results	Authors' conclusions/comments
hepatotoxic compounds. Toxicol Appl Pharmacol. Jan; 142(1):13-21.	with chloroform (CHCL3) or carbon tetrachloride (CCl4) by inhalation (0, 50, or 500 ppm x 6 hr), or by p.o. or i.p. administration (0, 0.105, or 1.675 mmol/kg).	For the p.o. route, ethanol increased the metabolism and toxicity of both compounds at either dose. For the i.p. route, however, ethanol increased the metabolism and toxicity of the high dose of CHCI3 only, but of both CCI4 doses.	
Wu G. 1997. Using a four-compartment closed model to describe inhalation of vaporised ethanol on 1-14C-pyruvate kinetics in mice. Arch Toxicol. 71(8):501-7.	A 1-14C-pyruvate kinetics model was created.	Although the results showed that the inhalation of vaporized ethanol increased the expired 14CO2, the compartmental analysis revealed that the increase of expired 14CO2 is mainly attributed to increased 1-14C-pyruvate transmembrane process.	The authors concluded that the developed model is useful for toxicokinetic analysis when blood is not easy to obtain. Moreover, the developed model can also be used to model two compartments as urine and feces when the toxin is not eliminated through air.
Pastino GM, Asgharian B, Roberts K, Medinsky MA, Bond JA. 1997. A comparison of physiologically based pharmacokinetic model predictions and experimental data for inhaled ethanol in male and female B6C3F1 mice, F344 rats, and humans. Toxicol Appl Pharmacol. Jul;145(1):147-57.	Development of a PBPK model for inhaled ethanol in rats, mice and humans.	During exposure to 600 ppm for 6 hr, steady-state blood ethanol concentrations (BEC) were reached within 30 min in rats and within 5 min in mice. Maximum BEC ranged from 71 µM in rats to 105 µM in mice. Exposure to 200 ppm ethanol for 30 min resulted in peak BEC of approximately 25 microM in mice and approximately 15 µM in rats. Peak BEC of about 10 µM were measured following exposure to 50 ppm in female rats and male and female mice, while blood ethanol was undetectable in male rats. The PBPK model accurately simulated BEC in rats and mice at all exposure levels, as well as BEC reported in human males in previously published studies. Simulated peak BEC in human	The authors concluded that inhalation of ethanol at or above the concentrations expected to occur upon refueling results in minimal BEC and are unlikely to result in toxicity.

Table 12 - Ethanol

Citation	Study methods	Results	Authors' conclusions/comments
		males following exposure to 50 and 600 ppm ranged from 7 to 23 µM and 86 and 293 µM, respectively.	
Wu G. 1997. Use of a five-compartment closed model to describe the effects of ethanol inhalation on the transport and elimination of injected pyruvate in the rat. Alcohol Alcohol. Sep-Oct;32(5):555-61.	Model of rat inhalation ethanol pharmacokinetics.	The compartmental analysis showed that the inhalation of vaporized ethanol can increase 2-[14C]pyruvate trans-membrane, trans-tissue processes and oxidation rate.	The author concluded that the model developed is useful for general pharmacokinetic and toxicokinetic analysis as well as for studies on ethanol.
Gaffney JS and Marley NA. 1997. Potential Air Quality Effects of Using Ethanol-Gasoline Fuel Blends: A Field Study in Albuquerque, New Mexico. Environ. Sci. Technol. 31 (11), pp 3053-3061.	Exposure study on ethanol fuels. Atmospheric concentrations of ozone, oxides of nitrogen, CO, peroxyacetyl nitrate (PAN), aldehydes, and organic acids were measured in the summer of 1993, before the use of ethanol fuels, and in the winters of 1994 and 1995, during the use of 10% ethanol fuel (>99%).	Data showed increased levels of peroxyacetyl nitrate (PAN) and aldehydes in winter. The formaldehyde/acetaldehyde ratio was 1.4, indicating an anthropogenic source, and PAN and acetaldehyde levels were anti-correlated over short time periods, indicating primary acetaldehyde emissions.	Use of ethanol fuel-blend (10%) led to higher atmospheric levels of PAN.
Coccini T, Fenoglio C, Maestri L, Costa LG, Manzo L. 1998. Effect of subchronic ethanol ingestion on styrene-induced damage to the	Subchronic ethanol treatment in rats exposed orally to styrene.	Rats exposed to 300 ppm styrene presented morphological alterations throughout the respiratory tract. Electron microscopy analysis showed diffuse cell	The authors concluded that the lack of effects of ethanol on styrene pulmonary toxicity after combined exposure may be due to the different

Table 12 - Ethanol

Citation	Study methods	Results	Authors' conclusions/comments
tracheal and pulmonary epithelium of the rat. J Apol Toxicol. Sep-Oct;18(5):349-56.		damage involving the tracheal, bronchiolar and alveolar epithelium. These abnormalities were accompanied by 40% depletion of GSH in the lung tissue and also 35% depletion in hepatic GSH in the absence of alteration of the GSH content in blood. Styrene metabolism was apparently induced by subchronic ethanol treatment, as indicated by an increased excretion of urinary mandelic (+140%, $p < 0.05$) and phenylglyoxylic (+50%) acids. However, repeated ethanol administration did not exacerbate the lung GSH depletion nor the damaging effect to the respiratory tract induced by the 2-week exposure to styrene alone.	tissue distribution of the cytochrome P-450 isoforms involved in the styrene biotransformation to styrene-7,8-oxide, and their different induction by ethanol.
Vassiliev V, Kalda A, Pokk P, Vali M, Zharkovsky A. 1998. The effects of the nitric oxide synthase inhibitor 7-nitroindazole on ethanol pharmacokinetics in rats after acute and chronic ethanol administration. Alcohol Alcohol. Nov-Dec; 33(6):609-15.	Acute and chronic ethanol exposure in rats exposed intraperitoneally to 2 or 4 g/kg, along with NOS inhibitors 7-NI (20, 40, 80, and 120 mg/kg, i.p.).	Ethanol at a dose of 4 g/kg, i.p. induced sleep in rats (sleep time: 117.2+/-30.7 min). Administration of the NOS inhibitors 7-NI (20 mg/kg, i.p.) and L-NOARG (20 mg/kg, i.p.) 30 min before ethanol significantly increased the duration of ethanol-induced sleep. L-NOARG also significantly increased the toxicity of ethanol as evidenced by increased post-experimental lethality. Ethanol at a dose of 2 g/kg (i.p.) did not induce sleep in vehicle-treated rats; however, the combined administration of ethanol (2 g/kg) and 7-NI at doses of 40, 80, and 120 mg/kg caused sleep, for 49.4+/-3.7, 204.0+/-13.3, and 447.5+/-62.8 min, respectively. L-NOARG (20 mg/kg) had no effect on ethanol concentrations in blood after acute ethanol administration (4 g/kg). 7-NI in lower doses (20 and 40 mg/kg) had	

Table 12 - Ethanol

Citation	Study methods	Results	Authors' conclusions/comments
		no effect and in higher doses (80 and 120 mg/kg) significantly slowed ethanol clearance during the 12 h after ethanol administration. The administration of 7-NI immediately after the end of ethanol exposure had a pronounced effect on ethanol pharmacokinetics; in 7-NI-treated rats the fall in ethanol concentrations was significantly slower as compared with vehicle-treated rats. In 7-NI-treated rats, blood-ethanol levels were higher at 3, 6, 9, and 12 h after the end of ethanol exposure.	
Perkins NC, Heard CM. 1999. In vitro dermal and transdermal delivery of doxycycline from ethanollmigliol 840 vehicles. Int. Pharm. Nov 15;190(2):155-64.	Experiments were performed to determine the permeation of doxycycline across excised full-thickness human skin and heat-separated epidermal membranes from saturated solutions in ethanol, 1:1 and 2:1 ethanol/M840.	Unusual burst behavior was observed using an ethanol vehicle, possibly as a result of the formation of dimers at saturation. Doxycycline permeated to a higher degree from ethanolic vehicles when M840 is present, suggesting that M840 is capable of enhancing the permeation of doxycycline. The flux across full-thickness skin was highest from a 2:1 ethanol:M840 vehicle (2.41 microg cm^{-2} h^{-1}), sufficient to deliver 282 microg/l using an area of application of 30 cm^2.	
Guppy LJ, Littleton JM. 1999. Damaging effects of the calcium paradox are reduced in isolated hearts from ethanol- dependent rats: paradoxic effects of dihydropyridine drugs. J Cardiovasc Pharmacol.	Rats exposed via inhalation for 6-10 days.	Hearts from ethanol-exposed rats were significantly protected against myocardial damage, as shown by a marked reduction in release of intracellular proteins (lactate dehydrogenase, creatine phosphokinase, and myoglobin) during the reperfusion phase.	The authors concluded that ong-term exposure to ethanol *in vivo* produces marked alterations in the toxic effects of changes in myocardial calcium concentration.

Table 12 - Ethanol

Citation	Study methods	Results	Authors' conclusions/comments
Dec;34(6):765-71.			
Daiker DH, Shipp BK, Schoenfeld HA, Klimpel GR, Witz G, Moslen MT, Ward JB Jr. 2000. Effect of CYP2E1 induction by ethanol on the immunotoxicity and genotoxicity of extended low-level benzene exposure. J Toxicol Environ Health A. Feb 11;59(3):181-96.	Mice exposed orally to ethanol (in diet at 4.1%) along with benzene inhalation exposure at 0.44 ppm or 4.4 ppm for 6 or 11 weeks.	A liquid diet containing 4.1% ethanol was used to induce hepatic CYP2E1 activity by 4-fold in female CD-1 mice. In ethanol treated mice exposed to benzene, no statistically significant alterations were found in spleen lymphocyte cellularity, subtype profile, or function (mitogen-induced proliferation, cytokine production, or natural killer cell lytic activity) after 6 wk of ethanol diet, 0.44 ppm benzene exposure, or both. No treatment-associated changes in either genotoxic endpoint were detected in animals exposed to 4.4 ppm benzene for 6 or 11 wk with or without co-exposure to ethanol.	The authors concluded an absence of genetic toxicity in CD-1 mice exposed to environmentally relevant levels of benzene with or without CYP2E1 induction.
Loquet G, Campo P Lataye R Cossec B, Bonnet P. 2000. Combined effects of exposure to styrene and ethanol on the auditory function in the rat. Hear Res. Oct;148(1-2):173-80.	Styrene and ethanol oral exposure in rats once/day (4g/kg).	The authors report that ethanol alone did not have any effect on auditory sensitivity, whereas styrene alone caused permanent threshold shifts and outer hair cell damage. Hearing and outer hair cell losses were larger after the exposure to both ethanol and styrene than those induced by styrene alone, indicating a clear potentiation of styrene ototoxicity by ethanol.	The authors concluded that ethanol alters styrene metabolism and can therefore be considered a modifying factor of styrene toxicokinetics.
Blasiak J, Trzeciak A, Malecka-Panas E, Drzewoski J, Wolewódzka M. 2000. In vitro genotoxicity of ethanol and acetaldehyde in human lymphocytes and the gastrointestinal tract mucosa cells.	Genotoxicity study (Comet assay) – DNA in human lymphocytes, gastric mucosa (GM) and colonic mucosa (CM) was	Ethanol caused DNA strand breaks, which were repaired during 4 hr, except when this agent was applied in GM cells at a concentration of 1 M. A dose-dependent decrease in the tail moment of all types of acetaldehyde-treated cells was observed. Similar results were obtained when a	The authors concluded that ethanol and acetaldehyde can contribute to cancers of the digestive tract.

Table 12 - Ethanol

Citation	Study methods	Results	Authors' conclusions/comments
Toxicol In Vitro. Aug;14(4):287-95.	investigated.	recognized DNA crosslinking agent, formaldehyde, was used. The authors pointed out that acetaldehyde may form crosslinks with DNA. These crosslinks were poorly repaired. CM cells showed the highest sensitivity of all cell types to ethanol than lymphocytes and GM cells. There were no differences in the sensitivity to acetaldehyde of all the cell types.	
Phillips BJ, Jenkinson P. 2001. Is ethanol genotoxic? A review of the published data. Mutagenesis. Mar;16(2):91-101.	Review of the genotoxicity of ethanol.	There is clear evidence that ethanol is not a bacterial or mammalian cell mutagen. Evidence from the use of ethanol as a vehicle control suggests that it is not mutagenic or clastogenic *in vitro*. Reported tests for chromosome aberration induction *in vivo* are all negative and only a minority of micronucleus tests are positive. Conflicting results have been reported for the dominant lethal assay, although an inter-laboratory study performed to OECD guidelines was negative. There is some evidence that ethanol induces SCE *in vivo* and can also act as an aneugen at high doses. Many *in vivo* studies were designed to model alcoholism and used very high doses, sometimes for long periods.	The authors concluded that some degree of genotoxicity may result from excessive alcohol drinking, but this is not considered relevant to any conceivable exposure obtainable by either inhalation or dermal exposure in the workplace.
Choi WS, Murthv GG, Edwards DA, Langer R, Klibanov AM. 2001. Inhalation delivery of proteins from ethanol suspensions. Proc Natl Aced Sci U S A. Sep 25;98(20):11103-7.	Inhalation delivery of proteins from their suspensions in absolute ethanol was explored both *in vitro* and *in vivo*.	Protein suspensions in ethanol of up to 9% (wt/vol) were readily aerosolized with a commercial compressor nebulizer. Enzyme suspensions in anhydrous ethanol retained their full catalytic activity for at least 3 weeks at room temperature. The bioavailability based on estimated deposited lung dose of insulin delivered by inhalation of ethanol	

Table 12 - Ethanol

Citation	Study methods	Results	Authors' conclusions/comments
		suspension aerosols was 33% (relative to an equivalent s.c. injection). Inhalation of ethanol in a relevant amount/time frame resulted in no detectable acute toxic effects on rat lungs or airways, as reflected by the absence of statistically significant inflammatory or allergic responses, damage to the alveolar/capillary barrier, and lysed and/or damaged cells.	
Schifter I, Vera M, Diaz L, Guzman E, Ramos F, Lopez-Salinas E. 2001. Environmental Implications on the Oxygenation of Gasoline with Ethanol in the Metropolitan Area of Mexico City. Environ. Sci. Technol. 35 (10), pp 1893-1901.	Exhaust regulated (CO, NO, and hydrocarbons) and toxic (benzene, formaldehyde, acetaldehyde, and 1,3-butadiene) emissions were evaluated for MTBE (5 vol %)- and ethanol (3, 6, and 10 vol %)-gasoline blends.	The most significant overall emissions variations derived from the use of 6 vol % ethanol (relative to a 5% MTBE base gasoline) were a16% decrease in CO, 28% reduction in formaldehyde, and 80% increase in acetaldehyde emissions. A 26% reduction in CO emissions from the oldest fleet (<MY 1991, without catalytic converter), which represents about 44% of the in-use light duty vehicles in Mexico City, can be attained when using 6 vol % ethanol-gasoline, without significant variation in hydrocarbons and NO emissions, when compared with a 5% vol MTBE-gasoline.	The authors concluded that, on the basis of the emissions results, an estimation of the change in the motor vehicle emissions of the metropolitan area of Mexico City was calculated for the year 2010 if ethanol were to be used instead of MTBE, and the outcome was a considerable decrease in all regulated and toxic emissions, despite the growing motor vehicle population.
MacDonald AJ, Rostami-Hodiegan A, Tucker GT, Linkens DA. 2002. Analysis of solvent central nervous system toxicity and ethanol interactions using a human population physiologically based kinetic and dynamic model. Requl Toxicol Pharmacol. Apr; 35(2 Pt 1):165-76.	PBPK models of inhaled m-xylene and orally ingested ethanol were developed and combined by a competitive enzyme (CYP2E1) inhibition model.	Probabilistic estimates of an individual experiencing CNS disturbances given exposure to the current UK occupational exposure standard (100 ppm time-weighted average over 8 h), with and without ethanol ingestion, were obtained. The probability of experiencing CNS effects given this scenario increased markedly and nonlinearly with ethanol dose.	The authors concluded that as CYP2E1-mediated metabolism of other occupationally relevant organic compounds may be inhibited by ethanol, simulation studies of this type should have an increasingly significant role in the chemical toxicity risk assessment.

Table 12 - Ethanol

Citation	Study methods	Results	Authors' conclusions/comments
Kampf G, Muscatiello M, Hantschel D, Rudolf M. 2002. Dermal tolerance and effect on skin hydration of a new ethanol-based hand gel. J Hosp Infect. Dec;52(4):297-301.	Humans exposed to an ethanol-based gel: 85% (w/w) repetitive occlusive patch test.	In the induction phase none of the 53 participants had a skin reaction. In the challenge phase one participant had a barely perceptible skin reaction, and one had mild erythema at one time point. Relative skin hydration on treated skin in comparison with an untreated control field was significantly higher after one week by 6.85% ($p = 0.0031$; paired t -test for dependent samples) and after two weeks by 4.47% ($p = 0.0153$).	The authors concluded that Sterillium Gel did not demonstrate a clinically relevant potential for dermal irritation or sensitization, and significantly increased skin hydration after repetitive use, and so could enhance compliance with hand hygiene among healthcare workers.
Irvine LF. 2003. Relevance of the developmental toxicity of ethanol in the occupational setting: a review. J Appl Toxicol. Sep-Oct; 23(5): 289-99.	Developmental toxicology review of the use of ethanol as an industrial chemical.		The author concluded that there is no evidence that industrial exposure to ethanol is a developmental toxicity hazard. Developmental toxicity may result from drinking alcoholic beverages, the threshold level for all aspects of which has yet to be defined. This is not, however, considered relevant to the low blood alcohol concentrations resulting from any conceivable inhalation or dermal exposure in the workplace or through the directed use of any consumer product containing ethanol.
Nadeau V, Lamoureux D, Beuter A, Charbonneau M, Tardif R. 2003. Neuromotor Effects of Acute Ethanol Inhalation Exposure in Humans: A Preliminary Study. Journal of Occupational Health Vol. 45, No. 4 215-222.	Humans exposed via inhalation to 0, 250, 500 and 1,000 ppm for 6 hours.	Reaction time, body sway, hand tremor and rapid alternating movements were measured before and after each exposure session. ETOH was not detected in blood nor in alveolar air when volunteers were exposed to 250 and 500 ppm, but at the end of exposure to 1,000 ppm, blood and alveolar air concentrations were 0.443	The authors concluded that acute exposure to ethanol at 1,000 ppm or lower or to concentrations that could be encountered upon refueling is not likely to cause any significant neuromotor alterations in healthy males.

Table 12 - Ethanol

Citation	Study methods	Results	Authors' conclusions/comments
		mg/100 ml and 253.1 ppm, respectively. The neuromotor tests did not show conclusively significant differences between the exposed and non-exposed conditions.	
Gusbers JHJ, Tielemans E, Brouwer DH, Van Hemmen JJ. 2004. Dermal Exposure During Filling, Loading and Brushing with Products Containing 2-(2-Butoxyethoxy)ethanol. Oxford Journals Medicine, The Annals of Occupational Hygiene. Volume 48, Issue 3, 219-227.	DEGBE (2-(2-butoxyethoxy ethanol) was used as a 'marker' substance to determine dermal exposure in humans to the products that workers were handling.	A total of 94 task-based measurements were performed, 30 on filling, 28 on loading and 36 on brushing, which resulted in potential dermal hand exposure to the handled product of 4.1-18,269 mg [geometric mean (GM) 555.4, n = 30], 0.3-27,745 mg and 11.3-733.3 mg for each of the scenarios, respectively. Potential whole body exposure to the product during filling and loading ranged from 1.67 to 155.0 and <LOD to 176.2.	
Tardif R, Liu L, Raizenne M. 2004. Exhaled ethanol and acetaldehyde in human subjects exposed to low levels of ethanol. Inhal Toxicol. Apr;16(4):203-7.	Human exposure via inhalation to 25, 100, or 1000 ppm for 6 hours.	The concentrations of acetaldehyde and ethanol in alveolar air measured after 2 h of exposure at 25 ppm were 0.06 ppm and 7.5 ppm, respectively. Overall, there was a significant correlation between ethanol exposure and ethanol ($R(2) = .92$, $p < .001$) and acetaldehyde ($R(2) = .99$, $p < .001$) in alveolar air. The ratios between acetaldehyde and ethanol in alveolar air after 4 h of exposure to 25.9 ppm, 101.7 ppm, and 990.8 ppm ethanol were 0.005, 0.008, and 0.006, respectively.	The authors concluded that inhalation of ethanol at low concentrations resulted in measurable levels of acetaldehyde in alveolar air and suggests that acetaldehyde may be used, with good toxicological relevance, as an indicator of exposure to low levels of ethanol in air.
Van der Merwe D and Riviere JE. 2005. Comparative studies on the effects of water, ethanol and water/ethanol mixtures on chemical partitioning into porcine		Partitioning of test substances was highest in water and was higher from 50% ethanol than from 100% ethanol, except for parathion, 4-nitrophenol, atrazine and propazine. Correlation existed between	

Table 12 - Ethanol

Citation	Study methods	Results	Authors' conclusions/comments
stratum corneum and silastic membrane. Toxicology in Vitro, Volume 19, Issue 1, February, 69-77.		molecular weight and partitioning in water, but not in ethanol and ethanol/water mixtures. Lipid order, as reflected in FT-IR spectra, was not altered.	
Chu I, Poon R, Valli V, Yaqminas A, Bowers WJ Seeqal R, Vincent R. 2005. Effects of an ethanol-gasoline mixture: results of a 4-week inhalation study in rats. J Appl Toxicol. May-Jun;25(3):193-9.	Rats exposed via inhalation to 6,130 ppm ethanol, 500 ppm gasoline or a mixture of 85% ethanol and 15% gasoline (by volume, 6,130 ppm ethanol and 500 ppm gasoline), 6 h a day, 5 days per week for 4 weeks.	Female rats (receiving mixture) showed growth suppression, which was reversed after 4 weeks of recovery. Increased kidney weight and elevated liver microsomal ethoxyresorufin-O-deethylase (EROD) activity, urinary ascorbic acid, hippuric acid and blood lymphocytes were observed and most of the effects were associated with gasoline exposure. Combined exposure to ethanol and gasoline appeared to exert an additive effect on growth suppression. Inflammation of the upper respiratory tract was observed only in the ethanol-gasoline mixture groups, and exposure to either ethanol or gasoline had no effect on the organ. Morphology in the adrenal gland was characterized by vacuolation of the cortical area. Although histological changes were generally mild in male and female rats and were reversed after 4 weeks, the changes tended to be more severe in male rats. Brain biogenic amine levels were altered in ethanol- and gasoline-treated groups. Gasoline appeared to suppress dopamine concentrations in the nucleus accumbens region co-exposed to ethanol.	The authors concluded that treatment with ethanol and gasoline, at the levels studied, produced mild, reversible biochemical hematological and histological effects, with some indications of interactions when they were co-administered.
Panchagnula R, Desu H, Jain A, Khandavilli S. 2005. Feasibility studies of dermal delivery of paclitaxel with binary combinations	Dermal delivery of paclitaxel with binary combinations of ethanol and	Maximum Kepidermis for PCL was observed with IPM, while PCL in EtOH/IPM (1:1) showed high partitioning into dermis. Maximum flux of PCL was observed with	

Table 12 - Ethanol

Citation	Study methods	Results	Authors' conclusions/comments
of ethanol and isopropyl myristate: role of solubility, partitioning and lipid bilayer perturbation. II Farmaco, Volume 60, Issues 11-12, November-December, 894-899.	isopropyl myristate: was studied using the rat skin.	EtOH/IPM (1:1). With a binary combination of EtOH/IPM (1:1) a higher concentration of PCL can be delivered to deeper layer of skin whereas with IPM higher concentration of PCL could be localized in the epidermis.	
Heaton MB, Paiva M, Madorsky I, Siler-Marsiolio K, Shaw G. 2006. Effect of bax deletion on ethanol sensitivity in the neonatal rat cerebellum. J Neurobiol. Jan;66(1):95-101.	Mice exposed via inhalation. Mice with a targeted deletion of the proapoptotic bax gene were used to determine whether elimination of this protein would mitigate ethanol toxicity.	Ethanol exposure during the peak period of cerebellar vulnerability resulted in substantial loss of Purkinje cells in wild-type animals, but not in bax knock-outs. Granule cells in the bax gene-deleted animals were not similarly protected from ethanol effects. Levels of ROS following acute ethanol exposure were appreciably enhanced in the wild-type animals but not in the bax knock-outs.	The authors concluded that Bax is important to ethanol-induced Purkinje cell death during critical neonatal periods, but that ethanol effects on granule cells may function at least partially independent of this apoptosis agonist.
Johns DO, Daniell WE, Shen DO, Kalman DA, Dills RL, Morgan MS. 2006. Ethanol-induced increase in the metabolic clearance of 1,1,1-trichloroethane in human volunteers. Toxicol Sci. Jul;92(1):61-70.	Moderate drinkers exposed via inhalation to 1,1,1-trichloroethane to 175 ppm for 2 hours. They were administered ethanol (0.35 g/kg bw) on each of the 7 days preceding the exposure.	Prior ethanol consumption resulted in a significant increase in apparent metabolic clearance of 1,1,1-trichloroethane (mean increase = 25.4%).	The authors concluded that ethanol consumption over time can affect the rate at which an organic solvent is cleared through metabolism in humans. For chemicals with toxic metabolic products, this inductive effect of ethanol consumption on the rate of biotransformation could be potentially harmful to exposed individuals.
Miller MA, Rosin A, Levsky ME, Patel MM, Gregory TJD, Crystal CS. 2006. Does the clinical use of	Humans exposed dermally to 5mL of 62% denatured	All had an initial blood ethanol level of less than 5 mg/dL. There were no noted adverse reactions during the study. Blood	The authors concluded that the use of ethanol-based hand sanitizers, when frequently used in accordance with

Table 12 - Ethanol

Citation	Study methods	Results	Authors' conclusions/comments
ethanol-based hand sanitizer elevate blood alcohol levels? A prospective study. American Journal of Emergency Medicine, Volume 24. Issue 7, Pages 815-817.	ethyl alcohol applied to both hands and rubbed until dry. This activity was repeated 50 times over 4 hours.	ethanol level upon completion of the 50 applications of EBHS was less than 5 mg/dl in all 5 study participants.	labeling, do not raise serum blood ethanol levels.
Kramer A, Below H, Bieber N, Kampf G, Toma CD, Huebner NO, Assadian O. 2007. Quantity of ethanol absorption after excessive hand disinfection using three commercially available hand rubs is minimal and below toxic levels for humans. BMC Infect Dis. Oct 11;7:117.	Volunteers applied three hand-rubs containing 95% (hand-rub A), 85% (hand-rub B) and 55% ethanol (hand-rub C; all w/w). Four mL were applied 20 times for 30 s, with 1 minute break between applications. For surgical hand disinfection, 20 mL of each hand rub was applied to hands and arms up to the level of the elbow 10 times for 3 minutes, with a break of 5 minutes between applications.	The median of absorbed ethanol after hygienic hand disinfection was 1,365 mg (A), 630 mg (B), and 358 mg (C). The proportion of absorbed ethanol was 2.3% (A), 1.1% (B), and 0.9% (C). After surgical hand disinfection, the median of absorbed ethanol was 1,067 mg (A), 1,542 mg (B), and 477 mg (C). The proportion of absorbed ethanol was 0.7% (A), 1.1% (B), and 0.5% (C). The highest median acetaldehyde concentration after 20 hygienic hand disinfections was 0.57 mg/L (hand-rub C, after 30 min), after 10 surgical hand disinfections 3.99 mg/L (hand-rub A, after 20 minutes).	The authors concluded that the overall dermal and pulmonary absorption of ethanol was below toxic levels in humans and allows the conclusion that the use of the evaluated ethanol-based hand-rubs is safe.
Jacobson MZ. 2007. Effects of ethanol (E85) versus gasoline vehicles on cancer and mortality in the United States. Environ Sci	A nested global-through-urban air pollution/weather forecast model is	E-85 (85% ethanol fuel, 15% gasoline) may increase ozone-related mortality, hospitalization, and asthma by about 9% in Los Angeles and 4% in the United States as	The model predicted that E-85 may increase ozone-related mortality and increased PAN. Cancer risk is not

Table 12 - Ethanol

Citation	Study methods	Results	Authors' conclusions/comments
Technol. Jun 1;41(11):4150-7.	combined with high-resolution future emission inventories, population data, and health effects data to examine the effect of converting from gasoline to E-85 on cancer, mortality, and hospitalization in the United States as a whole and Los Angeles in particular.	a whole relative to 100% gasoline. Ozone increases in Los Angeles and the northeast were partially offset by decreases in the southeast. E-85 also increased peroxyacetyl nitrate (PAN) in the U.S. but was estimated to cause little change in cancer risk. Due to its ozone effects, future E-85 may be a greater overall public health risk than gasoline. However, because of the uncertainty in future emission regulations, it can be concluded with confidence only that E-85 is unlikely to improve air quality over future gasoline vehicles. Unburned ethanol emissions from E-85 may result in a global-scale source of acetaldehyde larger than that of direct emissions.	expected to change.
Song CL, Zhou YC, Huang RJ, Wang YQ, Huang OF, LO G, Liu KM. 2007. Influence of ethanol-diesel blended fuels on diesel exhaust emissions and mutagenic and genotoxic activities of particulate extracts. J Hazard Mater. Oct 22;149(2):355-63.	Exposure and genotoxicity study: influence of ethanol addition on diesel exhaust emissions. The experiments were conducted on a heavy-duty diesel engine and five fuels were used, namely: E-0 (base diesel fuel), E5 (5%), E1-0 (10%), E-15 (15%) and E-20 (20%), respectively.	From the point of exhaust emissions, the introduction of ethanol to diesel fuel could result in higher brake-specific THC (BSTHC) and CO (BSCO) emissions and lower smoke emissions, while the effects on the brake-specific NOx (BSNOx) and particulate matter (BSPM) were not obvious. The PAH emissions showed an increasing trend with a growth of ethanol content in the ethanol-diesel blends. As to the biotoxicity, E-20 always had the highest brake-specific revertants (BSR) in both TA98 and TA100 with or without metabolizing enzymes (S9), while the lowest BSR were found in E-5 except that of TA98-S9. DNA damage data showed a lower genotoxic potency of E-10 and E-15 as a whole.	

Table 12 - Ethanol

Citation	Study methods	Results	Authors' conclusions/comments
Bird MD, Kovacs EJ. 2008. Organ-specific inflammation following acute ethanol and burn injury. J Leukoc Biol. 2008 Sep;84(3):607-13. Epub Mar 24.	Inflammatory responses at the wound site and in other distal organs after exposure to acute ethanol and burn injury in mice.	Increased mortality in mice given ethanol and burn injury paralleled elevated serum levels of proinflammatory cytokines, IL-6 and TNF-alpha, marked infiltration of leukocytes into the lung and gut, as well as immunosuppression at the sites of infection.	Ethanol resulted in higher mortality among mice with burn injury.
Rosano TG, Lin J. 2008. Ethyl glucuronide excretion in humans following oral administration of and dermal exposure to ethanol. J Anal Toxicol. Oct;32(8):594-600.	Repetitive daily exposures to hand sanitizer (60% ethanol) in human volunteers. Also oral exposure to a 24-g drink	EtG concentration ranged from < 10 to 114 microg/L in 88 first-morning void specimens. EtG excretion following a 24-g ethanol drink by 4 adults revealed maximum urine EtG concentrations of 12,200-83,200 microg/L. Oral ethanol use also showed an increase in the percent (molar equivalent) ethanol excreted as EtG with increasing oral ethanol doses.	
Li Y, Yu X, Wane Z, Wane H. 2008. Ethanol poisoning together with organophosphate exposure: a difficult clinical diagnosis because of physician anchoring. Alcohol Alcohol. Nov-Dec;43(6).	Case report of acute ethanol poisoning along with dermal exposure to organophosphorus.	Based on the history and a misinterpretation of the physical examination, the patient was treated as an organophosphorus poisoning. Ultimately, serum analysis helped clarify the diagnosis.	The author concluded that toxicologists should be aware of the error known as anchoring and take appropriate precautions to limit its occurrence.
Kirschner MH, Lang RA, Breuer B, Breuer M, Gronover CS, Zwinciers T, Böttrich JG, Arndt A, Brauer U, Hintzpeter M, Burmeister MA, Fauteck JD. 2009. Transdermal resorption of an ethanol- and 2-propanol-containing skin disinfectant. Langenbecks Arch Surq. Jan;394(1):151-7.	Dermal exposure to 20 ml of three different alcohol-containing disinfectants.	No clinically relevant enhancement of dermal absorption, with respect to ethanol and 2-propanol, could be observed within 1 h after application, neither when used as single preparations, nor in combination.	The authors concluded that the use of ethanol- and 2-propanol-containing disinfectants in the medical environment can be considered as safe.

Table 12 - Ethanol

Citation	Study methods	Results	Authors' conclusions/comments
Bevan RJ Slack RJ, Holmes P Levy LS. 2009. An assessment of potential cancer risk following occupational exposure to ethanol. J Toxicol Environ Health B Crit Rev. Mar;12(3):188-205.	Review of cancer risk from occupational exposure (inhalation and dermal).	Inhalatory exposure at the current occupational exposure limit (OEL) for the United Kingdom (1,000 ppm ethanol over an 8-h shift) was estimated to be equivalent to ingestion of 10 g ethanol (approximately 1 glass of alcohol) per day. However, in the occupational setting the dose-rate delivery of this amount of ethanol is low, allowing for its rapid and effective elimination, for the majority of individuals. Similarly, while dermal absorption in an occupational setting could potentially add to overall body ethanol burden, additional carcinogenic risk of such exposure is considered negligible.	The authors concluded that there appears little cause to suppose occupational exposure at or below the current OEL associates with any appreciable increase in risk of cancer. However, available occupational exposure data to confirm this view are currently limited.
Chaudhuri SR, Rachna M. Gajjar RM, Krantz WB, Kasting GB. 2009. Percutaneous absorption of volatile solvents following transient liquid exposures II. Ethanol. Chemical Engineering Science, Volume 64, Issue 8, 15 April, 1665-1672.	Human cadaver skin was exposed to four doses ranging from 6.33 to 50.6 pL/cm^2 (5-40 pL over an area of 0.79 cm^2).	Results not presented.	
Anderson GG. 2009. Ethanol fuel use in Brazil: air quality impacts. Energy Environ. Sci., 2, 1015-1037.	Review of air quality and emissions data in Brazil related to vehicle pollutants that may be impacted by large quantities of ethanol in the fuel.	The atmospheric concentrations of acetaldehyde (CH3CHO) and ethanol in Brazil are much higher than those in other areas of the world, while the concentrations of the single ring aromatic compounds and small carboxylic acids are more typical of observations elsewhere. Acetaldehyde and ethanol increase in vehicle emissions and nitrogen oxides (NOx) may increase when ethanol fuels are used. Both CH3CHO and NO, are very important contributors to photochemical air pollution and ozone (O3)	The author concludea that these issues must be evaluated for other areas of the world that are considering the use of high ethanol content vehicle fuels.

Table 12 - Ethanol

Citation	Study methods	Results	Authors' conclusions/comments
		formation. There are very significant O3 air quality problems in Brazil, most studied in the larger cities of Sao Paulo and Rio de Janeiro.	
Uittamo J, Siikala E, Kaihovaara P, Salaspuro M, Rautemaa R. 2009. Chronic candidosis and oral cancer in APECED-patients: production of carcinogenic acetaldehyde from glucose and ethanol by Candida albicans. Int J Cancer. Feb 1;124(3):754-6.	Comparison of the production of acetaldehyde (ACH) from ethanol (EtOH) and glucose by oral *Candida* isolates from autoimmune polyendocrinopathy-candidiasis-ectodermal dystrophy (APECED) patients with control isolates.	There was no significant difference in the ACH production from EtOH or glucose between the earlier and more recent isolates from 6 patients. There were some differences between the earlier and more recent isolates from 8 patients, mostly when incubated in glucose, but there was no obvious temporal trend nor were the differences significant.	The authors concluded that *C. albicans* isolated from APECED patients produced potentially mutagenic amounts of ACH when incubated in 100 mM glucose. The amount of ACH produced was significantly higher when compared to *C. albicans* strains isolated from groups of patients with oral cancer or from healthy controls.
Jaimes-Lizcano YA, Lawson LB, Papadopoulos KD. 2010. Oil-frozen W(1)/ONV(2) double emulsions for dermal biomacromolecular delivery containing ethanol as chemical penetration enhancer. J Pharm Sci. Oct 19. [Epub ahead of print]	Oil-frozen water-in-oil-in-water (W(1)/O/W(2)) double emulsions (DE) containing ethanol up to 40% (w/v) in the external aqueous W(2) phase were used in *in vitro* dermal experiments.	Fluorescein isothiocyanate-labeled bovine serum albumin (FITC-BSA) penetration of up to 86 pm into porcine skin occurred in the study, reaching the viable epidermis where the immunocompetent Langerhans cells are located.	The authors concluded that oil-frozen VV(1)/ONV(2) DE, with penetration-enhancing ethanol in the W(2) phase, can potentially be used for cutaneous vaccine delivery formulations.
Lane RA, Egli-Gany D, Brill FH, Böttrich JG, Breuer M, Breuer B, Kirschner MH. 2010. Transdermal absorption of ethanol- and 1-	Dermal exposure to 20 ml of two different alcohol-containing	No clinically relevant dermal absorption, with respect to ethanol and 1-propanol, could be observed within 1 h after application. Disinfectant-related mild local	The authors concluded that the use of the tested formulations containing ethanol and 1-propanol can be considered as safe.

Table 12 - Ethanol

Citation	Study methods	Results	Authors' conclusions/comments
propanol-containing hand disinfectants. Langenbecks Arch Surg. Dec 1. [Epub ahead of print]	disinfectants was applied with a 200-cm^2 gauze swab on a skin area.	skin erythema was observed in three cases.	
Kayani MA and Parry JM. 2010. The in vitro genotoxicity of ethanol and acetaldehyde. Toxicology in Vitro, Volume 24, Issue 1, February, 56-60.	Genotoxicity study to evaluate the ability of ethanol and its metabolite acetaldehyde to induce chromosomal changes using *in vitro* CBMN assay (Cytokinesis Blocked Micronucleus assay.	Both ethanol and acetaldehyde produced statistically significant ($p < 0.05$) dose-dependent increases in MN induction as compared with the controls over the dose range tested. Kinetochore analysis proved that the MN induced in ethanol were originated by an aneugenic mechanism, whereas in the case of acetaldehyde most of the MN had originated by a clastogenic mechanism.	The authors concluded that ethanol is itself genotoxic, at least *in vitro*, and produces genotoxic effects mainly through an aneugenic mechanism whereas its metabolite acetaldehyde is a clastogen.
Ginnebaugh DL, Liang J, Jacobson MZ. 2010. Examining the temperature dependence of ethanol (E85) versus gasoline emissions on air pollution with a largely-explicit chemical mechanism. Atmospheric Environment, Volume 44, Issue 9, March, 1192-1199.	Use of a model to examine the temperature dependence of ethanol and gasoline exhaust chemistry.	Accounting for chemistry and dilution alone, the average ozone concentrations through the range of temperatures tested are higher with E-85 than with gasoline by ~7 part per billion volume (ppbv) at higher temperatures (summer conditions) to ~39 ppbv at low temperatures and low sunlight (winter conditions) for an area with a high nitrogen oxide (NOx) to non-methane organic gas (NMOG) ratio.	The authors concluded that E-85's effect on health through ozone formation becomes increasingly more significant relative to gasoline at colder temperatures due to the change in exhaust emission composition at lower temperatures. Acetaldehyde and formaldehyde concentrations are also much higher with E-85 at cold temperatures, which is a concern because both are considered to be carcinogens.
Ranzer MJ, Chen L, DiPietro LA. 2011. Fibroblast function and wound breaking strength is	Human dermal fibroblasts exposed to ethanol (100	At 24, 48, and 72 hours after acute ethanol exposure (8 hours duration), fibroblasts displayed a significant impairment in	The authors concluded that these studies revealed that a single exposure to ethanol prior to injury can

Table 12 - Ethanol

Citation	Study methods	Results	Authors' conclusions/comments
impaired by acute ethanol intoxication. Alcohol Clin Exp Res. Jan;35(1):83-90.	mg/dl) and then examined for proliferative capacity and mRNA production. Also, in *in vivo* mouse studies, the wound breaking strength, LOX activity, collagen, and hyaluronic acid (HA) contents of wounds (100 mg/dl ethanol) were examined.	proliferative capacity (up to 50% at 24 hours $p < 0.001$). fter ethanol exposure, fibroblasts produced less collagen I and LOX mRNA, but more collagen III mRNA than control fibroblasts ($p < 0.05$). Ethanol exposure *in vivo* caused a reduction in wound breaking strength of up to 40% when compared to control mice ($p < 0.01$). LOX activity, collagen, and HA contents in the wounds of ethanol-exposed mice were significantly reduced ($p < 0.01$).	cause a significant decrease in wound breaking strength and that ethanol directly impairs fibroblast function, leading to decreased collagen production. They also suggested that results provided a possible explanation for how acute ethanol exposure might increase in wound complications and wound failure.
Reisfield GM, Goldberger BA, Crews BO, Pesce AJ, Wilson GR, Teitelbaum SA, Bertholf RL. 2011. Ethyl glucuronide, ethyl sulfate, and ethanol in urine after sustained exposure to an ethanol-based hand sanitizer. J Anal Toxicol. Mar;35(2):85-91.	Dermal exposure to hand sanitizer (62% ethanol) every 5 min for 10 h on three consecutive days.	Ethyl glucuronide was undetectable in all pre-study urine specimens, but two pre-study specimens had detectable ethyl sulfate (73 and 37 ng/mL). None of the pre-study specimens had detectable ethanol. The maximum ethyl glucuronide and ethyl sulfate concentrations over the course of the study were 2,001 and 84 ng/mL, respectively, and nearly all ethyl glucuronide - and ethyl sulfate -positive urine specimens were collected at the conclusion of the individual study days.	The authors concluded that ethyl glucuronide cutoffs do not distinguish between ethanol consumption and incidental exposures, particularly when urine specimens are obtained shortly after sustained use of ethanol-containing hand sanitizer. They also indicated that ethyl sulfate may be an important complementary biomarker in distinguishing ethanol consumption from dermal exposure.

Table 12 - Ethanol

Welfare Effects Studies

Citation	Study methods	Results	Authors' conclusions/comments
Oliveira-Filho EC, Grisolia CK, Paumoartten FJ. 2009. Effects of endosulfan and ethanol on the reproduction of the snail Biomphalaria tenagophila: a multigeneration study. Chemosphere. Apr;75(3):398-404. Epub Jan 12.	Ecotoxicity study on freshwater snails exposed to 19.8, 198, 1980 mg/L for 8 weeks.	At 198 mg/L, ethanol reduced fecundity of F(0) and F(1), an effect that was apparently aggravated by exposure over successive generations. Ethanol drastically reduced the proportion of hatchings among F(2) embryos. No-observed-effect-concentrations for ethanol were 19.8 mg/L for reduction in fecundity and <19.8 mg/L for developmental toxicity (hatching retardation).	Ethanol reduced the ability of freshwater snails to reproduce.
Bhanu SV and Philip B. 2011. Effect of Ethanol on Branchial Adenosine Triphosphatases in Oreochromis mossambicus (Peters). Toxicol Int. Jan;18(1):27-30.	Ecotoxicity study in fish.	Ethanol brought about a decrease in body weight, followed by significant inhibition in total ATPase, Na (+)/K(+) ATPase, Ca(2+) ATPase, and Mg(2+) ATPase activities.	
Chen TH, Wanq YH, Wu YH. 2011. Developmental exposures to ethanol or dimethylsulfoxide at low concentrations alter locomotor activity in larval zebrafish: implications for behavioral toxicity bioassays. Aquat Toxicol. Apr;102(3-4):162-6. Epub Feb 2.	Ecotoxicity study on fish – control, 0.01, 0.1, and 1% ethanol.	Hatchability was not affected. The 1% ethanol group suffered 89% mortality during 108-120 hour-post-fertilization. No developmental defects at the 0.01 and 0.1% concentrations occurred, but significantly higher deformity rates occurred with 1% ethanol. Hyperactivity and less tortuous swimming paths were observed in all ethanol concentration groups.	The authors suggested that data of behavioral toxicity bioassays using ethanol as a carrier solvent should be interpreted cautiously, because at low concentrations, ethanol could alter locomotor activity of larval zebrafish without causing any observable developmental defects.

Table 13 – Isopropylcyclopentane

Welfare Effects Studies

Citation	Study methods	Results	Authors' conclusions/comments
P.A. Vieira, R.B. Vieira, F.P. de Franca and V.L. Cardoso 2007. Biodegradation of effluent contaminated with diesel fuel and gasoline. Journal of Hazardous Materials, Volume 140, Issues 1-2, 9 February, Pages 52-59	Effects of fuel concentration (diesel and gasoline), nitrogen concentration and culture type on the biodegradation of synthetic effluent similar to what was found at inland fuel distribution terminals. Cultures were obtained from a lake with a history of petroleum contamination and were named culture C1 (collected from surface sediment) and C2 (collected from a depth of approximately 30 cm).	Of the parameters studied, the ones that had the greatest influence on the removal of total petroleum hydrocarbons (TPH) were a nitrogen concentration of 550 mg/L and a fuel concentration of 4% (v/v) in the presence of culture C1. The biodegradability study showed a TPH removal of 90 ± 2% over a process period of 49 days. Analysis using gas chromatography identified 16 hydrocarbons. The aromatic compounds did not degrade as readily as the other hydrocarbons that were identified.	No specific mention of isopropylcyclopentane in this study.

Table 14 - Methacrolein

Health Effects Studies

Citation	Study methods	Results	Authors' conclusions/comments
P.G. Gervasi, L. Citti, M. Del Monte, V. Longo and D. Benetti 1985. Mutagenicity and chemical reactivity of epoxidic intermediates of the isoprene metabolism and other structurally related compounds	Mutagenicity study using *Salmonella typhimurium* and isoprene intermediates 2- methyl-1,2,3,4-diepoxybutane and 1,2,3,4-diepoxybutane.	The compound 2- methyl-1,2,3,4-diepoxybutane, chemically analogous to the well known carcinogenic 1,2,3,4-diepoxybutane, was found to be as mutagenic as the latter. The mutagenic activities of oxiranes were correlated to their alkylating powers towards nicotinamide and to their half-lives for spontaneous hydrolysis.	The relationship between alkylating power and mutagenicity was found to hold for the stable epoxides that react mainly by an SN2 substitution mechanism.
Benamira M and Marnett LJ. 1992. The lipid peroxiation of product 4-hydroxynonenal is apotent inducer of the SOS response. Mutat Res. Nov; 293 (1): 1-10.	Bacterial mutagenesis study of 4-hydroxynonenal, acrolein, crotonaldehyde, methacrolein, and malondialdehyde in *Salmonella typhimurium* strain TA1535/pSK1002.	This study measured the ability of chemicals to induce expression of the SOS-regulated umu operon. 4-hydroxynonenal was the most potent SOS-inducer, with maximal induction observed at concentrations of 0.1-1 µM. Acrolein, crotonaldehyde and methacrolein induced little increase over background umu expression.	
Larsen ST and Nielsen GD. 2000. Effects of methacrolein on the respiratory tract in mice" Toxicol Lett. 2000 Apr 3; 114(1-3):197-202.	Acute inhalation of 2 to 26 ppm methacrolein in mice.	Irritation of the upper respiratory tract caused a concentration-dependent decrease in respiratory rate in the tested range. During exposure, the sensory irritation response maintained the same level, i.e. no desensitization occurred. At 10.4 ppm methacrolein respiratory rate was reduced by 50% (RD50). The extrapolated threshold for the respiratory depressing effect, RDO, was 1.3 ppm.	The sensory irritation effect of methacrolein was compared with results from closely related compounds in order to elucidate the mechanism of the interaction between methacrolein and the sensory irritant receptor. Only a minor airflow limitation occurred in the lower respiratory tract, suggesting that the main effect of methacrolein was sensory irritation.

Table 14 - Methacrolein

Citation	Study methods	Results	Authors' conclusions/comments
Doyle M, Sexton KG, Jeffries H, Bridge K, Jaspers I. 2004. Effects of 1,3-butadiene, isoprene, and their photochemical degradation products on human lung cells. Environ Health Perspect. Nov; 112(15): 1488-95.	Gene expression is human lung A549 cells was measured with 1,3-butadiene (BD), isoprene (ISO) and their photochemical degradation products (acrolein, acetaldehyde, formaldehyde, methacrolein, and methyl vinyl ketone)	Cytotoxicity and interleukin-8 (IL-8) gene expression was used as a marker for inflammation. Results indicated that although BD and ISO alone caused similar cytotoxicity and IL-8 responses compared with the air control, their photochemical products significantly enhanced cytotoxicity and IL-8 gene expression, suggesting that once ISO and BD are released into the environment, reactions occurring in the atmosphere transform these hydrocarbons into products that induce potentially greater adverse health effects than the emitted hydrocarbons by themselves.	Data suggested that, based on the carbon concentration or per carbon basis, biogenic ISO transforms into products with proinflammatory potential similar to that of BD products.
Nøjgaard JK, Christensen KB, Wolkoff P 2005. The effect on human eye blink frequency of exposure to limonene oxidation products and methacrolein. Toxicol Lett. Apr 10; 156(2):241-51.	Study in which 10 male subjects (average age: 43 yrs) were exposed to limonene oxidation products (LOPs), including methacrolein, for 20 mins in the non-dominant eye at 20% relative humidity (RH), while viewing an educational film.	Mean BF (blinking frequency) increased significantly during exposure to LOPs and methacrolein compared to the baseline of clean air, and the findings coincided with weak eye irritation symptoms. LOEL for methacrolein was 286 ppb and a 10-min-old LOPs mixture of initially 92 ppb limonene and 101 ppb ozone (O3), which increased the BF comparably by 18% (p=0.001) and 17% (p=0.003), respectively. The increase in BF was smaller, although not significantly different, during exposure to LOPs at 50% RH to 20% RH in mixtures prepared from ca. 350 ppb limonene and 300 ppb O3.	LOPs may cause trigeminal stimulation and possibly eye irritation at O3 and limonene concentrations which are close to high-end values measured in indoor settings. The effects may be exacerbated by low RH.
Norman Y. Kado, Robert A. Okarnoto, Paul A. Kuzmicky, Reiko Kobayashi, Alberto Ayala,	Chemical and biological analyses of toxicant emissions from	The 2000 model year CNG-fueled vehicle had the highest emissions of 1,3-butadiene, benzene, and carbonyls (e.g.,	The emissions of toxic compounds from an in-use CNG transit bus (without an oxidation catalyst) and

Table 14 - Methacrolein

Citation	Study methods	Results	Authors' conclusions/comments
Michael E. Gebel, Paul L. Rieger, Christine Maddox, and Leo Zafonte 2005. Emissions of Toxic Pollutants from Compressed Natural Gas and Low Sulfur Diesel-Fueled Heavy-Duty Transit Buses Tested over Multiple Driving Cycles. Environ. Sci. Technol., 2005, 39 (19), pp 7638-7649	compressed natural gas (CNG) and low sulfur diesel fueled heavy-duty transit buses tested over 3 different cycles using a chassis dyanometer. The CNG bus had no after-treatment, and the diesel bus was tested first equipped with an oxidation catalyst (DC) and then with a catalyzed diesel particulate filter (DPF). Emissions were also tested for mutagenic activity	formaldehyde) of the three vehicle configurations tested in this study. The 1998 model year diesel bus equipped with an OC and fueled with low-sulfur diesel had the highest emission rates of PM and PAHs. The highest specific mutagenic activities and the highest mutagen emission rates were from the CNG bus in strain TA98 tested over the New York Bus (NYB) driving cycle. The 1998 model year diesel bus with DPF had the lowest VOCs, PAH, and mutagenic activity emission. In general, the NYB driving cycle had the highest emission rates (g/mi), and the Urban Dynamometer Driving Schedule (ODDS) had the lowest emission rates for all toxics tested over the three transient test cycles investigated. Also, transient emissions were, in general, higher than steady-state emissions.	from a vehicle fueled with low-sulfur diesel fuel (equipped with DPF) were lower than from the low-sulfur diesel fueled vehicle equipped with OC. All vehicle configurations had generally lower emissions of toxics than an uncontrolled diesel engine. Tunnel backgrounds (measurements without the vehicle running) were measured throughout this study and were helpful in determining the incremental increase in pollutant emissions. Also, the on-site determination of VOCs, especially 1,3-butadiene, helped minimize measurement losses due to sample degradation after collection.
Seaman VY, Charles MJ, Cahill TM. 2006. A sensitive method for the quantification of acrolein and other volatile carbonyls in ambient air. Anal Chem. 2006 Apr 1; 78(7):2405-12.	Using a mist-chamber method to determine concentrations of acrolein and other carbonyls in air with short sampling times (10 min) at three sites in northern California reflecting hemispheric background concentrations, biogenic-dominated regions, and urban environments. The	The resulting acrolein concentrations were 0.056, 0.089, and 0.29 $\mu g/m^3$, respectively, which are all above the EPA Reference Concentration of 0.02 $\mu g/m^3$. The minimum detection limit of 0.012 $\mu g/m^3$ is below that of other published methods. The resulting methacrolein concentrations were <0.027, 0.048, 0.044 $\mu g/m^3$. The minimum detection limit of methacrolein .007 $\mu g/m^3$.	

Table 14 - Methacrolein

Citation	Study methods	Results	Authors' conclusions/comments
	carbonyls were then liberated from the bisulfite, derivatized, and quantified by gas chromatography/electron capture negative ionization mass spectrometry.		
Al Rashidi M, Shihadeh A, Saliba NA 2008. Volatile aldehydes in the mainstream smoke of the narghile waterpipe. Food Chem Toxicol. Nov;46(11):3546-9.	Study that identified and quantified volatile aldehydes in the gas and particle phases of mainstream narghile smoke generated using a popular type of flavored ma'ssel tobacco mixture.	Using a standardized smoking machine protocol consisting of 171 puffs, 2.6s puff duration and 17s inter puff interval, the average yields of formaldehyde, acetaldehyde, acrolein, propionaldehyde and methacrolein were 630, 2,520, 892, 403, and 106 µg/smoking session, respectively. None of the aldehydes identified in this study were found in the particulate phase of the smoke, except for formaldehyde for which the partitioning coefficient was estimated as Kp = 3.3 x 10(-8) µg/m^3.	Given previously reported lung absorption fractions of circa 90% for volatile aldehydes, the yields measured in this study, according to the authors, were sufficient to induce various diseases depending on the extent of exposure, and on the breathing patterns of the smokers.
Daher N, Saleh R, Jaroudi E, Sheheitli H, Badr T, Sepetdjian E, Al Rashidi M, Saliba N, Shihadeh A. 2010 Atmos Environ. Jan 1; 44 (1):8-14.	To investigate and compare emissions of ultrafine particles (UFP, <100 nm), carcinogenic polyaromatic hydrocarbons (PAH), volatile aldehydes, and carbon monoxide (CO) for cigarettes and narghile (shisha, hookah) waterpipes. Sidestream cigarette and waterpipe smoke	The authors reported that a single waterpipe use session emitted in the sidestream smoke approximately four times the carcinogenic PAH, four times the volatile aldehydes, and 30 times the CO of a single cigarette. Accounting for exhaled mainstream smoke, and given a habitual smoker smoking rate of 2 cigarettes per hour, during a typical one-hour waterpipe use session, a waterpipe smoker likely generates ambient carcinogens and toxicants equivalent to 2-10 cigarette smokers, depending on the	

Table 14 - Methacrolein

Citation	Study methods	Results	Authors' conclusions/comments
	was captured and aged in a 1 m^3 Teflon-coated chamber operating at 1.5 air changes per hour (ACH). Measured aldehydes consisted of formaldehyde, acetaldehyde, acrolein, methacrolein, and propionaldehyde.	compound in question.	
Carl Renan Estrellan and Fukuya lino 2010. Toxic emissions from open burning. Chemosphere, Volume 80, Issue 3, June, Pages 193-207	Review was compiled on the data from recent actual and simulation studies on toxic emissions from open burning and categorized into sources, broadly as biomass and anthropogenic fuels.	Emission factors, in mass of pollutant per mass of material being burned, and actual concentrations, in mass of pollutant per unit volume, have been compared based on source classifications. In addition to gaseous emissions, this review presented updated data on emissions to air in the form of particulate matter, and emissions to soil and water environment. Data from forest fires, accidental fires such as vehicle fires, house fires, and unintentional landfill fires were included in this review as well as combustion involving traditional and recreational activities.	No specific mention of methacrolein in this review.

Table 14 - Methacrolein

Welfare effects studies on Methacrolein

Citation	Study methods	Results	Authors' conclusions/comments
Robert O. Bray, Carl L. Wambolt and Rick G. Kelsey 1991. Influence of sagebrush terpenoids on mule deer preference. Journal of Chemical Ecology, Volume 17, Number 11, 2053-2062	Mule deer (8) ingestion of p-cymene, 1,8-cineole, methacrolein (two concentrations), and the nonvolatile crude terpenoid fraction (NVCTF) of various taxa of chopped alfafa hay.	Compound influence on preference, in order of increasing deterrence, was as follows: 50% methacrolein, mountain big sagebrush NVCTF, methacrolein, basin big sagebrush NVCTF, p- cymene, Wyoming big sagebrush NVCTF, black sagebrush NVCTF, and 1,8-cineole. Methacrolein appeared to be an important preference determinant among big sagebrush subspecies, and p-cymene between black sagebrush and big sagebrush. The NVCTFs containing sesquiterpene lactones as one of their constituents were closely related to the preference of all four taxa.	Future studies of animal preference for sagebrush should consider all of the potential defensive chemicals in the foliage.
Tomasz Gierczak, James B. Burkholder, Ranajit K. Talukdar, A. Melloukl, S. B. Barone and A. R. Ravishankarab 1997. Atmospheric fate of methyl vinyl ketone and methacrolein. Journal of Photochemistry and Photobiology A: Chemistry, Volume 110, Issue 1, 15 October, Pages 1-10	Rate coefficients for the reaction of OH with methyl vinyl ketone (MVK) and methacrolein between 232 k and 378 k.	The UV absorption cross-sections of MVK and methacrolein were measured over the wavelength range 250-395 nm using a diode array spectrometer. Absolute quantum yields for loss of MVK and methacrolein were measured at 308, 337, and 351 nm. The MVK quantum yield was both pressure and wavelength dependent.	Atmospheric loss rate calculations using these results showed that the primary loss process for both MVK and methacrolein was the reaction with OH radicals throughout the troposphere.
Akira Tani, Seita Tobe, and Sachie Shimizu 2010. Uptake of Methacrolein and Methyl Vinyl Ketone by Tree Saplings and Implications for Forest Atmosphere. Environ. Sci. Technol., 44 (18), pp 7096-7101	Uptake rate of methacrolein by leaves of three different *Quercus* species, *Q. acutissima*, *Q. myrsinaefolia*, and *Q. phillyraeoides*.	Uptake rates of methacrolein and croton aldehydes were found to be higher than those of the two ketones. In particular, the rate of MEK uptake for *Q. myrsinaefolia* was exceptionally low. The ratio of intercellular to fumigated concentrations, Ci/Ca, for MACR and CA was found to be low (0-0.24), while the ratio for the two ketones	The loss rate by tree uptake was the highest for both methacrolein and MEK, suggesting that tree uptake provides a significant sink.

Table 14 - Methacrolein

Citation	Study methods	Results	Authors' conclusions/comments
	Rates of uptake of croton aldehyde (CA) and methyl ethyl ketone (MEK) were also investigated for comparison.	was 0.22-0.90. The loss rate by tree uptake was the highest, followed by the reaction with OH radicals, even at a high OH concentration (0.15 pptv) both for methacrolein and MEK.	

Table 15 – Methanol

Health Effects Studies

Citation	Study Methods	Results	Authors' Conclusions/Comments
Smith MS. 1983. Solvent toxicity: isopropanol, methanol, and ethylene glycol. Ear Nose Throat J Mar;62(3):126-35.	Review of methanol toxicity.	Methanol itself is only slightly inebriating. Not until methanol is converted into formate do the serious effects of methanol on the GI, ophthalmologic, metabolic and central nervous systems occur. Delayed ocular damage is the toxic fingerprint of methanol.	
Clark CR, Dutcher JS, McClellan RO, Naman TM, Seizinger DE. 1983. Influence of ethanol and methanol gasoline blends on the mutagenicity of particulate exhaust extracts. Archives of Environmental Contamination and Toxicology, Volume 12, Number 3, 311-317.	Genotoxicity study: *Salmonella* mutagenicity test.	Dichloromethane extracts of the exhaust particles from all tests were mutagenic in Salmonella typhimurium strains TA 100 and TA 98. The extracts were less mutagenic in the nitroreductase deficient strains TA 98NR and TA 98DNPR suggesting that nitro substituted polycyclic aromatic hydrocarbons may be responsible for part of the mutagenicity.	Extracts of methanol exhaust was genotoxic however the authors state that hydrocarbons may be responsible for part of the mutagenicity.
Tephly TR and Martin KE. 1984. Methanol metabolism and toxicity. Food Science and Technology Bulletin: Functional Foods, Vol 12, 111-140.	Review study.	Authors stated that in humans biochemical reactions produce metabolites that are clearly toxic.	Review of methanol toxicity.
Finger W and Stettmeier H. 1984. Postsynaptic actions of ethanol and methanol in crayfish neuromuscular junctions. Pflügers Archiv European Journal of Physiology, Volume 400, Number 2, 113-120.	Ethanol and methanol in crayfish.	Both ethanol and methanol reduced the elementary currents (i) that flow through channels operated by quisqualate in a concentration-dependent manner but did not affect the apparent mean open time of the channels estimated from power spectra. Concentrations of 0.26 mol/l ethanol or 1 mol/l methanol, respectively,	The authors concluded that in crayfish opener muscles ethanol seems to selectively depress excitatory postsynaptic currents.

Table 15 – Methanol

Citation	Study Methods	Results	Authors' Conclusions/Comments
		reduced i e-fold.	
Black KA, Eells JT, Noker PE, Hawtrey CA, Tephly TR. 1985. Role of hepatic tetrahydrofolate in the species difference in methanol toxicity. Proc Natl Acad Sci USA. June; 82(11): 3854-3858.	Study in rats and monkeys. The susceptibility of various species to methanol toxicity is inversely related to the rate of tetrahydrofolate (H4folate)-dependent formate oxidation. Thus, the levels of various folate derivatives and folate-dependent enzyme activities present in the livers of monkeys, which are sensitive to methanol, and rats, which are not, were compared in order to investigate the biochemical basis of this species difference.	Hepatic H4folate levels in monkeys were 60% of those in rats, and formylated-H4folate derivatives were 2-fold higher in monkeys than in rats. No significant difference between monkeys and rats in the levels of total hepatic folate or 5-methyl-H4folate was observed. The activities of formyl-H4folate synthetase and formyl-H4folate dehydrogenase were 4- and 2-fold higher, respectively, in monkeys than in rats. There was no significant difference between monkeys and rats in methionine synthetase activity. Dihydrofolate reductase activity in monkeys was 20% of that in rats. 5,10-Methylene-H4folate reductase (NADPH) activity in monkeys was 40% and 25% of that in rats when the rates of the forward and reverse reactions, respectively, were compared. Serine hydroxymethyltransferase activity was 2-fold higher in monkeys than in rats.	The authors concluded that the differences in the activities of methylene-H4folate reductase and serine hydroxymethyl-transferase between monkeys and rats may have contributed to the difference in hepatic H4folate levels. The 40% lower level of hepatic H4folate in monkeys, as compared to rats, relateed well to the 50% lower maximal rate of formate oxidation in monkeys. Thus, the species difference in susceptibility to methanol may be explained by the difference in the level of hepatic H4folate.
Infurna R and Weiss B. 1986.	Oral study in rat.	No maternal toxicity was apparent as	The authors concluded that prenatal

Table 15 – Methanol

Citation	Study Methods	Results	Authors' Conclusions/Comments
Neonatal behavioral toxicity in rats following prenatal exposure to methanol. Teratology. Jun;33(3):259-65.	Rats were divided into three groups (N = 10). Two of the groups consumed drinking solutions of 2% methanol (MeOH) instead of distilled water either on gestational days 15-17 (MEeOH 1) or 17-19 (MEeOH 2)	measured by weight gain, gestational duration, and daily fluid intake. Litter size, birth weight, and infant mortality did not differ among the three groups. Postnatal growth and date of eye opening were unaffected. MeOH pups required longer than controls to begin suckling on postnatal day 1. On postnatal day 10, they required more time to locate nesting material from their home cages.	MeOH exposure induced behavioral abnormalities early in life that are unaccompanied by overt toxicity.
Andrews LS Clary JJ Terrill JB, Bolte HF. 1987. Subchronic inhalation toxicity of methanol. J Toxicol Environ Health. 20(1-2):117-24.	Subchronic inhalation study in rats and monkeys. Animals were treated with 0, 500, 2000, and 5000 ppm methanol vapor for 6 h/d, 5 d/w, for 4 wk.	The only treatment-and dose-related effect noted was that of mucoid nasal discharge in rats, which was considered reflective of upper respiratory tract irritation. No consistent treatment-related effects were found for organ or body weights or for histopathologic or ophthalmoscopic examinations.	The authors concluded that these findings support the use of the present American Council of Governmental Industrial Hygienists threshold limit value (TLV) of 200 ppm and short-term exposure limit (STEL) of 250 ppm for exposure to methanol vapor.
Kavet R and Nauss KM. 1990. The Toxicity of Inhaled Methanol Vapors. CRC Crit Rev Toxicol, 21:21-50.	Review of methanol vapor toxicity.	Acute methanol toxicity evolves in a well-understood pattern and consists of an uncompensated metabolic acidosis with superimposed toxicity to the visual system. The toxic properties of methanol are rooted in the factors that govern both the conversion of methanol to formic acid and the subsequent metabolism of formate to carbon dioxide in the folate pathway. In short, the toxic syndrome sets in if formate generation continues at a rate that exceeds its rate of metabolism.	The authors concluded that current evidence indicates that formate accumulation will not challenge the metabolic capacity of the folate pathway at the anticipated levels of exposure to automotive methanol vapor.

Table 15 – Methanol

Citation	Study Methods	Results	Authors' Conclusions/Comments
Cunningham ML, Burka LT, Matthews HB. 1990. The interaction of methanol, rat-liver S9 and the aromatic amine 2,4-diaminotoluene produces a new mutagenic compound. Mutat Res. Aug;244(4):273-7.	Genotoxicity study: Ames/*Salmonella* assay.	The authors reported a rapid and quantitative conversion of the mutagenic and carcinogenic aromatic amine 2,4-diaminotoluene (2,4-DAT) to a single product. This product was only produced in the presence of methanol, and not other organic solvents. Isolation of this product showed that it was highly mutagenic in *Salmonella* TA98 with S9 activation. Characterization of the product of the interaction of methanol and 2,4-DAT indicated that methanol is activated to a reactive intermediate, probably formaldehyde, by the 9000 X g supernatant used in the Ames/*Salmonella* assay. The formaldehyde subsequently reacts with 2,4-DAT to form the mutagenic product, identified as bis-5,5'(2,4,2',4'-tetraaminotolyl)methane.	The authors concluded that the results of this study demonstrate that methanol may be an inappropriate solvent for mutation and metabolism studies of aromatic amines and possibly other chemicals, and that solvent-xenobiotic interactions may in some cases lead to the misinterpretation of results.
Teo TR. 1991. The toxicity of methanol. Life Sci, 48(11):1031-41.	Review of methanol toxicity in humans and animals.	Methanol toxicity in humans and monkeys is characterized by a latent period of many hours followed by a metabolic acidosis and ocular toxicity. The metabolic acidosis and blindness is apparently due to formic acid accumulation in humans and monkeys, a feature not seen in lower animals.	
Eelis JT. 1991. Methanol-induced visual toxicity in the rat. J Pharmacol Exp Ther. Apr;257(1):56-63.	Study of methanol exposure in rats.	Methanol-intoxicated rats developed formic acidemia, metabolic acidosis and visual toxicity within 36 hr of methanol administration analogous to the human methanol poisoning syndrome.	The authors concluded that alterations in the electroretinogram occurred at formate concentrations lower than those associated with other visual changes and provide functional evidence of direct retinal

Table 15 – Methanol

Citation	Study Methods	Results	Authors' Conclusions/Comments
			toxicity in methanol poisoning.
Liesivuori J and Savolainen H. 1991. Methanol and formic acid toxicity: biochemical mechanisms. Pharmacol Toxioal. Sep,69(3):157-63.	Review of mechanisms of toxicity of methanol.	Metabolism of methanol, methyl ethers, esters and amides give rise to formic acid. This acid is an inhibitor of the mitochondria! cytochrome oxidase causing histotoxic hypoxia. The body burden of formate in methanol poisoning is high enough to cause acidosis, and other clinical symptoms. The acidosis causes e.g. dilatation of cerebral vessels, facilitation of the entry of calcium ions into cells, loss of lysosomal latency and deranged production of ATP. The latter effect seems to impede parathormonedependent calcium reabsorption in the kidney tubules. Urinary acidification is affected by formic acid. Its excretion causes continuous recycling of the acid by the tubular cell CI-/formate exchanger. This sequence of events may partially explain an accumulation of formate in urine.	The authors concluded that occupational exposure to vapors of methanol and formic acid can be quantitatively monitored by urinary formic acid determinations. Formic acid toxicity may prove a suitable model for agents causing histotoxic hypoxia.
Black F and Gabele P. 1991. Impact of methanol and CNG fuels on motor-vehicle toxic emissions. Environmental Protection Agency, Jan 01, Technical Report No. PB-92-110378/XAB.	Emissions study: Methanol and CNG vs. gasoline.	Results suggested that at equivalent ambient temperatures and average speeds, motor vehicle toxic emissions are generally reduced with methanol and compressed natural gas (CNG) fuels relative to those with gasoline, except for formaldehyde emissions, which may be elevated. As with gasoline, tailpipe toxic emissions with methanol and compressed natural gas fuels generally increase when ambient temperature or average speed decreases	The results suggested that overall toxic emissions are reduced among vehicles using methanol or CNG vs. gasoline.

Table 15 – Methanol

Citation	Study Methods	Results	Authors' Conclusions/Comments
		(the sensitivity to these variables is greater with methanol than with compressed natural gas).	
Molinia DV and Swan MA. 1991. Effect of ethanol and methanol on the motility of Saccostrea commercialis sperm and sperm models. Molecular Reproduction and Development, Volume 30, Issue 3, 241-249.	A study with *S. commercialis* sperm.	Methanol and ethanol at concentrations of 2.5% and 5% (v/v), respectively, were found to significantly ($P < 0.001$) decrease the radius of curvature and track velocity of *S. commercialis* sperm.	The authors concluded that ethanol is dependent on the pH of the sperm model used.
Downie A, Khattab TM, Malik MI, Samara IN. 1992. A case of percutaneous industrial methanol toxicity. Occup Med (Londj. Feb;42(1):47-9.	Case report: individual wore a breathing apparatus but no protective clothing at a petrochemical complex.	After 2-3 hours working in the confined space of the tank, he worked on deck and continued to wear his methanol-soaked clothing which eventually dried out. Visual symptoms of acute methanol toxicity presented some 8 hours after exposure. The appropriate treatment (with ethanol provided by the ship bond) was carried out in a hospital and the individual recovered completely.	Methanol was acutely toxic.
Maejima K, Suzuki T Niwa K, Numata H, Maekawa A, Nagase S, Ishinishi N. 1992. Toxicity to rats of methanol-fueled engine exhaust inhaled continuously for 28 days. J Toxicol Environ Health. Oct;37(2):293-312.	Inhalation exposure in rats to exhaust generated by an M85 methanol-fueled engine (methanol with 15% gasoline) without catalyst for 8 h/d, 7 d/wk for 7, 14, 21, or 28 d.	Concentration- and time-dependent yellowing of the fur was prominent in all treated groups. Concentration-dependent increases in the erythrocyte count, hematocrit, hemoglobin concentration, formaldehyde in plasma, and carboxyhemoglobin in the erythrocytes, and decrease in serum alkaline phosphatase activity were seen after all exposure periods. Lesions were found in the nasal cavity and lungs after 7 d of exposure.	The authors concluded that these results may suggest that the lesions observed in the nasal cavity of rats exposed to methanol-fueled engine exhaust were mainly caused by formaldehyde, although other components in the exhaust also may have affected nasal cavity and/or lungs to a lesser extent.

Table 15 – Methanol

Citation	Study Methods	Results	Authors' Conclusions/Comments
		Squamous metaplasia of the respiratory epithelium of level 1 lining of the nasoturbinate and/or maxilloturbinate and infiltration of neutrophils into the submucosa, and decreases of Clara cells in the terminal bronchiolus and of cilia in the bronchiolar epithelium, were observed in the high-concentration group (carbon monoxide, 94 ppm; formaldehyde, 6.9 ppm; methanol, 17.9 ppm; nitrogen oxides, 52.7 ppm; nitrogen dioxide, 10.6 ppm). The histopathological extents of several lesions increased slightly with the exposure time. Slight squamous metaplasia and hyperplasia of the respiratory epithelium at level 1 were also observed in the medium-concentration group (one in three of the high-concentration group). No histopathological changes were found in the olfactory epithelium of the nasal cavity. In the low-concentration group (one in nine of the high-concentration group), no marked histopathological changes in these organs were observed.	
ATSDR. 1993. Methanol toxicity. Am Fam Physician. Jan;47(1):163-71.	Review of methanol toxicity.	Toxic metabolites can cause fatality after a characteristic latent period. Methanol is well absorbed following inhalation, ingestion or cutaneous exposure. It is oxidized in the liver to formaldehyde, then to formic acid, which contributes to the profound metabolic acidosis occurring in acute methanol poisoning. The metabolic products of methanol can produce a	

Table 15 – Methanol

Citation	Study Methods	Results	Authors' Conclusions/Comments
		syndrome of delayed-onset acidosis, obtundation, visual disturbance and death.	
Rogers JM, Mole ML, Chernoff N, Barbee BD, Turner CI, Logsdon TR, Kavlock RJ. 1993. The developmental toxicity of inhaled methanol in the CD-1 mouse, with quantitative dose-response modeling for estimation of benchmark doses. Teratology. Mar;47(3):175-88.	Inhalation exposure of pregnant CD-1 mice to 1,000, 2,000, 5,000, 7,500, 10,000, or 15,000 ppm methanol for 7 hr/day on days 6 -15 of gestation.	One dam died in each of the 7,500, 10,000, and 15,000 ppm methanol exposure groups, but no dose-response relationship was evident for maternal death. The sham-exposed and food-deprived controls as well as all methanol exposed dams gained less weight than did unexposed dams fed ad libitum, but methanol did not exacerbate this effect. Significant increases in the incidence of exencephaly and cleft palate were observed at 5,000 ppm and above, increased embryo/fetal death at 7,500 ppm and above (including an increasing incidence of full-litter resorptions), and reduced fetal weight at 10,000 ppm and above. A dose-related increase in cervical ribs or ossification sites lateral to the seventh cervical vertebra was significant at 2,000 ppm and above.	The authors concluded that the NOAEL for the developmental toxicity in this study was 1,000 ppm.
Pamies RJ, Sugar D, Rives LA Herold AH. 1993. Methanol intoxication. How to help patients who have been exposed to toxic solvents. Postgrad Med. Jun;93(8):183-4, 189-91, 194.	Discussion of how to treat patients exposed to methanol.	Typical symptoms of methanol intoxication include lethargy, vertigo, vomiting, blurred vision, and decreased visual acuity. Treatment focuses on prevention of methanol conversion to its toxic metabolites, correction of metabolic acidosis, and elimination of the toxic substances from the system. Ethanol and bicarbonate administration and hemodialysis have been effective.	

Table 15 – Methanol

Citation	Study Methods	Results	Authors' Conclusions/Comments
Andrews JE, Ebron-McCoy M, Logsdon TR, Mole LM, Kavlock RJ, Rogers JM. 1993. Developmental toxicity of methanol in whole embryo culture: a comparative study with mouse and rat embryos. Toxicology. Aug 27;81(3):205-15.	Inhalation exposure in rats and mice. Differential sensitivity was studied in whole embryo culture (WEC). Rat embryos were explanted and cultured in 0, 2, 4, 8, 12 or 16 mg MeOH/ml rat serum for 24 h. Mouse embryos were explanted and cultured in 0, 2, 4, 6 or 8 mg MeOH/ml culture medium (75% rat serum, 25% Tyrode's salt solution) for 24 h.	Results in rats: Embryonic development of the 2 and 4 mg MeOH/ml groups was not significantly different from the controls whereas the higher concentrations resulted in a concentration related decrease in somite number, head length and developmental score. The 12 mg/ml dose resulted in some embryolethality as well as dysmorphogenesis, while the highest dose was embryolethal. MeOH was dysmorphogenic *in vitro* in rat embryos at a MeOH concentration comparable to that reported in maternal serum following teratogenic *in vivo* exposures. Results in mice: Embryonic development in the 2 mg/ml MeOH group was not significantly different from the controls but all higher concentration groups had a significant decrease in developmental score and crown-rump length. The high concentration group also suffered 80% embryolethality.	The authors concluded that mouse embryos were affected at MeOH concentrations which were not dysmorphogenic or embryotoxic in the rat, suggesting that the higher sensitivity of the mouse to the developmental toxicity of inhaled methanol is due, at least in part, to greater intrinsic embryonal sensitivity of this species to methanol.
Roeggla G, Wagner A, Frossard M Roegdla H. 1993. Marked variability in methanol toxicity. Am Fam Physician. Oct;48(5):731.	Letter to the editor re: on treatment of patients with methanol intoxication.	With methanol toxicity, the usual indications for dialysis are a peak methanol level greater than 50 mg per dL, metabolic acidosis not immediately correctable with bicarbonate therapy, visual impairment and renal failure. The authors suggest that chronic alcoholics also be considered for hemodialysis because of the very high risk	

Table 15 – Methanol

Citation	Study Methods	Results	Authors' Conclusions/Comments
		of death. These patients should be treated only in emergency departments capable of performing rapid hemodialysis.	
Bolon B, Dorman DC, Janszen O, Morgan KT, Welsch F. 1993. Phase-specific developmental toxicity in mice following maternal methanol inhalation. Fundam Appl Toxicol. Nov;21(4):508-16.	Inhalation study in pregnant CD-1 mice in which animals were treated 6 hr/day to 5,000, 10,000 or 15,000 ppm methanol.	Examination of near-term fetuses revealed embryotoxicity (increased resorptions, reduced fetal weights, and/or fetal malformations) at 10,000 and 15,000 ppm, while 3-day exposures at 5,000 ppm yielded no observable adverse effects. Terata included neural and ocular defects, cleft palate, hydronephrosis, deformed tails, and limb (paw and digit) anomalies. Neural tube defects and ocular lesions occurred after methanol inhalation between neural tube development and closure, while limb anomalies were induced only during potential neural tube reopening; cleft palate and hydronephrosis were observed after exposure during either period.	The authors concluded that these findings were consistent with prior reports that maternal methanol inhalation at high levels induced developmental toxicity in a concentration-dependent manner.
Aufderheide TP, White SM, Brady WJ, Stueven HA. 1993. Inhalational and percutaneous methanol toxicity in two firefighters. Ann Emerg Med. Dec;22(12):1916-8.	Report of 2 cases of adult inhalation of methanol.	Both patients complained only of a mild headache at the time of the emergency department evaluation and had normal physical examinations, normal anion gaps, and peak methanol levels of 23 and 16 mg/dL, respectively.	
Frenia ML and Schauben JL. 1993. Methanol inhalation toxicity. Ann Emerg Med. Dec;22(12):1919-23.	Report of 7 cases of patients who intentionally inhaled GARB-MEDIC carburetor cleaner containing toluene	Patients arrived at the emergency department with central nervous system depression, nausea, vomiting, shortness of breath, photophobia, and/or decreased visual acuity. Measured blood methanol levels ranged from 50.4 to 128.6 mg/dL.	

Table 15 – Methanol

Citation	Study Methods	Results	Authors' Conclusions/Comments
	(43.8%), methanol (23.2%), methylene chloride (20.5%), and propane (12.5%).	Blood formic acid levels were 120, 193, and 480 micrograms/mL, respectively, in three patients. Ophthalmic examinations revealed hyperemic discs and decreased visual acuity in one patient. One individual was found pulseless with several GARB-MEDIC cans nearby. Attempts at revival were unsuccessful.	
Lee EW, Garner CD, Terzo TS. 1994. Animal model for the study of methanol toxicity: comparison of folate-reduced rat responses with published monkey data. J Toxicol Environ Health. Jan;41(1):71-82.	Three groups of Long-Evans rats with different levels of liver folate were prepared and examined for formate accumulation after methanol administration (3.5 g/kg).	Oral exposure: The folate-reduced (FR) rats prepared by feeding a folate-deficient diet with 1% succinylsulfathiazole yielded blood formate levels equivalent to those found in methanol-intoxicated humans and developed signs of the visual system toxicity. The area under the concentration vs. time curve for blood formate in FR rats was 2.5-fold greater than that in monkeys when 2.0 g/kg methanol was administered. Inhalation exposure: FR rats exposed to 3,000 ppm methanol, 20 h/d, could not survive more than 4 d. On the other hand, monkeys exposed to 3,000 ppm, 21 h/d, out-lived 20 d.	The authors concluded that these results indicated FR rats are more sensitive to methanol challenges than monkeys, and suggest that the FR rat could be a congruous animal model for evaluating the health effects of methanol in humans.
Maelima K, Suzuki T, Numata H, Maekawa A, Naciase S, Ishinishi N. 1994. Subchronic (12-week) inhalation toxicity study of methanol-fueled engine exhaust in rats. J Toxicol Environ Health.	Rats were exposed via inhalation to exhaust from M85 fuel without catalyst for 8 h/d, 6 d/wk for 4, 8, or 12 wk.	Concentration- and time-dependent increase in carboxyhemoglobin in the erythrocytes and a decrease in cytochrome P-450 in the lungs were observed in all treated groups. Significant increases in plasma formaldehyde were observed in all treated groups. No change of plasma folic	The authors concluded that further study on more long-term inhalation of lower concentrations of exhaust might be needed.

Table 15 – Methanol

Citation	Study Methods	Results	Authors' Conclusions/Comments
Mar;41(3):315-27.		acid was observed in any group, and no methanol or formic acid was detected in the plasma in any animals. Exposure-related changes were found only in the nasal cavity of the high-concentration group observed after 4 wk of exposure, and the incidences and degrees of these lesions increased slightly with the exposure time. No changes were found in the olfactory epithelium of the nasal cavity.	
Poon R, Chu I, Biarnason S Potvin M, Vincent R Miller RB Valli VE. 1994. Inhalation toxicity study of methanol, toluene, and methanol/toluene mixtures in rats: effects of 28-day exposure. Toxicol Ind Health, May-Jun;10(3):231-45.	Rats exposed via inhalation to 300 ppm or 3,000 ppm for six hrs per day, five days/week for four weeks.	The thyroid gland in females appeared to be a target organ, although the changes were confined to a mild, and occasionally moderate, reduction in follicle size. No changes were observed in liver weights, hepatic mixed-function oxidases, or serum aspartate transaminase activities, and only minimal changes occurred in liver histopathology.	The authors concluded that these data indicated exposure to methanol produced mild biochemical effects and histological changes in the thyroid and nasal passage.
Poon R, Chu I, Bjarnason S, Vincent R, Potvin M, Miller RB, Valli VE. 1995. Short-term inhalation toxicity of methanol, gasoline, and methanol/gasoline in the rat. Toxicol Ind Health. May-Jun;11(3):343-61.	Rats exposed via inhalation to methanol (2,500 ppm), gasoline (3,200 ppm), and methanol/gasoline (2,500/3,200 ppm, 570/3,200 ppm) 6 hours per day, 5 days per week for 4 weeks.	Depression in body weight gain and reduced food consumption were observed in male rats, and increased relative liver weight was detected in rats of both sexes exposed to gasoline or methanol/gasoline mixtures. Rats of both sexes exposed to methanol/gasoline mixtures had increased relative kidney weight and females exposed to gasoline and methanol/gasoline mixtures had increased kidney weight. Decreased serum glucose and cholesterol were detected in male rats exposed to gasoline and methanol/gasoline mixtures.	The authors concluded that gasoline was largely responsible for the adverse effects, the most significant of which included depression in weight gain in the males, increased liver weight and hepatic microsomal enzyme activities in both sexes, and suppression of uterine eosinophilia. No apparent interactive effects between methanol and gasoline were observed.

Table 15 – Methanol

Citation	Study Methods	Results	Authors' Conclusions/Comments
		Decreased hemoglobin was observed in females inhaling vapors of gasoline and methanol/gasoline at 570/3,200 ppm.	
Medinsky MA and Dorman DC. 1995. Recent developments in methanol toxicity. Toxicol Lett. Dec;82-83:707-11.	Review of recent developments of methanol toxicity.	The rate at which rodents detoxify formate is more rapid than that of primates. Formate, an endogenous biological substrate, is detoxified by metabolism to CO_2 via a tetrahydrofolate-(THF) dependent pathway. Species with high hepatic THF levels, such as rodents, are less sensitive to the neurotoxic effects of large methanol doses compared with species with low THF levels, such as primates. Healthy human volunteers exposed at rest or during exercise to 200 ppm methanol for 6 h or exposed to 20 mg/kg orally have elevated blood levels of methanol, but blood formate concentrations are not significantly increased above endogenous concentrations.	The authors concluded that limited studies in non-human primates with low THF levels exposed to 900 ppm methanol for 2 h have shown that concentrations of methanol-derived formate in blood remain below endogenous levels. Thus, human populations may not be at added risk of neurotoxic effects resulting from exposure to low levels of methanol.
Ward KW, Perkins RA, Kawagoe JL, Pollack GM. 1995. Comparative toxicokinetics of methanol in the female mouse and rat. Fundam Appl Toxicol. Jul;26(2):258-64.	Mice and rats exposed via the oral or IV route to single dose of 2.5 g/kg methanol.	The disposition of methanol was nonlinear in both species. Data obtained after i.v. administration of methanol to mice were well described by a one-compartment model with Michaelis-Menten elimination. Blood methanol concentration-time data after oral administration could be described by a one-compartment (mice) or two-compartment (rats) model with Michaelis-Menten elimination from the central compartment and biphasic absorption from the gastrointestinal tract.	When normalized for body weight, mice evidenced a higher maximal elimination rate than rats (Vmax = 117 +/- 3 mg/hr/kg vs. 60.7 +/- 1.4 mg/hr/kg for rats). The contribution of the fast absorption process to overall methanol absorption also was larger in the mouse than in the rat.

Table 15 – Methanol

Citation	Study Methods	Results	Authors' Conclusions/Comments
Perkins RA, Ward KW, Pollack GM. 1995. Comparative Toxicokinetics of Inhaled Methanol in the Female CD-1 Mouse and Sprague-Dawley Rat. Fundamental and Applied Toxicology Volume 28, Issue 2, December 245-254.	Mice exposed via inhalation to 2,500, 5,000, and 10,000 ppm methanol vapor for 8 hours.	Total 8-hr ventilation decreased slightly with increasing exposure concentration. The fraction of inhaled methanol absorbed (0.85 ± 0.14) did not vary statistically with exposure concentration. Model predictions for the mouse were compared to a previously developed inhalation toxicokinetic model for the rat. The comparison demonstrated that at similar methanol vapor concentrations, mice evidenced a two- to three-fold higher blood methanol concentration than rats, despite the fact that the apparent Vmax for methanol elimination in the mouse is two-fold larger than that in the rat.	The authors concluded that these data may have significant implications in understanding species differences in methanol-induced teratogenic effects.
Franzblau A, Batterman S, D'Arcy JB, Sargent NE, Gross KB, Schreck RM. 1995. Breath monitoring of inhalation and dermal methanol exposure. Applied Occupational & Environmental Hygiene, Vol. 10, no. 10, 833-839.	Inhalation or dermal exposure in humans to various concentrations of methanol for 8 hours.	The results demonstrated that blood and breath concentrations of methanol were disproportional for varying periods of time during and following cessation of methanol exposure, depending on the route of exposure (dermal versus inhalation).	The concentration of methanol in exhaled air was found to be not proportional to blood methanol.
Rogers JM and Mole ML. 1997. Critical periods of sensitivity to the developmental toxicity of inhaled methanol in the CD-1 mouse. Teratology. Jun;55(6):364-72.	Inhalation exposure of pregnant CD-1 mice to 10,000 ppm MeOH or filtered air for 7 hr/day on 2 consecutive days during gestation days (gd) 6-13, or to single day (7-hr) exposures	Cleft palate, exencephaly, and skeletal defects were the fetal anomalies observed in this mouse strain. Cleft palate occurred with 2-day exposures on gd 6-7 through gd 11-12 (peak on gd 7-8), and with 1-day exposure on gd 5 through gd 9 (peak on gd 7). Exencephaly occurred with 2-day exposures on gd 6-7 through gd 8-9 (peak gd 6-7) or 1-day exposure on gd 5 through	The authors concluded that gastrulation and early organogenesis represent a period of increased embryonal sensitivity to methanol.

Table 15 – Methanol

Citation	Study Methods	Results	Authors' Conclusions/Comments
	to 10,000 ppm MeOH during gd 5-9.	gd 8 (peak on gd 7). Skeletal elements malformed included the exoccipital (peak gd 6-7, gd 5), atlas (peak gd 6-7, gd 5,6), axis (peak gd 6-7, gd 7), cervical vertebra 7 with a rib (peak gd 6-7, gd 7), and lumbar vertebra 1 with a rib (peak gd 7-8, gd 7). An increased incidence of fetuses with 25 presacral vertebrae (normal = 26) was observed with methanol exposure on gd 5, whereas an increased incidence of fetuses with 27 presacral vertebrae was observed with MeOH exposure on gd 7.	
Arredondo Y, Moreno-Marias M, Pleixats R, Palacin C, Raga MM, Castello JM, Ortiz JA. 1997. Preparation, antimicrobial evaluation, and mutagenicity of [2-hydroxyary1]-[1-methyl-5- nitro-1H-2-imidazolyl] methanols, [5-tert-Buty1- 2-methylaminopheny1]41- methy1-5-nitro-1H-2-imidazolyl]methanol, and [2-Hydroxyary1]-[1-methyl-5-nitro-1H-2-imidazolyl] ketones. Bioorganic & Medicinal Chemistry Volume 5, Issue 10, October 1959-1968.	Mutagenic properties of various methanol compounds were evaluated.	Results not presented in abstract.	It seems like methanol was not specifically evaluated in this study.
Batterman SA and Franzblau A. 1997. Time-resolved cutaneous absorption and permeation rates of methanol in human volunteers. Int Arch Occup Environ Health.	Human (12 volunteers) dermal exposure to methanol – one hand for durations of 0-16min	The pre-exposure methanol concentration in blood was 1.7 +/- 0.9 mg/l, and subjects had statistically different mean concentrations. The maximum methanol concentration in blood was reached 1.9	The authors concluded that while variability in blood concentrations and absorption rates approached a factor of two, differences between individuals were not statistically

Table 15 – Methanol

Citation	Study Methods	Results	Authors' Conclusions/Comments
70(5):341-51.	in a total of 65 sessions.	mg/l 1.0 h after exposure. Delivery rates from skin into blood lagged exposure by 0.5 h, and methanol continued to enter the systemic circulation for 4 h following exposure. The maximum concentration of methanol in blood following an exposure to one hand lasting approximately 20 min is comparable to that reached following inhalational exposures at a methanol concentration of 200 ppm, the threshold limit value-time weighted average (TLV-TWA).	significant. The derived absorption and permeation rates provided information regarding kinetics and absorbed dose that can help to interpret biological monitoring data and confirm mathematical models of chemical permeation.
Gattas, GJF, Cardoso LA, Faria MM [Ethics and Occupational Medicine-FMUSP, Sao Paulo (Brazil)] [and others]. 1997. Mutagenic effect of methanol in gas station operators from Sao Paulo/Brazil. Environmental and Molecular Mutagenesis; Volume 29; Issue: Suppl.28; Conference: 28.	Human subjects were exposed to a fuel called MEG (a mix of 33% methanol, 60% ethanol and 7% gasoline). In November 1991, for economical reasons, the MEG fuel was introduced in big cities. Micronuclei (MN) frequency was evaluated in three different periods: before MEG introduction (1989), and twice after MEG utilization (1992 and 1995).	Statistical analysis through non-parametric tests revealed a highly significant increase (P=0.001) in the frequency of MN before (1.38/2,000 cells) and immediately after methanol introduction (3.0/2,000 cells). The frequency of MN returned to normal (1.4/2,000 cells), in the third evaluation when the exposure to methanol decreased.	The authors concluded that these results should represent genetic risk for individuals under occupational exposure and for the population as a whole when methanol has been introduced on a large scale in fuel.
Poon R, Park G Viau C Chu I,	Rats inhalation	No clinical signs of toxicity were observed	Based on the marked increase in

Table 15 – Methanol

Citation	Study Methods	Results	Authors' Conclusions/Comments
Potvin M, Vincent R, Valli V. 1998. Inhalation toxicity of methanol/gasoline in rats: effects of 13-week exposure. Toxicol Ind Health. Jul-Aug;14(4):501-20.	exposure to 85% methanol, 15% gasoline: 50/3, 500/30, and 5,000/300 ppm for 6 hours per day, 5 days per week, for 13 weeks	in the treatment group and their growth curves were not significantly different from the control. Except for decreased forelimb grip strength in high dose females, no treatment-related neurobehavioural effects (4-6 hours post inhalation) were observed. At necropsy, the organ-to-body-weight ratios for the liver, spleen, testes, thymus and lungs were not significantly different from the control group. There were no treatment-related effects in the hematological endpoints and no elevation in serum formate levels. Minimal serum biochemical changes were observed with the only treatment-related change being decreased creatinine in the females. A dose-related increase in urinary ascorbic acid was detected in males after 2, 4 and 8 weeks of exposure, but not after the 12th week, and in females only at week 2. Increased urinary albumin was observed in treated males starting at the lowest dose and at all exposure periods, but not in females. A treatment-related increase in urinary beta 2-microglobulin was detected in males at week 2 only.	urinary ascorbic acid and albumin in the high-dose males and the decreased forelimb grip strength in the high dose females, the authors concluded that the no observed adverse effect level (NOAEL) of methanol/gasoline vapor is 500/30 ppm.
Ruppová K, Wsólová L, Urbanciková M, Slamenová D. 2000. Comparison of three in vitro assays at evaluation of IC50 of acetylsalicylic acid, ferrous sulfate, amitriptyline, methanol, isopropanol and ethylene glycol in	Cytotoxicity study/*in vitro* assays of human cancer cells. Short-term exposure to 2,000, 2,500, 2,750, 3,000, 4,000 and 5,000 mM; and long-	The cytotoxic effect of short-term treatment was evident at concentrations higher than 2,000 mM by the PE test (plating efficiency) and at concentrations higher than 3,000 mM by the BM test (Bradford's method - microprotein assay). After a 24-h exposure the cytotoxic effect was more evident by all	The authors concluded that the cytotoxicity of long-term treatment of cells with methanol correlated well with the published data obtained by similar methods.

Table 15 – Methanol

Citation	Study Methods	Results	Authors' Conclusions/Comments
human cancer cells. Neoplasma. 47(3):172-6.	term exposure to 1,000, 1,250, 1,500, 1,750, 2,000 and 2,500 mM.	used methods: by the BM test and by the NRU test (frame neutral red uptake – alternative to ocular irritancy test) at concentrations higher than 1,250 mM and by the PE test at concentrations higher than 1,500 mM. The obtained iC50s (inhibitory concentrations) of short-term treatment were about two times higher than those of long-term treatment.	
Gattas GJ Cardoso Lde A, Medrado-Faria Mde A, Saldanha PH. 2001. Frequency of oral mucosa micronuclei in gas station operators after introducing methanol. Occup Med (Lond). Mar;51(2):107-13.	Frequency of oral mucosa micronuclei in gas station operators exposed to vapors from fuel containing 33% methanol, 60% ethanol, and 7% gasoline.	The frequency of micronuclei (MN) was evaluated before and 1 year after a mixed fuel called MEG, which contains 33% methanol, 60% ethanol and 7% gasoline, was introduced. The third evaluation, 3 years later, represents a period where the number of cars using alcohol fuel had decreased drastically and the pump and operator exposure to MEG became very low. The frequency of MN observed in 76 employees in 1992 (mean = 3.62 +/- 0.39) was significantly increased ($P < 0.001$) as compared with 76 operators exposed in 1989 (mean = 1.41 +/- 0.26) and 129 exposed in 1995 (mean = 1.20 +/- 0.15).	The authors concluded that these findings could indicate a mutagenic hazard of the MEG occurring in those with occupational exposure.
Huang YS, Held GA, Andrews JE, Rogers JM. 2001. (14)C methanol incorporation into DNA and proteins of organogenesis stage mouse embryos in vitro. Reprod Toxicol. Jul-Aug;15(4):429-35.	Gestational day 8 CD-1 mouse embryos were grown for 24 h in culture medium (CM) with 0, 4, or 8 mg MeOH + 20 microCi (14)C-MeOH/mL.	Methylation of a selected gene, Hoxc-8, was assessed by using methylation-specific restriction enzymes. The (14)C activity was found superimposed over the DNA-containing fraction, indicating incorporation. DNA from embryos treated with 4 mg MeOH/mL CM gave the highest incorporation of (14)C-MeOH (8 mg/mL	The authors concluded that methanol is teratogenic in mice *in vivo* and causes dysmorphogenesis in cultured organogenesis stage mouse embryos. They also concluded that these results indicated that methyl groups from (14)C-MeOH were incorporated into mouse embryo DNA and protein

Table 15 – Methanol

Citation	Study Methods	Results	Authors' Conclusions/Comments
		was growth-inhibiting). Methylation of Hoxc-8 appeared to be increased in embryos treated with 4 mg MeOH/mL CM, but not in embryos treated with 8 mg MeOH/mL. Lack of incorporation of methylation at the higher concentration may be due to the failure of embryos to grow at this concentration of MeOH. Incorporation of (14)C-MeOH into specific proteins was observed but the labeling specificity was not methanol dose-related.	and that the results further suggested that methanol exposure may increase genomic methylation under certain conditions which could lead to altered gene expression.
Zabrodskii PF and Germanchuk VG. 2001. [Effect of ethanol on methanol immunotoxicity]. [Article in Russian]. Eksp Klin Farmakol. Sep-Oct;64(5):40-2.	Acute methanol poisoning in rats.	Ethanol reduced nonspecific resistance of the organism and decreases activity of the native killer cells, antibody production mainly with respect to the T-dependent antigen, antibody-mediated cell cytotoxicity, and the formation of delayed-type hypersensitivity associated with methanol poisoning.	The authors concluded that ethanol serves as an antidote of methanol.
Bouchard M, Brunet RC, Droz P-O, Carrier G. 2001. A Biologically Based Dynamic Model for Predicting the Disposition of Methanol and Its Metabolites in Animals and Humans. Oxford Journals, Life Sciences & Medicine, Toxicological Sciences, Volume 64, Issue 2, 169-184.	A multicompartment biologically based dynamic model was developed to describe the time evolution of methanol and its metabolites in the whole body and in accessible biological matrices of rats, monkeys, and humans following different exposure	The average pulmonary absorption fraction of methanol was estimated to be 0.60 in rats, 0.69 in monkeys, and 0.58-0.82 in human volunteers. The corresponding average elimination half-life of absorbed methanol through metabolism to formaldehyde was estimated to be 1.3, 0.7-3.2, and 1.7 h. Saturation of methanol metabolism appeared to occur at a lower exposure in rats than in monkeys and humans. Also, the main species difference in the kinetics was attributed to a metabolism rate constant of whole body	The model, adapted to kinetic data in human volunteers exposed acutely to methanol vapors, predicted that 8-h inhalation exposures ranging from 500 to 2,000 ppm, without physical activities, are needed to increase concentrations of blood formate and urinary formic acid above mean background values reported by various authors (4.9-10.3 and 6.3-13 mg/liter, respectively).

Table 15 – Methanol

Citation	Study Methods	Results	Authors' Conclusions/Comments
	scenarios.	formaldehyde to formate estimated to be twice as high in rats as in monkeys. Inversely, in monkeys and in humans, a larger fraction of body burden of formaldehyde is rapidly transferred to a long-term component.	
Chao M-R, Lin T-C, Chao H-R, Chang F-H and Chen C-B. 2001. Effects of methanol-containing additive on emission characteristics from a heavy- duty diesel engine. The Science of The Total Environment, Volume 279, Issues 1-3, 12 November, 167-179.	Effect of methanol-containing additive (MCA) on the regulated emissions of hydrocarbons (HC), carbon monoxide (CO), nitrogen oxides (NO_x), particulate matter (PM), as well as the unregulated carbon dioxide (CO_2) and polycyclic aromatic hydrocarbons (PAHs) from a diesel engine; 0, 5, 8, 10 and 15% MCA by volume.	Results showed that MCA addition slightly decreased PM emissions but generally increased both THC and CO emissions. Decrease in NO_x emissions was found common in all MCA blends. As for unregulated emissions, CO_2 emissions did not change significantly for all MCA blends, while vapor-phase and particle-associated PAHs emissions in high load and transient cycle tests were relatively low compared to the base diesel when either 5 or 8% MCA was used. This may be attributed to the lower PAHs levels in MCA blends. Finally, the particle-associated PAH emissions also showed trends quite similar to that of the PM emissions in this study.	MCA addition decreased PM and NO_x emissions while it increased total HC and CO emissions.
Lin TC and Chao MR. 2002. Assessing the influence of methanol-containing additive on biological characteristics of diesel exhaust emissions using microtox and mutatox assays. Sci Total Environ. Feb 4;284(1-3):61-74.	Microtox and Mutatox assays, respectively, were used to evaluate the acute toxicity and genotoxicity of crude extracts from diesel engine exhaust of	Microtox results showed that MCA additive moderately lowered the toxicity levels of particle-associated (SOF) samples, but generally increased the vapor- phase (XOC) associated toxicity. A strong correlation was found between XOC-associated toxicity and total hydrocarbon (THC) concentrations, while only a slight	The authors concluded that the genotoxicity data did not parallel the Microtox results in this study, indicating that potentially long-term genotoxic agents may not be revealed by short-term toxicity assays.

Table 15 – Methanol

Citation	Study Methods	Results	Authors' Conclusions/Comments
	fuel with a methanol-containing additive (MCA). Five additive levels (0, 5, 8, 10 and 15% of MCA by volume) were used.	link was found between SOF-associated toxicity and particulate matter (PM) concentrations. For Mutatox test results, when either 5 or 8% MCA used, XOC and SOF-associated genotoxicity in both steady-state and hot-start transient cycle tests were relatively lower compared to those of the base diesel. The genotoxic potential of XOC samples was significantly increased after treatment with an exogenous metabolic activation system (S9). On the contrary, the genotoxic potential of SOF samples without S9 metabolic activation was generally higher than those with S9.	
Velez LI, Kulstad E, Shepherd G, Roth B. 2003. Inhalational methanol toxicity in pregnancy treated twice with fomepizole. Vet Hum Toxicol. Feb;45(1):28-30.	Case report of pregnant woman with a long history of inhalant abuse.	In both admissions, she was treated with fomepizol (4-MP), a newly available alcohol dehydrogenase antagonist for treatment of ethylene glycol poisoning.	
Kostic MA and Dart RC. 2003. Rethinking the toxic methanol level. J Toxicol Clin Toxicol 41(6):793-800.	Review of methanol poisoning literature.	A total of 70 articles (173 patients) met inclusion criteria. Only 22 of these patients presented for care within 6 hr of ingestion with an early methanol level. All but 1 patient was treated with an inhibitor of alcohol dehydrogenase (ADH). A clear acidosis developed only with a methanol level > or = 126 mg/dL. The patient that did not receive an ADH inhibitor was an infant with an elevated early methanol level (46 mg/dL) that was given folate alone and never became acidotic.	The authors concluded that the small amount of data regarding patients arriving early showed that 126 mg/dL was the lowest early blood methanol level ever clearly associated with acidosis and that the data were insufficient to apply 20 mg/dL as a treatment threshold in a nonacidotic patient arriving early for care. Prospective studies are necessary to determine if such patients may be managed without antidotal therapy or

Table 15 – Methanol

Citation	Study Methods	Results	Authors' Conclusions/Comments
			dialysis.
Server A, Hovda KE, Nakstad PH, Jacobsen D, Dullerud R, Haakonsen M. 2003. Conventional and diffusion-weighted MRI in the evaluation of methanol poisoning. Acta Radiol. Nov;44(6):691-5.	Case report of methanol-poisoned patients.	Conventional MRI showed symmetrical, bilateral increased signal in the lentiform nuclei, involving predominantly putamina, but also extending into the corona radiata, centrum semiovale and subcortical white matter. Diffusion-weighted MRI showed decreased diffusion, which most probably reflects cytotoxic edema. In the other patient, fluid attenuated-inversion recovery (FLAIR) and T2-weighted images showed hyperintensity in the putamina, characteristic of post-necrotic changes. Analysis of proteinuria suggested that proximal tubular cells may be preferentially affected. Results of histopathological evaluation of the kidney on a limited sample size (n = 5) were inconclusive but suggestive of possible hydropic changes in the proximal tubule secondary to methanol toxicity.	The authors concluded that acute renal injury may be associated with other signs of severity in methanol poisoning, but it is almost always reversible in survivors. Indicators of acute renal injury were identified. The pathophysiology of this acute renal injury is multifactorial and far more complex than shock-related tubular necrosis.
Ferrari LA, Arado MG, Nardo CA, Giannuzzi L. 2003. Post-mortem analysis of formic acid disposition in acute methanol intoxication. Forensic Science International Volume: 133, Issue: 1-2, 152-158.	Case report of 15 cases of fatal methanol intoxication. Formic acid in blood and tissues was analyzed by head space gas chromatography. Methanol poisoning cases were classified in three groups	Formic acid concentration was between 0.03 and 1.10 g/l in the samples under study. A good correlation between blood and brain, but poor between blood and the remaining tissues was found. The best correlation among organs was found between lung and kidney for all groups (r^2 = 0.91, 0.84 and 0.87, corresponding to groups 1, 2 and 3, respectively). LI parameter was used to estimate formic acid incidence on the lethality of methanol	The authors concluded that the results showed the importance of performing formic acid analysis to diagnose severe methanol intoxication in post-mortem cases.

Table 15 – Methanol

Citation	Study Methods	Results	Authors' Conclusions/Comments
	according to survival time: more than 3 days (group 1), up to 3 days (group 2) and few hours (group 3).	poisoning cases. LI >100 indicated a severe intoxication and short survival time if the victim was assisted with ethanol therapy and hemodialysis was not applied. With regard to victims who received no therapeutic treatment and died in few hours, LI was in the range 40-100. LI was below 40 for individuals that survived more than 3 days and hemodialysis was not performed.	
Verhelst D, Moulin P, Haufroid V, Wittebole X, Jadoul M, Hantson P. 2004. Acute renal injury following methanol poisoning: analysis of a case series. Int J Toxicol. Jul-Aug;23(4):267-73.	Case report of methanol-poisoned patients (n=25).	Clinical pathological signs of acute renal injury were found in 15 patients. In comparison with the 10 other patients taken as a control group, the patients who developed renal injury had a lower blood pH value on admission, a higher serum osmolality, and a higher peak formate concentration.	Methanol poisoning resulted in renal toxicity.
Burbacher TM, Shen DD, Lalovic B, Grant KS, Sheppard L, Doris D, Ellis S, Liberato N. 2004. Chronic maternal methanol inhalation in nonhuman primates (Macaca fascicularis): Exposure and toxicokinetics prior to and during pregnancy. Neurotoxicology and Teratology Volume 26, Issue 2, March-April 201-221.	Inhalation exposure in monkeys to 0 (control), 200, 600, or 1,800 ppm methanol vapor for approximately 2.5 h/day, 7 days/week throughout breeding and pregnancy.	Average blood methanol concentrations at 30 min post-exposure were 5, 11, and 35 pg/ml across all four toxicokinetic studies in the 200, 600 and 1,800 ppm groups, respectively. Blood concentrations in the 200 ppm group were barely above basal (pre-exposure) blood methanol concentrations or those observed in the control group (approximately 3 pg/ml). Nonlinear elimination kinetics were observed in most of the 1,800 ppm group females. There was a decrease in elimination half- life (7-20%) and an increase in clearance (30%) after 3-months	

Table 15 – Methanol

Citation	Study Methods	Results	Authors' Conclusions/Comments
		of daily MeOH exposure compared to the initial exposure. There were no statistically significant changes in the first-order blood methanol half-life or clearance during pregnancy, but the mean distribution volume per kilogram body weight decreased by 22% and 17% in the 600 and 1,800 ppm groups. Plasma formate levels did not differ between the methanol and control exposure groups. Plasma formate and serum folate concentrations increased slightly over the course of this study in both the exposed and control groups but these increases were not related to methanol. exposure.	
Belson M and Morgan BW. 2004. Methanol toxicity in a newborn. J Toxicol Ciro Toxicol. 42 (5):673-7.	Case report of a human newborn poisoning from maternal exposure.	A son was delivered by emergent C-section (birthweight 950 g, Apgars 1 and 3) and required aggressive resuscitation. During his hospital course, acidosis (initial pH 6.9) persisted despite fluid, blood, and bicarbonate administration. His mother also had persistent metabolic acidosis despite fluids, bicarbonate, and dopamine. Results of other laboratory tests on the mother included undetectable ethanol and salicylates and an osmolar gap of 41. An ethanol drip was initiated for the mother 36 h after admission when a methanol level of 54 mg/dL was reported. When consulted on hospital day 3, the regional poison center recommended hemodialysis for the mother and administering fomepizole and testing the methanol level of the newborn	

Table 15 – Methanol

Citation	Study Methods	Results	Authors' Conclusions/Comments
		(61.6 mg/dL). Because the infant developed a grade 4 intraventricular bleed, no further therapy was offered, and he died on day 4. His mother died on day 10.	
Zhanq ZZ, Lianq Y, Ran Y, Gou XJ, Lin C, Cai C, Wu D. 2005. [Comparative study on the mutagenicity of the gasoline-fueled vehicle exhaust and methanol-fueled vehicle exhaust]. Wei Sheno Yan Jiu. Mar;34(2):163-6.	Ames assay and A549 cell micronucleus test *in vitro* were applied to compare the mutagenicity of gasoline-fueled vehicle exhaust and methanol-fueled vehicle exhaust.	Gasoline-fueled vehicle exhaust expressed evident mutagenicity in the Ames assay (TA98 strain) with or without rat liver-derived metabolic activation system (S9). The mutagenicity increased obviously when S9 was used and showed a good dose-response relationship. However, the methanol-fueled vehicle exhaust did not show any mutagenic potential in the Ames assay (TA98, TA100 strains) with or without rat liver-derived metabolic activation system (S9). The rate of A549 cell micronucleus formation could be induced by the gasoline-fueled vehicle exhaust. For the methanol-fueled vehicle exhaust, there were no significant differences in the rate of A549 cell micronucleus formation between the tested groups and control.	The authors concluded that the gasoline-fueled vehicle exhaust had potential mutagenicity and the methanol-fueled vehicle exhaust did not show any potential mutagenicity.
Llano Y, Zhan L, Zhanq Z, Zhanq H, Zeng X, Gou X, Lin C Cai C, Shao X, Shao G, Wu D. 2005. [Genotoxicity comparison between gasoline- and methanol-fueled exhaust by TK gene mutation assay]. Shenq Wu Yi Xue Goon Chenq Xue Za Zhi. Apr;22(2):347-50.	Gasoline and methanol fuel exhaust were sampled and the L5178Y thymidine kinase (TK) gene mutation assay was used to investigate their genotoxicity at the same dose range,	The results showed that the genotoxicity of gasoline-fueled exhaust is stronger than that of methanol-fueled exhaust, while the cytotoxicity of methanol-fueled exhaust is stronger than that of gasoline-fueled exhaust at the tested dose range.	The authors concluded the study demonstrated that the L5178Y TK gene mutation assay was more sensitive than the micronucleus and comet assays.

Table 15 – Methanol

Citation	Study Methods	Results	Authors' Conclusions/Comments
	and compared with the micronucleus and comet assays.		
Zhang ZZ, Zhang Q, Lianq Y, Li CQ, Lai CC, Huang WB, Wu DS. 2005. [Comparative study on the genotoxicity of gasoline-fueled vehicle exhaust and methanol-fueled vehicle exhaust]. [Article in Chinese]. Sichuan Da Xue Xue Bao Yi Xue Ban. Mar;36(2):249-52.	Genotoxicity study: comparison of gasoline exhaust with methanol exhaust.	Gasoline-fueled vehicle exhaust exerted stronger cytotoxicity to A549 cells in both 2-h and 24-h exposure times, compared with the methanol exhaust. In the A549 cell micronucleus test *in vitro*, at doses 0.025, 0.05, 0.1 and 0.2 L/ml, the micronucleated cell (MNC) rates were 2.65%, 4.35%, 4.95% and 5.85% respectively, which were higher than those (1.30% and 1.35%) of controls ($P<0.01$). For the methanol-fueled vehicle exhaust, there was no significant difference in the rate of A549 cell micronuclei between the test groups and control group. In the comet assay, gasoline-fueled vehicle exhaust could induce A549 cell DNA damage. The rate of caudate cell and the length of DNA migration increased with the escalation of dosing level. However, the methanol-fueled vehicle exhaust did not show any DNA damage to A549 cells.	The authors concluded the results demonstrated that gasoline-fueled vehicle exhaust can induce DNA and chromosome damage and it has a distinct genotoxicity, whereas the methanol-fueled vehicle exhaust does not show any potential genotoxicity in both tests.
Ernstgard L, Shibata E, Johanson G. 2005. Uptake and Disposition of Inhaled Methanol Vapor in Humans. Oxford Journals Life Sciences & Medicine Toxicological Sciences, Volume 88, Issue 1, 30-38.	Human (4 male, 4 female) inhalation exposure study.	The relative uptake of methanol was about 50% (range 47-53%). Methanol in blood increased from a background level of about 20 to 116 and 244 pM after 2-h exposure at 0, 100 ppm (131 mg/m^3) and 200 ppm (262 mg/m^3), respectively. Saliva showed substantially higher levels than blood immediately after exposure. This	The authors concluded that the study indicated nonsaturated, dose-proportional kinetics of methanol up to 200 ppm for 2 h. No gender differences were detected. Similar, parallel patterns were seen with regard to the methanol time courses in blood, urine, and saliva, whereas

Table 15 – Methanol

Citation	Study Methods	Results	Authors' Conclusions/Comments
		difference disappeared in a few minutes; thereafter the concentrations and time courses in blood, urine, and saliva were similar, with half times of 1.4, 1.7, and 1.3 h, respectively. The post-exposure decrease of methanol in exhaled air was faster, with a half time of 0.8 h. The methanol concentrations were approximately twice as high in all four types of biological samples at 200 compared to 100 ppm. No increase in urinary formic acid was seen in exposed subjects.	the concentration in exhaled air decreased markedly faster. Thus, apart from blood and urine, saliva also seems suitable for biomonitoring of methanol exposure.
Reed MD, Blair LF, Burling K, Daly I, Gigliotti AP, Gudi R, Mercieca MD, McDonald JD, O'Callaghan JP, Seilkop SK, Ronskoh NL, Wagner VO, Kraska RC. 2006. Health effects of subchronic exposure to diesel-water-methanol emulsion emission. Toxicol Ind Health. Mar;22(2):65-85.	Inhalation exposure in rats to a diesel-water-methanol emulsion emission at 125, 250 and 500 microgram total PM (particulate matter) /m^3 for 6 h/day, five days/week for the first 11 weeks and seven days/week thereafter.	Overall, effects observed were mild. Emulsion combustion emissions were not associated with neurotoxicity, reproductive/developmental toxicity, or *in vivo* genotoxicity. Small decreases in serum cholesterol in the 500-microgram/m^3 exposure group were observed. Positive mutagenic responses in several strains of *Salmonella typhimurium* were observed subsequent to treatment with emulsion emissions subfractions.	The authors concluded that, based on cholesterol results, the 250-microgram/m^3 exposure level was the no observed effect level. In general, biological findings in exposed rats and bacteria were consistent with exposure to petroleum diesel exhaust in the F344 rat and Ames assays.
Givens ML, Kalbfleisch K, Bryson S. 2006. 266: Inhalation of Methanol-Containing Products: A Significant Source of Possible Toxicity. Annals of Emergency Medicine, Volume 48, Issue 4, Supplement 1, October 80-81.			No abstract provided. Likely a review of products containing other chemicals along with methanol.
Bebarta VS, Heard K, Dart RC.	Observational study	Four patients had a methanol level greater	The authors concluded that these

Table 15 – Methanol

Citation	Study Methods	Results	Authors' Conclusions/Comments
2006. Inhalational abuse of methanol products: elevated methanol and formate levels without vision loss. American Journal of Emergency Medicine, Volume 24 Issue 6, 725-728.	of 7 human subjects who abused a methanol-containing hydrocarbon. products.	than 24 mg/dL and 2 had an anion gap greater than 17 mEq/L. The mean formic acid level was 71 µg/mL, and 1 patient had a level considered high enough to induce retinal toxicity (>200µg/mL). No patient had an abnormal ophthalmologic examination. All patients were treated with intravenous folate, Two received alcohol dehydrogenase blockade, and no patient received hemodialysis or intravenous bicarbonate. All patients' acidosis resolved within 4 hours. The methanol and formic acid levels were lower than those reported after methanol ingestion.	preliminary data suggested inhalant abusers of methanol products may have significantly elevated methanol and formic acid levels, but are at low risk for methanol-induced complications of visual dysfunction and refractory acidosis.
Zhang Z, Che W, Liana Y, Wu M, Li N, Shu Y, Liu F, Wu D. 2007. Comparison of cytotoxicity and genotoxicity induced by the extracts of methanol and gasoline engine exhausts. Toxicol In Vitro. Sep;21(6):1058-65.	The genotoxicity and cytotoxicity of organic extracts of condensate, particulate matters (PM) and semivolatile organic compounds (SVOC) of gasoline and absolute methanol engine exhaust were examined by using the MTT assay, micronucleus assay, comet assay and Ames test.	Gasoline engine exhaust exhibited stronger cytotoxicity to human lung carcinoma cell lines (A549 cell) than methanol engine exhaust. Furthermore, gasoline engine exhaust increased micronucleus formation, induced DNA damage in A549 cells and increased TA98 revertants in the presence of metabolic activating enzymes in a concentration-dependent manner. In contrast, methanol engine exhaust failed to exhibit these adverse effects.	The authors concluded that methanol may be used as a ~~c~~leaner" (more suitable?) fuel for automobile engines.
Sefidbakht S, Rasekhi AR, Karnali K, Haghighi AB, Salooti A,	Report of 9 cases of methanol poisoning.	Bilateral hemorrhagic or nonhemorrhagic necrosis of the putamina, diffuse white	The authors concluded that a good knowledge of the radiological findings

Table 15 – Methanol

Citation	Study Methods	Results	Authors' Conclusions/Comments
Meshksar A, Abbasi HR, Moghadami M, Nabavizadeh SA. 2007. Methanol poisoning: acute MR and CT findings in nine patients. Neuroradiology, Volume 49, Number 5, 427-435.		matter necrosis, and subarachnoid hemorrhage were among the radiological findings. Various patterns of enhancement of basal ganglia lesions were found including no enhancement, strong enhancement, and rim enhancement.	in methanol poisoning is necessary for radiologists. The present enabled the authors to include in a single report most of the radiological findings that have been reported previously.
Reddy NJ, Lewis LD Gardner TB, Osterlinq W, Eskey CJ Nierenberq DW. 2007. Two cases of rapid onset Parkinson's syndrome following toxic ingestion of ethylene glycol and methanol. Clin Pharmacol Ther. Jan;81(1):114-21.	Report of 2 cases of rapid onset Parkinson's syndrome following ingestion of either ethylene glycol or methanol.	Both patients survived their toxic ingestions, but then developed acute Parkinson's syndrome within 10 days of the ingestion. However, the patient who ingested methanol developed respiratory muscle stiffness/weakness, which responded poorly to anti-Parkinsonian drug therapy. Treatment with carbidopa/levodopa improved cogwheel rigidity and bradykinesia in both patients.	The authors conclude that acute Parkinsonism is one of the lesser-recognized complications of both ethylene glycol and methanol poisoning.
Wallage HR and Watterson JH. 2008. Formic acid and methanol concentrations in death investigations. J Anal Toxicol Apr ,32 (3).241-7.	Examination of 12 deaths from methanol poisoning.	Six individuals were found deceased, and their postmortem methanol and formic acid concentrations ranged from 84 to 543 mg/dL and 64 to 110 mg/dL, respectively. In the other six individuals, hospital treatment such as bicarbonate, ethanol infusion, and hemodialysis were administered. Antemortem methanol and formic acid concentrations ranged from 68 to 427 mg/dL and 37 to 91 mg/dL, respectively, whereas corresponding postmortem methanol and formic acid	The authors concluded that hospital treatment of formic acid toxicity resulted in significantly reduced postmortem methanol and formic acid concentrations.

Table 15 – Methanol

Citation	Study Methods	Results	Authors' Conclusions/Comments
		levels ranged from undetectable to 49 mg/dL and undetectable to 48 mg/dL, respectively.	
Kleiman R, Nickle R, Schwartz M. 2009. Medical toxicology and public health—Update on research and activities at the centers for disease control and prevention, and the agency for toxic substances and disease registry. Journal of Medical Toxicology, Volume 5, Number 3, 158-164.	Discussion of methanol toxicity, exposure and treatment.	People with potential exposure to methanol in any form need to wear the appropriate personal protective equipment, and they need to be familiar with proper detection methods. Medical professionals need to understand the metabolism of methanol and why knowledge of this metabolism is important for the diagnosis and management of methanol toxicity. Several methods of treatment exist for methanol toxicity.	
Wallace EA and Green AS. 2009. Methanol toxicity secondary to inhalant abuse in adult men. Olin Toxicol (Phila). Mar;47(3):239-42.	Retrospective chart review of adults with positive serum volatile screen for methanol and history of carburetor cleaning fluid fume inhalation.	Sixty-five cases presented with nausea/vomiting; 27 with intoxication or altered mental status; 21 with specific visual complaints. All visual symptoms resolved before discharge and all patients survived without sequelae.	The authors concluded that intentional inhalation of methanol fumes may produce toxicity.
Cruzan G. 2009. Assessment of the cancer potential of methanol. Crit Rev Toxicol. 39(4):347-63.	Review of the carcinogenic potential of methanol.	Genotoxicity studies do not suggest carcinogenic activity from methanol exposure. No increased neoplasms were found in the NEDO rat and mouse inhalation studies, even at air levels (up to 1,000 ppm for >19 hours/day, 7 days/week) that caused 10-fold increased blood methanol levels. The maximum dose level was 600 mg/kg/day.	The authors concluded that the data from genotoxicity studies, the inhalation and drinking water oncogenicity studies of methanol in rats and mice, and mode of action considerations support a conclusion that methanol is not likely to be carcinogenic in humans.

Table 15 – Methanol

Citation	Study Methods	Results	Authors' Conclusions/Comments
McCallum GP, Siu M, Sweetinci JN, Wells PG. 2011. Methanol exposure does not produce oxidatively damaged DNA in lung, liver or kidney of adult mice, rabbits or primates. Toxicol Appl Pharmacol. Jan 15;250(2):147-53.	Mice, rabbits, and monkeys treated with MeOH (2.0 g/kg ip) and 6h later assessed for oxidative damage to DNA, measured as 8-oxo-2'-deoxyguanosine (8-oxodG) by HPLC with electrochemical detection.	The authors reported no MeOH-dependent increases in 8-oxodG in lung, liver or kidney of any species. Chronic treatment of CD-1 mice with MeOH (2.0 g/kg ip) daily for 15 days also did not increase 8-oxodG levels in these organs. These results were corroborated in DNA repair-deficient oxoguanine glycosylase 1 (Ogg1) knockout (KO) mice, which accumulated 8-oxodG in lung, kidney and liver with age, but exhibited no increase following MeOH, despite a 2-fold increase in renal 8-oxodG in Ogg1 KO mice following treatment with a ROS-initiating positive control (i.e., the renal carcinogen potassium bromated – $KBrO_3$ – at 100 mg/kg ip).	The authors conclude that These observations suggest that MeOH exposure does not promote the accumulation of oxidatively damaged DNA in lung, kidney or liver, and that environmental exposure to MeOH is unlikely to initiate carcinogenesis in these organs by DNA oxidation.
McCallum GP Siu M, Ondovcik SL, Sweeting JN, Wells PG. 2011. Methanol exposure does not lead to accumulation of oxidative DNA damage in bone marrow and spleen of mice, rabbits or primates. Mol Carcinog. Mar;50(3):163-72.	Mice, rabbits, and monkeys treated with MeOH (2.0 g/kg ip) and assessed for tissue oxidative DNA damage 6 h post-dose, measured as 8-hydroxy-2'-deoxyguanosine (8-oxodG).	The authors reported no MeOH-dependent increases in 8-oxodG in bone marrow or spleen of any species. Chronic treatment of CD-1 mice with MeOH (2.0 g/kg ip) daily for 15 d also did not increase 8-oxodG levels in these organs. Further studies in the DNA repair deficient oxoguanine glycosylase 1 (Oggl) knockout (KO) mice supported these findings. Fibroblasts from Ogg1 KO mice accumulated 8oxodG following acute exposure to the renal carcinogen potassium bromate (KBrO(3) at 2.0 mM) but did not accumulate 8oxodG following exposure to 125 mM MeOH 6 h post-treatment. Oggl KO mice accumulated 8-oxodG in bone marrow and spleen with age but not following exposure	The authors concluded that Me0H exposure dis not promote the accumulation of oxidative DNA damage in bone marrow and spleen, and it is unlikely that human environmental exposure to MeOH would lead to lymphomas via this mechanism.

Table 15 – Methanol

Citation	Study Methods	Results	Authors' Conclusions/Comments
		to MeOH. In addition, free radical-mediated hydroxynonenal-histidine protein adducts were not enhanced by MeOH in primate bone marrow or spleen, or in rabbit bone marrow or mouse spleen, although modest increases were observed in rabbit spleen and mouse bone marrow.	
Macallister SL, Choi J, Dedina L, O'Brien PJ. 2011. Metabolic mechanisms of methanol/formaldehyde in isolated rat hepatocytes: Carbonyl-metabolizing enzymes versus oxidative stress. Chem Biol Interact. May 30;191(1-3):308-14. Epub Jan 26.	Study in rats – Accelerated Cytotoxicity Mechanism Screening (ALMS) technique to determine molecular basis for hepatotoxicity.	Methanol had little toxicity towards rat hepatocytes in 95% oxygen, even at 2M concentration, whereas 50 mM was the estimated LC50 when treatment was for 2 hr in 1% oxygen (this oxygen concentration was the estimated physiological concentration in the centrilobular region of the liver). Cytotoxicity was attributed to increased NADH levels caused by methanol metabolism, catalyzed by ADH1, resulting in reductive stress, which reduced and released ferrous iron from Ferritin causing oxygen activation. Hepatocyte protein carbonylation induced by formaldehyde (HCHO) formation was also increased about 4-fold when methanol was oxidized by the Fenton-like system (Fe(II)/H2O) and correlated with increased cytotoxicity.	The authors concluded that methanol at 1% oxygen involved activation of the Fenton system to form HCHO (formic acid). However, at higher oxygen levels, radicals generated through Fe(II)/H2O2 can oxidize methanol/HCHO to form pro-oxidant radicals and lead to increased oxidative stress through protein carbonylation and ROS (reactive oxygen species) formation which ultimately causes cell death.

Table 15 – Methanol

Welfare Effects Studies

Citation	Study methods	Results	Authors' conclusions/comments
Poirier SH, Knuth ML, Anderson-Buchou CD, Brooke LT, Lima AR, Shubat PJ. 1986. Comparative Toxicity of Methanol and N,N-Dimethylformamide to Freshwater Fish and Invertebrates. Bulletin of Environmental Contamination and Toxicology, Volume 37, Number 1, 615-621.	Ecotoxicity study of freshwater fish and invertebrates.	Median lethal LC50 concentrations reported in the study were as follows: In Rainbow trout: 24-h: 20,300 mg/L; 48-h: 20,100 mg/L; 96-h: 20,100 mg/L. In Fathead minnow: 24-h: 29,700 mg/L; 48-h: 29,700 mg/L; 96-h: 29,400 mg/L In Bluegill: 24-h: 19,100 mg/L; 48-h: 19,100 mg/L; 96-h: 15,400 mg/L Median effect (EC50) concentrations reported in the study were as follows: In Rainbow trout: 24-h: 13,200 mg/L; 48-h: 13,200 mg/L; 96-h: 13,000 mg/L In Fathead minnow: 24-h: 29,700 mg/L; 48-h: 29,700 mg/L; 96-h: 28,900 mg/L In Bluegill: 24-h: 16,100 mg/L; 48-h: 16,000 mg/L; 96-h: 12,700 mg/L	These value all indicate very low ecotoxicity.
Helmstetter A, Gamerdinger AP, Pruett RJ. 1996. Acute Toxicity of	Ecotoxicity study of blue mussels using	Approximate LC50 of 2% methanol was	This indicates very low excotoxicity.

Table 15 – Methanol

Citation	Study methods	Results	Authors' conclusions/comments
Methanol to Mytilus edulis. Bulletin of Environmental Contamination and Toxicology, Volume 57, Number 4, 675-681.	0%, 1%,2%,3%,5% and 10% methanol/seawater (v/v) solutions.	reported to be 15,900 mg/L.	
Liu XH, Pan H, Mazur P. 2003. Permeation and toxicity of ethylene glycol and methanol in larvae of Anopheles gambiae. J Exp Biol. Jul;206(Pt 13):2221-8.	The study applied proton NMR to measure the permeation of two cryoprotective agents (CPAs), ethylene glycol (EG) and methanol, into 1st instar *Anopheles* (mosquito) larvae.	After just 1-hr exposure to 1.5 mol/L methanol, the larval methanol concentration reached its maximum which was only 75% of the theoretical maximum. Toxicity data showed that larval survival remained at 91% and 95% after 4-hr and 1-hr exposure to 1.5 mol/L EG and 1.5 mol/L methanol, respectively, at which time the larval concentration of EG and methanol had risen to 1.21 mol/L and 1.13 mol/L, respectively.	The authors concluded that CPAs such as EG and methanol permeated *Anopheles* larvae to up to 81% and 75% of equilibrium, respectively, before the exposure becomes toxic.
Kavirai A, Bhunia F, Saha NC. 2004. Toxicity of methanol to fish, crustacean, oligochaete worm, and aquatic ecosystem. Int J Toxicol. Jan-Feb;23(1):55-63.	Ecotoxicity study in fish and aquatic invertebrates.	Ninety-six-hour acute toxicity tests revealed the cladoceran crustacea *Moina micrura* as the most sensitive to methanol (LC50 = 4.82 g/L), followed by the freshwater teleost *Oreochromis mossambicus* (LC50 = 15.32 g/L) and the oligochaete worm *Branchiura sowerbyi* (LC50 = 54.89 g/L). The fish, when exposed to lethal concentrations of methanol, showed difficulties in respiration and swimming. The oligochaete body wrinkled and fragmented under lethal exposure of methanol, and a significant reduction in the appetite of fish when exposed to 736.10 mg/L or higher concentrations of methanol was observed. Chronic toxicity bioassays (90 days) in	The authors concluded that 23.75 mg/L was the no-observed-effect concentration (NOEC) of methanol to freshwater aquatic ecosystem.

Table 15 – Methanol

Citation	Study methods	Results	Authors' conclusions/comments
		outdoor enclosures showed a reduction in growth, maturity index and fecundity of fish at 47.49 mg/L or higher concentrations of methanol. Primary productivity, phytoplankton population, and alkalinity of water were also reduced at these concentrations. Chronic exposure to 1,527.60 mg/L methanol resulted in damages of the epithelium of primary and secondary gill lamellae of the fish.	
Baltz DM, Chesney EJ, Tarr MA, Kolok AS, Bradley MJ. 2005. Toxicity and sublethal effects of methanol on swimming performance of juvenile Florida pompano. Transactions of the American Fisheries Society, Vol. 134/3, pp. 730-740.	Ecotoxicity study in fish (Florida pompano *Trachinotus carolinus*)	A 24-h static exposure test identified the median lethal concentration as 1.28% (volume per volume) at 30 practical salinity units and 25°C. The mean critical swimming speed (U_{crit}) of juveniles was evaluated before and after exposure to a 1.07% concentration and showed that U_{crit} was significantly reduced ($P < 0.0002$) in post-exposure trials. After exposure and a 17-h recovery period in clean seawater, the mean 6.5% decline in performance of the treatment group contrasted sharply with a mean increase of 4.0% in the control group.	The authors concluded that conditioning, training effects, or both were significantly surpassed by the negative sublethal effects of methanol exposure.

Table 16 - Styrene

Health Effects Studies

Citation	Study methods	Results	Authors' conclusions/comments
Watanabe T, Endo A, Sato K, Ohtsuki T Mivasaka M, Koizumi A, Ikeda M. 1981. Mutagenic Potential of Styrene in Man. Ind Health;19(1):37-45.	Cytogenic changes and cell-cycle kinetics; cultured lymphocytes of 16 occupationally-exposed workers; exposed at 2-211 ppm.	The styrene-exposed workers showed no increase in chromosomal aberrations or sister-chromatid exchange frequencies, while a slight decrease in the mitotic index and a slight inhibition of cell growth were detected.	No correlation was evident between the cytogenetic findings and styrene exposure intensity as examined by the repeated air analysis, the personal sampling method, or the urinalysis for metabolites.
Conner, MK, Alarie, Y & Dombroske RL 1981. Styrene causes SCE in mice. Food and Cosmetics Toxicology Volume 19, Page 516	Mice exposed to104, 387, 591 or 922 ppm for 6 hr daily on 4 consecutive days.	Dose-dependent increases in SCE frequencies were observed at concentrations of 387 ppm and above in all three types of cells in hepatectomized mice and in both the alveolar macrophages and bonemarrow cells in non-hepatectomized mice. In addition, at the highest concentration, increases in frequency were also found to be dependent on length of exposure. Since there was no significant difference between the SCE frequencies in bone-marrow cells or alveolar macrophages of hepatectomized mice and the frequencies in non-hepatectomized animals exposed to similar concentrations, the observed effects were not thought to be due to the biotransformation of styrene to styrene oxide. Hepatectomized mice were, however, more sensitive than non-hepatectomized mice to the cellular toxicity of styrene. This was indicated by a decrease in the proportion of second division relative to first division metaphases which was observed in all three cell types of hepatectomized mice	This suggests a loss in the styrene detoxification mechanism in the hepatectomized mice.

Table 16 - Styrene

Citation	Study methods	Results	Authors' conclusions/comments
		exposed for 4 days to 922 ppm and in the regenerating liver cells after 4 days of exposure of 591 ppm.	
Norpoa H, Vainio H. 1983. Genetic toxicity of styrene and some of its derivatives. Scand J Work Environ Health. Apr;9(2 Spec No):108-14.	Review.	Styrene, an important plastic monomer, was mutagenic after metabolic activation in several test systems. Probably because of an unfavorable activation: inactivation ratio, some mutagenicity assays have not, however, found styrene mutagenic. Styrene is converted by microsomal monooxygenases *in vivo* to styrene-7,8-oxide, which is a well-known mutagen. Arene oxides have also been proposed as the reactive metabolites of styrene, but the significance of these compounds is not yet fully understood. Only few derivatives of styrene have been tested for mutagenicity. The results are characterized by difficulties in metabolic activation. Many styrene-7,8-oxide analogues substituted at the phenyl ring are electrophilic reactants and mutagenic *in vitro*. Human whole-blood lymphocyte cultures have a peculiar feature, i.e, styrene and many of its analogues substituted at the ring or vinyl chain induce sister chromatid exchanges in the cultured cells without exogenous metabolizing systems. T his activation is brought about by erythrocytes present in the cultures and probably results from the conversion of styrenes to styrene-7,8-oxides.	
Andersen ME and Ramsey JC 1983. A physiologically-based	A PBPK model consisting of a series of	The model has 4 tissue groups: highly perfused organs, excluding liver; muscle	

Table 16 - Styrene

Citation	Study methods	Results	Authors' conclusions/comments
description of the inhalation pharmacokinetics of styrene monomer in rats and humans. Toxicology Letters, Volume 18, Supplement 1, 28 August-3 September 1983, Page 140	mass-balance differential equations to describe the time course of styrene in blood and fat. Rats were treated with 80, 200, 600 or 1,200 ppm for 6 hours and then were killed various times during exposure and during the 18-hr period after exposure.	and skin; fat and marrow; and organs with high capacity to metabolize styrene. Biochemical constants for styrene metabolism (V = 3.6 mg/hr; K = 0.3 mg/L) were estimated from pseudo-steady state exposure data The most important constants were V and the blood flow, volume, and partition coefficient in the fat. The successful rat model was scaled to predict styrene kinetics in humans. Predicted behavior agreed well with published data from two different human inhalation experiments. Intravenous and oral kinetics of styrene in rats were simulated by adding appropriate differential equations to the general P-B inhalation model and compared to published data. In these simulations, oral uptake from a water vehicle was adequately represented by simple first-order input; in contrast, uptake from a corn oil vehicle was more complex with apparent contributions from zero-order input. When rats were exposed to styrene at 600 or 1,200 ppm for 24 hr. there was induction of styrene metabolism during exposure. With the 600 ppm exposure, a computerized fitting procedure indicated a 3-4-fold induction beginning after 10.6 hr and increasing with a rate constant of 0.32 hr.	
Ramsey JC and Andersen ME 1984. A physiologically based description of the inhalation pharmacokinetics of styrene in	Physiologically-based pharmacokinetic modeling study: a series of mass-balance	The simulated behavior of styrene is particularly sensitive to changes in the constants describing the fat tissue group, and to the maximum metabolic rate	These results showed that physiologically-based pharmacokinetic models provide a rational basis with which (1) to

Table 16 - Styrene

Citation	Study methods	Results	Authors' conclusions/comments
rats and humans. Toxicol Appl Pharmacol. 1984 Mar 30;73(1)159-75.	differential equations which quantify the time course of styrene concentration within four tissue groups: highly perfused organs; moderately perfused tissues such as muscle; slowly perfused fat tissue; and organs with high capacity to metabolize styrene (principally liver). Rats were exposed via inhalation to 80, 200, 600, or 1,200 ppm.	described by Vmax. The constants used to simulate the fate of styrene in rats were scaled up to represent humans. At < 200 ppm, the ratio of styrene concentration in blood to inhaled air is controlled by perfusion-limited metabolism. At 200 ppm, this ratio is controlled by the blood:air partition coefficient and is not linearly related to the ratio attained at lower (nonsaturating) exposure concentrations.	explain the relationship between blood concentration and air concentration of an inhaled chemical, and (2) to extrapolate this relationship from experimental animals to humans. Model simulations showed that styrene metabolism was saturated at inhaled concentrations above approximately 200 ppm in mice, rats, and humans.
Andersen ME, Gams ML, Ramsey JC 1984. Inhalation pharmacokinetics: evaluating systemic extraction, total in vivo metabolism, and the time course of enzyme induction for inhaled styrene in rats based on arterial blood:inhaled air concentration ratios. Toxicol Appl Pharmacol. Mar 30;73(1):176-87	Naïve rats exposed via inhalation to styrene pre-treatment for 6 hrs (dose not specified). Rats also pretreated with pyrazole (320 mg/kg 1/2 hr before styrene exposure) and phenobarbital (80 mg/kg/day for 4 d before styrene exposure) to estimate the dynamics of the induction process. Styrene treatment with 400, 600, or 1,200 ppm for 24 hrs following pretreatments.	Using extraction ratios and several physiological constants, metabolic constants were evaluated for styrene metabolism *in vivo*. In naive rats, the maximum velocity of metabolism was 10.0 mg/kg/hr, and Km was of the order of 0.2 mg/liter. Pretreatment with pyrazole essentially abolished *in vivo* styrene metabolism. Pretreatment with phenobarbital increased Vmax about six fold. Prior exposure to styrene (1,000 ppm for 6 hr/day on each of 4 days before experimentation) increased Vmax by a factor of 2. At 400 ppm, induction began after a lag of 15.5 hr, had a half-life of 3.5 hr, and reached 2.7 times the Vmax in naive rats. At 600 ppm treatment, induction began after 10.6 hr, proceeded with a half-life of 2.2 hr, and increased	Induction complicates kinetic modeling of continuous inhalation with soluble, well-metabolized vapors because it is time- and concentration- dependent. These methods should prove useful for studying the *in vivo* metabolism of other soluble, well-metabolized vapors and for examining the time course of induction of the metabolizing enzymes for these chemicals.

Table 16 - Styrene

Citation	Study methods	Results	Authors' conclusions/comments
		Vmax by 3.4 times. At 1,200 ppm: induction began earlier, 4.6 hr, and reached a greater value, 4.4 times Vmax, but had a half-life similar to that at 600 ppm. No induction occurred in 48-hr exposure to 200 ppm.	
Wieczorek H, Piotrowski JK 1985. Evaluation of low exposure to styrene. I. Absorption of styrene vapours by inhalation under experimental conditions. Int Arch Occup Environ Health;57(1):57-69.	Styrene exposure via inhalation to 20 to 200 mg/m^3 in human volunteers (6 men and 1 woman.	Average retention of styrene vapors in the respiratory tract was 71%. The yield of styrene metabolism measured within 24 h was 39 and 17% for mandelic acid and phenylglyoxylic acid, respectively. The determination of mandelic acid in urine collected immediately after the exposure was applied as an exposure test. The excretion rate of this metabolite assured the best correlation with the absorbed dose. The relative standard deviations of the test related to actual dose level varied, depending on the analyzed concentration range, from 0.21 to 0.33.	Quantitative interpretation of the test is possible for styrene concentrations in the air exceeding 20 mg/m^3. The concentration amounting to 100 mg/m^3 (TLV) corresponds with the mandelic acid excretion rate of 15 mg per hour.
Wieczorek H 1985. Evaluation of low exposure to styrene. II. Dermal absorption of styrene vapours in humans under experimental conditions. Int Arch Occurs Environ Health; 57(1):71-5.	Styrene exposure in 4 human volunteers via the dermal route – 1,300 to 3,200 mg/m^3	The increase in the levels of mandelic and phenylglyoxylic acids in urine after exposure was strongly noticeable. The dermal vapor absorption coefficient (alpha) was calculated; for styrene it was ca. 0.022 m^3/h. It was calculated that the dermal absorption of the styrene vapors contributed about 5% to the amount absorbed in the respiratory tract under the same conditions.	
Głośnicka R, Dziadziuszko H 1986. Mutagenic action of styrene	Styrene (100, 200, 250, 300, 400, and 500	Styrene oxide had an analogical effect on bacteria except that it was much better	Results showed mild genotoxic activity of styrene.

Table 16 - Styrene

Citation	Study methods	Results	Authors' conclusions/comments
and its metabolites. II. Genotoxic activity of styrene, styrene oxide, styrene glycol and benzoic acid tested with the SOS chromotest. Bull Inst Marit Trop Med Gdynia;37(3-4):295-302.	µg/ml) and styrene oxide (100, 200, 250, 300, 800, 1,000, 1,400, and 1,600 µg/ml) mutagenicity studies in *Salmonella agona* and *E.coli*.	tolerated by them. This compound, when in 800 and 1,600 µg/ml concentrations, inhibited the growth of bacteria at an about 2 x 10^8 level for six hours, whereupon after 24 hours their number increased up to about 10^9 bacteria per 1 ml. The lethal dose of styrene oxide for the strain of *S.agona* was 3,200 µg/ml. There were also parallel microscopic observations of *S.agona* bacteria. Preparations were stained with carbol fuchsine and examined microscopically. After five hours cultivation in the presence of styrene at 400 µg/ml concentration, minute spherical mini-cells were observed. In the preparations made of five-hour bacteria cultures in the presence of styrene oxide (400 µg/ml), the bacteria took the form of long filaments, with no apparent intercellular septa, which suggested certain analogies with the response to DNA-injuring factors, observed with the strains of *E.coli*. Styrene had a slight effect on induction of *E.coli* SOS system. The highest non-toxic dose (100 µg/ml) resulted in releasing negligible amounts of β-galactosidase. The induction factor for this concentration was 1.16. With 200 µg/ml concentration, there was an evidence of alkaline phosphatase production inhibition and a reduction to a half, compared with the control group in relation to β-galactosidase secretion.	
Paterson S, Mackay D 1986. A pharmacokinetic model of styrene		The physiologically-based pharmacokinetic model of J. C. Ramsey	It is suggested that pharmacokinetic fugacity models can complement

Table 16 - Styrene

Citation	Study methods	Results	Authors' conclusions/comments
inhalation with the fugacity approach. Toxicol Appl Pharmacol. Mar 15;82(3):444-53		and M. E. Andersen (1984, Toxicol. Appl. Pharmacol. 73, 159-175) of styrene inhalation in rats, with extrapolation to humans, was reformulated with the chemical equilibrium criterion of fugacity instead of concentration to describe compartment partitioning. Fugacity models have been used successfully to describe environmental partitioning processes which are similar in principle to pharmacokinetic processes. The fugacity and concentration models are mathematically equivalent and produce identical results. The use of fugacity provides direct insights into the relative chemical equilibrium partitioning status of compartments, thus facilitating interpretation of experimental and model data. It can help to elucidate dominant processes of transfer, reaction and accumulation, and the direction of diffusion. Certain model simplifications become apparent in which compartments which remain close to equilibrium may be grouped. Maximum steady-state tissue concentrations for a known exposure may be calculated readily.	conventional concentration models and may facilitate linkage to fugacity models describing environmental sources, pathways, and exposure routes.
Sikov MR, Cannon WC, Carr DB, Miller RA, Niemeier RW, Hardin BD 1986. Reproductive toxicology of inhaled styrene oxide in rats and rabbits. J Appl Toxicol. Jun;6(3):155-64	Styrene oxide treatment in female rats via inhalation – 0, 100 or 300 ppm for 7 hrs, 5d/week for 3 weeks. Also, inhalation treatment in female rabbits – 0, 15 or 50	In rats at 100 ppm, extensive mortality occurred. At 300 ppm, rapidly lethal occurred. Exposures were terminated in this latter group and the group was eliminated from further study. In rabbits, up to 50 ppm produced mortality. Pregnant animals were examined for toxic changes including altered tissue weights	These results indicated that inhalation exposures at these concentrations produced reproductive and development toxicity, as well as maternal toxicity.

Table 16 - Styrene

Citation	Study methods	Results	Authors' conclusions/comments
	ppm for 7 hrs through 24 days of gestation (dg). Rats (0, 100 ppm groups) were then mated and exposed to 0 or 100 ppm styrene oxide daily through 18 dg. The rats were killed at 20 dg and the rabbits at 30 dg.	and histopathologic effects. Litters were evaluated using several measures of embryotoxicity, and live fetuses were examined for external, visceral, and skeletal malformations. Exposure during gestation appeared to increase preimplantation loss in rats, and tended to increase the incidence of resorptions in rabbits. In both species, fetal weights and crown-rump lengths were reduced by gestational exposure. The incidences of ossification defects of the sternebrae and occipital bones were increased by gestational exposure of rats to styrene oxide.	
Malek RF, Daisey JM, Cohen BS 1986. The effect of aerosol on estimates of inhalation exposure to airborne styrene.	Four spraying experiments of polyester resin were performed during which 64 samples were collected and analyzed for styrene air concentrations.	The four spraying experiments showed that aerosols represented 30% (+/- 3%) of the total air concentration of styrene.	The contribution of aerosols to inhalation exposure needs to be considered in other industrial situations where spray processes are used.
Murphy JH 1987. The effect of aerosol on estimates of inhalation exposure to airborne styrene. Am Ind Hyg Assoc J. Jan;48 (1):A14, A16.			Commentary; not an actual study.
Pryor GT, Rebell CS, Howd RA. 1987. Hearing loss in rats caused by inhalation of mixed xylenes and styrene. J Appl Toxicol.	Xylene and styrene exposure in male weanling Fischer rats – 800, 1,000, and 1,200	Both xylenes and styrene caused marked hearing loss as assessed by behavioral (conditioned avoidance) and electrophysiologic (brainstem auditory-	Both styrene and xylene demonstrated ototoxic potential.

Table 16 - Styrene

Citation	Study methods	Results	Authors' conclusions/comments
Feb;7(1):55-61	ppm daily for 14 hrs/day for 6 weeks for mixed xylenes and 3 weeks for styrene. Also exposure to xylenes for 3 days for 4 hrs to 1,700 ppm or for 8 hrs to 1,450 ppm to compare the results with those obtained with toluene under comparable exposure schedules.	evoked response) methods. Both solvents appeared to be more potent ototoxicants than toluene, as indicated by effective concentration, effective durations of exposure, and the range of frequencies affected.	
Conti B, Maltoni C, Perino G, Ciliberti A 1988. Long-term carcinogenicity bioassays on styrene administered by inhalation, ingestion and injection and styrene oxide administered by ingestion in Sprague-Dawley rats, and para-methyistyrene administered by ingestion in Sprague-Dawley rats and Swiss mice. Ann N Y Aced Sci. ;534:203-34.	Rats exposed to styrene via inhalation at 0, 10, 25, 100, and 300 ppm, 4 hours daily, 5 days weekly, for 52 weeks. Also exposed via gavage to 0, 50, and 250 ppm in olive oil, once daily, 4-5 days weekly, for 52 weeks. Also exposed via intraperitoneal injection to 0 or 50 mg in olive oil 4 times at 2-month intervals. Also exposed once via subcutaneous injection to 0 and 50 mg in olive oil. Also, styrene oxide was administered to Sprague-Dawley rats by gavage as styrene at 0, 50, and 250 mg/kg b.w.	The study was terminated when the survival rate reached 50% in at least one experimental group. Styrene, when given by inhalation, was found to cause an increase in total (benign and malignant) and malignant mammary tumors. Styrene oxide produced a high incidence of tumors in the forestomach (papillomas, acanthomas, and in situ and invasive squamous cell carcinomas). Para-methylstyrene was not shown to be carcinogenic.	Styrene and styrene oxide were found to be carcinogenic.

Table 16 - Styrene

Citation	Study methods	Results	Authors' conclusions/comments
	in olive oil, once daily, 4-5 d/wk, for 52 wks. Also, para-methylstyrene exposure in Sprague-Dawley rats via gavage to 0, 10, 50, 250, and 5,000 mg/kg b.w. Also exposure in Swiss mice to 0, 10, 50, 250 mg/kg b.w., in olive oil, once daily, 5 d weekly, for 108 wks and 78 wks.		
R.K.S. Dogra, S. Khanna, S.N. Srivastava, L.J. Shukla and R. Shanker 1989. Styrene-induced immunomodulation in mice. International Journal of Immunopharmacology, Volume 11, Issue 5, Pages 577-586	Styrene exposure in male mice via the oral route to 0.02, 0.03, 0.05 of the LD50/animal/day (LD50 = 1 g/kg) daily for 5 days.	No overt toxicity in lymphoid organs or on hematologic parameters was observed. At the tested dose levels styrene produced a mild reduction in the organ weight of adrenal and spleen and slight reduction in the cellular viability of lymph nodes. There was a dose-dependent suppression in the humoral immune response (IgM-producing PFC5 of spleen and serum anti-SRBC HA titre) to SRBC. The proliferative response to the B-cell mitogen, LPS, however, revealed a significant increase in the incorporation of 3HT with the middle and lowest doses of styrene. The results of cell- mediated immunity appeared somewhat unexpected and more complex as exposure resulted in a dose-dependent enhancement in the cutaneous DTH reaction to SRBC together with increased blastogenic response of splenic lymphocytes to phytohaemagglutinin (PHA). Additionally, there was significant	The study identified the immunotoxic potential of styrene which acts differently on various arms of the rodent's immune system

Table 16 - Styrene

Citation	Study methods	Results	Authors' conclusions/comments
		impairment in the functional activity (NBT reduction, attachment and phagocytic indices) of nonadherent and adherent peritoneal exudate cells.	
Truchon G, Gerin M Brodeur J 1990. Urinary excretion of mandelic, phenylglyoxylic, and specific mercapturic acids in rats exposed repeatedly by inhalation to various concentrations of styrene vapors. Can J Physiol Pharmacol. May;68(5):556-61.	Styrene vapor exposure in Sprague-dawley rats via inhalation to 25, 50, 100, or 200 ppm, 6 h/day, 5 days/week, for 4 consecutive weeks. Concentrations were varied from day to day according to a random pattern allowing treated animals to be exposed five times to each concentration of styrene. Urinary metabolites were analyzed from samples collected during exposure (0-6 h) and after exposure (6-24 h) – mandelic acid; phenylglyoxylic acid; and two mercapturic acids, N-acetyl-S-(1-phenyl-2-hydroxyethyl)-L-cysteine (M1) and N-acetyl-S(2-phenyl-2-hydroxyethyl)-L-cysteine (M2). Various parameters of renal toxicity and hepatic microsomal and	The results showed that there was a very good relationship between the excretion of all four styrene metabolites and the degree of daily exposure to styrene over the entire period of urine collection, with correlation coefficients ranging from 0.82 to 0.98. The correlation was poor for mandelic acid during the 0-6 h period. There was no evidence that repeated exposure to styrene caused renal toxicity, nor induced hepatic microsomal enzyme activities, but cytosolic glutathione S-transferase activity was increased moderately by 1.5 times. Thus, under conditions of exposure to styrene likely to be found in the workplace, all four metabolites measured were good indicators of styrene exposure throughout the length of the experiment.	The data suggested that measurement of these metabolites offers the possibility to monitor internal exposure to a toxic electrophilic compound more directly.

Table 16 - Styrene

Citation	Study methods	Results	Authors' conclusions/comments
	cytosolic enzyme activities were also measured.		
Elovaara E Vainio H, Aitio A. 1990. Pulmonary toxicity of inhaled styrene in acetone-, phenobarbital- and 3-methylcholanthrene-treated rats. Arch Toxicol. 1990;64(5):365-9.	Styrene vapor exposure in rats via inhalation – 5 h per day, three times to 500 cm^3/m^3 (2,100 mg/m^3) – and a single 24-hr exposure to acetone, phenobarbital, or 3-methylcholanthrene – followed by styrene exposure (24 h).	At 2,100 mg/m^3, pulmonary changes in glutathione (GSH) indicated by the concentration of non-protein sulphydryls showed a decrease of 43% in rats. Only a marginal decrease was observed in the pulmonary cytochrome P450 oxidative metabolism. Following a single 24-h inhalation exposure, the decreases in GSH were 66% in lung but only 16% in liver. On the other hand, a multifold increase in the disposition of thioether compounds was found in urine. Pulmonary cytochrome P450-dependent metabolism was decreased, shown by low residual activities of 7-ethoxyresorufin (less than 20%), 7-ethoxycoumarin (53%) and 7-pentoxyresorufin 0-dealkylases (76%). Epoxide hydrolase and GSH S-transferase enzyme activities which catalyze styrene detoxification were not decreased. Pretreated rats showed pulmonary effects different from each other and from those of styrene alone. Acetone potentiated the lung effect and elevated 1.5-fold urine thioether output. Inducer pretreatment seemed to be a factor aggravating styrene toxicity; in effect this was clearest in acetone-induced rats	In general, GSH depletion accompanied by inhibition of cytochrome P450-dependent oxidative drug metabolism were the earliest biochemical lesions manifested in styrene-exposed lung.
Elovaara E Engstrom K, Nakaiima T, Park SS, Gelboin HV, Vainio H	Effect of various cytochrome P-450	In acetone-pretreated rats, PGA and MA and thioether formation were elevated 30	These studies showed that styrene inhalation induced principally

Table 16 - Styrene

Citation	Study methods	Results	Authors' conclusions/comments
1991. Metabolism of inhaled styrene in acetone-, phenobarbital- and 3-methylcholanthrene-pretreated rats: stimulation and stereochemical effects by induction of cytochromes P45011E1, P4501IB and P4501A. Xenobiotica. May;21(5):651-61.	inducers, namely acetone, phenobarbital (PB) and 3-methylcholanthrene (MC), on the PK of styrene metabolism was studied – styrene inhalation exposure in rats to 500 cm^3/m^3 (2100 mg/m3) for 24 hrs. Styrene metabolism was studied measuring phenylglyoxylic acid (PGA), the enantiomers of mandelic acid (MA), and total thioethers.	to 50%. The R/S ratio of MA enantiomers was about two in all styrene-exposed groups except PB-pretreated rats, which showed a ratio of four. Styrene metabolism in liver microsomes measured *in vitro* was increased by styrene 140%, acetone plus styrene by 190%, methylcholanthrene plus styrene by 180% and phenobarbital plus styrene by 250%. N-Nitrosodimethylamine demethylation (NDMAD) and 7-pentoxyresorufin dealkylation (PROD) in liver microsomes were enhanced 100 to 150% by styrene inhalation. The metabolism of 7-ethoxyresorufin was not significantly enhanced. Monoclonal antibodies to P-450 1A1, 1A2, 11B1 and 11E1 were utilized to identify cytochrome P- 450s by Western blot analysis.	cytochrome P4501E1, whereas styrene given by gavage at a high narcotic dosage induced both P45011E1 (NDMAD, 60%) and P4501IB (PROD, 3,000%). The authors concluded that cytochrome P45011E1 induction had a major impact on styrene metabolism and that P45011B1 induction yielded an altered MA metabolite enantiomer ratio.
Kligerman AD, J Bryant, JA Campbell, BW Collins, CL Doerr, GL Erexson, P Kwanyuen and DL Morgan 1992. Cytogenetic studies of mice exposed to styrene by inhalation. Mutation Research/Environmental Mutagenesis and Related Subjects Volume 271, Issue 2, 1992, Page 173		The European Environmental Mutagen Society poster abstract of the 21st annual meeting, 25-31 August 1991, Prague (Czechoslovakia).	English translation not available.
Dypbukt JM Costa LG, Manzo L, Orrenius S, Nicotera P 1992. Cytotoxic and genotoxic effects of styrene-7,8-oxide in neuroadrenergic Pc 12 cells.	Cytotoxic and genotoxic effects of styrene-7,8-oxide (SO) in Pc-12 cells treated with 0.5-1 µM, 30 µM, and 100	At 0.5-1 µM, there occurred a rapid increase in cytosolic Ca2+, depletion of intracellular glutathione and ATP, DNA damage and loss of cell viability. At ≤ 100 µM, no loss of cell viability was reported	These results indicated that non-lethal concentrations of SO can cause modifications that compromise the ability of Pc-12 cells to respond

Table 16 - Styrene

Citation	Study methods	Results	Authors' conclusions/comments
Carcinooenesis. 1992 Mar;13(3):417-24.	µM.	nor was cell growth rate affected. At 30 and 100 µM, SO stimulated the formation of alkali-sensitive, DNA single-strand breaks (SSB). DNA SSB were fully repaired when cells exposed to 30 µM SO were subsequently incubated for 3 h in fresh medium, whereas DNA repair was only partial after exposure to 100 µM SO. When cells exposed to 30 or 100 µM SO were incubated with the inhibitors of repair synthesis 1-beta-D-arabinofuranosyl-cytosine (AraC) and hydroxyurea (HU), SSB accumulated, indicating the involvement of the excision- repair system in the removal of DNA lesions. An SO adduct with guanine at the N7 position was detected in the DNA extracted from treated cells. SO did not induce the formation of double-strand breaks, interstrand cross-links, or DNA- protein cross-links. Although cells exposed to 30 or 100 µM SO underwent normal cell division, latent DNA damage was retained for up to 14 subsequent replicative cycles. In addition, SO-treated cells partially lost their normal ability to differentiate in response to nerve growth factor (NGF) stimulation. NGF failed to induce differentiation in cells that had replicated for 20 generations after exposure to 100 µM SO. Spontaneous differentiation stimulated by high-density culture was also inhibited in SO-treated cells.	to NGF and differentiate.
Kligerman AD, Allen JW, Bryant MF, Campbell JA, Collins BW,	Styrene exposure in female B6C3F1 mice	There was a significant concentration-related elevation of SCE frequency in	

Table 16 - Styrene

Citation	Study methods	Results	Authors' conclusions/comments
Doerr CL, Erexson GL, Kwanyuen P, Morgan DL 1992. Cytogenetic studies of mice exposed to styrene by inhalation. Mutat Res. Jul;280(1):35-43.	via inhalation to 0, 125, 250 or 500 ppm. One day after the final exposure, peripheral blood, spleen, and lungs were removed and cultured for analysis of micronucleus (MN) induction, chromosome breakage, and SCE induction.	lymphocytes from the spleen and the peripheral blood as well as in cells from the lung. However, no statistically significant concentration-related increases were found in the frequency of chromosome aberrations in the cultured splenocytes or lung cells, and no significant increases in MN frequencies were observed in binucleated splenocytes or normochromatic erythrocytes in peripheral blood smears.	
Checkoway H, Costa LG, Camp J, Coccini T, Daniell WE, Dills RL 1992. Peripheral markers of neurochemical function among workers exposed to styrene. Br J Ind Med. Aug;49(8):560-5	Occupational exposure to styrene at concentrations ranging from 1 to 160 ppm in styrene workers (60) compared to 18 reference workers. Biomarkers of neurochemical function measured were sigma receptor binding in lymphocytes, monoamine oxidase type B (MAO-B) activity in platelets, and serotonin uptake by platelets. Also, blood styrene concentration was measured via exposure index to account for the use of protective equipment and dermal uptake. For blood exposure groups	The prevalence of headache, dizziness, light headedness, fatigue, irritability, memory loss, and feeling "drunk" at work increased with increasing blood styrene concentration. No effect on sigma receptor binding was seen. A slight positive correlation was found for uptake of serotonin, which has been used as an exposure related effect indicator in previous studies of workers exposed to solvents. The MAO-B activity decreased with increasing blood styrene concentration; the mean (SE) MAO-B values for the four groups were 34.2 (3.0), 28.1 (5.3), 20.1 (4.8), and 16.9 (7.7) pmol/10^7 cells/min. The MAO-B activity also correlated negatively with the number of reported nervous system symptoms, whereas no associations were seen between prevalence of symptoms and either serotonin uptake or sigma receptor binding.	The findings for MAO-B activity were consistent with previously reported experimental data, and suggested that MAO-B may be a useful marker of styrene neurotoxicity.

Table 16 - Styrene

Citation	Study methods	Results	Authors' conclusions/comments
	were defined as reference, 0.05, 0.05-0.19, and greater than or equal to 0.20 micrograms/ml.		
Preston RJ, Abernethy DJ. 1993. Studies of the induction of chromosomal aberration and sister chromatid exchange in rats exposed to styrene by inhalation. IARC Sci Publ; (127):225-33.	Styrene exposure in male Fischer 344 rats via Inhalation to 150, 500 or 1,000 ppm for 6 h/day on 5 days/wk for 4 wks. Ethylene oxide, a 150 ppm group, was included in an attempt to establish the usefulness of rat lymphocytes for cytogenetic analysis in this protocol of long-term exposure by inhalation. Peripheral blood samples were drawn at 1, 2, 3 and 4 weeks of exposure and at 4 weeks after the end of exposure. Cultures were established, and sister chromatid exchange and chromosomal aberrations were analyzed.	The frequency of chromosomal aberrations was not increased over that in the air controls in the animals exposed to styrene or ethylene oxide at any of the sampling times. Styrene did not induce SCE at any of the concentrations or sampling times; however, the frequency of SCE was increased following exposure to ethylene oxide at all sampling times, with a positive exposure-response relationship with time of exposure as the variable.	
Norppa H, Sorsa M. 1993. Genetic toxicity of 1,3-butadiene and styrene. IARC Sci Publ.	Review of styrene and 1,3-butadiene toxicity.	1,3-Butadiene and styrene (vinyl benzene) are indirect genotoxins, which require metabolic activation to an epoxide form in	

Table 16 - Styrene

Citation	Study methods	Results	Authors' conclusions/comments
(127):185-93.		order to bind covalently to DNA. Styrene 7,8-oxide, the active metabolite of styrene, is a carcinogen in rodents and has been shown to be genotoxic in most *in vitro* test systems and at various genetic endpoints. The few studies available on the genotoxicity of styrene 7,8-oxide *in vivo* have yielded negative or (in mice) weakly positive results. Styrene is not usually genotoxic *in vitro* in assays employing a microsomal preparation from rat liver for metabolic activation, but positive effects have been obtained when other sources of metabolic activation, such as human erythrocytes, were provided. *In vivo*, styrene has been found repeatedly to be weakly genotoxic in the assay for sister chromatid exchange, especially in mice. Cytogenetic damage (usually chromosomal aberrations) has been reported in many studies of workers, mainly from the reinforced plastics industry where ambient concentrations of styrene may be high (50-100 ppm), while most negative findings are associated with exposure to lower levels.	
Chakrabarti S Duhr MA, Senecal-Quevillon M, Richer 1993. Dose-dependent genotoxic effects of styrene on human blood lymphocytes and the relationship to its oxidative and metabolic effects. Environ Mol Mutagen;22(2):85-92	Genotoxic studies of styrene – sister chromatid exchanges (SCEs), cell cycle, and cell survival were analyzed. *In vitro* human blood lymphocytes were cultured for 72 hr in the	At up to 200 µM, both the SCE frequency and the cell cycle length were increased linearly with increasing concentrations without addition of any exogenous metabolizing system. At > 200 µM, no further increase in genotoxic response occurred. The range of concentrations (10-200 µM) at which increase of cell cycle length due to styrene was observed	Based on the relationship between the formation of different metabolic events and the genotoxicity of styrene, it may be possible that the genotoxic properties of styrene in human blood lymphocytes may be mediated initially not only by the formation of the presumably reactive styrene 7,8-oxide, but also by that of

Table 16 - Styrene

Citation	Study methods	Results	Authors' conclusions/comments
	presence of different concentrations of styrene (0-1,000 µM). Twenty-four hours before harvest, BrdU (5 µg/ml) was added to assess the increase in SCEs and cell cycle delay	did not impair the viability of the cells, suggesting that such cell cycle delay is a genotoxic-related event and not caused by cytotoxicity. *In vitro* metabolic transformation of styrene in whole-blood lymphocyte cultures without the presence of any exogenous metabolic activation system showed the formation of a reactive intermediate, styrene 7,8-oxide, to be capacity-limited, as verified from a nonlinear increase in the formation of styrene glycol. The same phenomenon of saturation has also been observed with regard to other metabolic effects due to styrene in whole blood lymphocytes in culture, such as a dose-dependent increase in lipid peroxidation and depletion of blood lymphocyte glutathione	a reactive oxygen species as well. However, the present data were not sufficient enough to definitely identify the role of reactive oxygen species in such toxicity and therefore it warrants further study.
Jégaden D, Amann D, Simon JF, Habault M, Leoctux B, Galopin P 1993. Study of the neurobehavioural toxicity of styrene at low levels of exposure. Int Arch Occup Environ Health;64(7):527-31.	Three psychometric tests were performed twice daily in dockyard workers (30) exposed to styrene and in 30 control workers. Concentrations of styrene were < TLV-TWA of 50 ppm.	The results were usually better in the evening than in the morning in both groups, which proves the lack of acute intoxication at the end of the day at this level of exposure. On the other hand, all of the tests conducted on the exposed subjects are significantly less good than those on the controls.	The results suggest the existence of minor but significant organic mental disorders in the subjects exposed to a mean dose of 30 ppm in this study. These results are inconsistent with those of several recent studies.
Löf A, Johanson G.1993. Dose-dependent kinetics of inhaled styrene in man. IARC Sci Publ. 127):89-99.	Styrene vapor exposure in 2 volunteers (1 male, 1 female) for 2 h at 50 Watts of activity to 26, 77, 201 and 386 ppm. Styrene was measured in arterialized capillary	Non-linear relationships between the level of exposure to styrene and the concentration of styrene in arterial blood and 0-5 hr cumulative excretion of mandelic acid indicated metabolic saturation. A PBPK model was used to estimate the maximum metabolic rate	According to authors, this is the first time that dose-dependent kinetics of styrene has been shown in humans

Table 16 - Styrene

Citation	Study methods	Results	Authors' conclusions/comments
	blood during and after exposure by head-space GC, and the levels of mandelic acid in urine were analyzed by high-performance LC.	(Vmax) of styrene from data on blood styrene. According to the model, the Vmax is 2.9 mmol/hr, and metabolic saturation occurs at concentrations of 100-200 ppm styrene, depending on the level of physical activity.	
Kligerman AD, Allen JW, Erexson GL, Morgan DL 1993. Cytogenetic studies of rodents exposed to styrene by inhalation. IARC Sci Publ. (127):217-24.	Styrene exposure in B6C3F1 female mice and Fischer 344 rats via inhalation to125, 250 and 500 ppm for 6 h/day for 14 consecutive days. One day after the final exposure, murine peripheral blood lymphocytes, spleen and lungs were removed, and the cells were cultured for analysis of chromosomal aberrations, micronucleus induction and SCE. Peripheral blood smears were scored for MN induction in normochromatic erythrocytes. For the rats, peripheral blood lymphocytes were cultured for analyses and were examined for DNA strand breakage under alkaline	There were mall but statistically significant concentration-related increases in the frequency of sister chromatid exchange were seen in both mice and rats in all cell types examined. No statistically significant concentration-related increase in chromosomal aberration or micronucleus induction frequencies were observed in either species, and there was no significant increase in DNA strand breakage in peripheral blood lymphocytes from exposed rats.	These results indicated that styrene was a weak inducer of sister chromatid exchange *in vivo* when administered to rodents by inhalation

Table 16 - Styrene

Citation	Study methods	Results	Authors' conclusions/comments
	conditions. Bone-marrow smears were made from femurs of rats for analysis of MN induction in normochromatic erythrocytes.		
Charles S. Rebert, William K. Boyes, Gordon T. Pryor, David J. Svensgaard, Kara M. Kassay, G. Ross Gordon and Natasha Shinsky 1993. Combined effects of solvents on the rat's auditory system: Styrene and trichloroethylene International Journal of Psychophysiology, Volume 14, Issue 1, January, Pages 49-59	Styrene and trichloroethylene exposure in male Long Evan rats at solvent pair (STY/TCE) concentrations of 0/3,000, 250/2,250, 500/1,500, 750/750, and 1000/0 ppm for 8 h/day for 5 days.	Hearing loss was studied by recording the brainstem auditory evoked response (BAER). Decreased BAER amplitude, indicative of hearing loss, was correlated with blood levels of total solvent.	The effects were as predicted by a linear dose-addition model, indicating neither synergistic nor antagonistic interactions at the concentrations studied.
Morgan DL, Mahler JF, O'Connor RW, Price HC Jr, Adkins B Jr. 1993. Styrene inhalation toxicity studies in mice. I. Hepatotoxicity in B6C3F1 mice. Fundam Appl Toxicol. 1993 Apr;20(3):325-35	Styrene exposure via inhalation in B6C3F1 mice to 0, 125, 250, or 500 ppm styrene, 6 hr/day, for up to 14 d.	Centrilobular hepatic necrosis and deaths occurred after one exposure to 500 ppm or two exposures to 250 ppm. Mortality and hepatotoxicity were not increased by additional exposures, and in surviving mice, regeneration and repair of initial hepatic injury occurred in spite of continued exposure for 14 days. A marked sex difference was observed, with male mice significantly more susceptible to styrene toxicity than females. A nonlinear dose-response was observed where mortality in male and female mice was greater in the 250 ppm dose group	Severe congestion and necrosis of the liver was present in moribund mice; hepatic congestion and serum alanine aminotransferase and sorbitol dehydrogenase were significantly greater in moribund animals.

Table 16 - Styrene

Citation	Study methods	Results	Authors' conclusions/comments
		than that in the 500 ppm dose group.	
Morgan DL, Mahler JP, Dill JA, Price HC Jr, O'Connor RW, Adkins B Jr. 1993. Styrene inhalation toxicity studies in mice. II. Sex differences in susceptibility of B6C3F1 mice. Fundam Appl Toxicol. Oct; 21(3):317-25.	Styrene exposure in mice via inhalation to 0, 125, 250, or 500 ppm for 6 hr/day for up to 3 days.	Styrene exposure caused increased mortality and hepatotoxicity (centrilobular necrosis, increased serum liver enzymes) in males and females after one or two exposures to 250 and 500 ppm. Hepatic GSH levels were decreased in a dose-dependent manner in males and females. After one exposure, GSH levels in males rebounded above controls in all dose groups. After three exposures to 125 or 250 ppm males appeared to maintain GSH levels, GSH was still decreased in the 500 ppm group. GSH levels in females were decreased after each exposure in all dose groups to lower levels than in males, and did not rebound above controls	Hepatic GSH levels were decreased in a dose-dependent manner in males and females.
Morgan DL, Mahler JF, Dill JA, Price HC Jr, O'Connor RW, Adkins B Jr. 1993. Styrene inhalation toxicity studies in mice. III. Strain differences in susceptibility. Fundam Appl Toxicol. 1993 Oct;21(3):326-33.	Styrene exposure in B6C3F1, C57BL/6, Swiss, and DBA/2 mice (8 weeks old) via inhalation to 0, 125, 250, or 500 ppm 6 hr/day, for 4 days (20/sex/dose). Styrene uptake and styrene-7,8-oxide (SO) formation were measured in blood. An estimate of SO detoxification by conjugation with GSH was obtained by	In general, mortality, increased liver weights, and hepatocellular necrosis were observed in the 250 and 500 ppm dose groups for all strains and both sexes. Mortality, increased liver weights, and hepatocellular necrosis were greatest in B6C3F1 and C57BL/6 mice in the 250 ppm dose group and in males; hepatotoxicity was similar in both strains. Swiss mice exhibited dose-dependent increases in mortality, liver weights, and in hepatocellular necrosis, with only slight sex differences at early time points. Hepatotoxicity in DBA/2, B6C3F1, and C57BL/6 strains was greater at 250 than 500 ppm; however, toxicity was less	Blood styrene and SO levels did not correlate well with strain differences in toxicity.

Table 16 - Styrene

Citation	Study methods	Results	Authors' conclusions/comments
	measuring hepatic GSH depletion.	severe in DBA/2 than in other strains based on absence of mortality in either sex and less extensive liver necrosis at both 250 and 500 ppm.	
McConnell EE, Swenberq JA. 1994. Review of styrene and styrene oxide long-term animal studies. Crit Rev Toxicol;24 Suppl:S49-55	Review of toxicity studies on styrene and five on styrene oxide in an effort to evaluate the potential carcinogenic activity. The styrene studies included inhalation exposure (rats, mice, guinea pigs, and rabbits), intragastric gavage (rats and mice), drinking water (rats), and intraperitoneal injection (rats), while styrene oxide exposure was via intragastric gavage (rats and mice) or skin painting (mice).	There was no convincing evidence of carcinogenic activity of styrene in animals, although many of the studies were considered inadequate. Styrene oxide was carcinogenic to the forestomach of both sexes of rats and mice after gavage exposure and was associated with an increase in liver neoplasms in male mice in one study. No carcinogenic activity was observed in mice after dermal exposure (skin paint).	None of the studies of styrene or styrene oxide reported here were well suited for extrapolating potential carcinogenic activity of either compound to humans because all have deficiencies in design, conduct, interpretation, or utilized a less than ideal route of exposure. A chronic state-of-the-art inhalation study is needed to evaluate this aspect of hazard assessment
Holz O, Scherer G, Brodtmeier S, Koops F, Warncke K, Krause T, Austen A, Ancierer J, Tricker AR, Adikofer F et al. 1995. Determination of low level exposure to volatile aromatic hydrocarbons and genotoxic effects in workers at a styrene plant. Occup Environ Med.Jun;52(6):420-8.	25 healthy workers were examined for cytogenetic effects in peripheral white blood cells. Concentrations of aromatic hydrocarbons determined from active air sampling in all areas of the factory were styrene, 73-3,540 µg/m^3 (< 0.01-0.83 ppm); ethylbenzene, 365-	No genotoxic effects related to exposure were detected by DNA adducts or DNA single strand breaks and sister chromatid exchange. The only effect related to exposure was an increase in kinetochore positive micronuclei in peripheral lymphocytes; the frequency of total micronuclei in peripheral lymphocytes did not change. Smoking was confirmed by measurement of plasma cotinine, and no confounding effect was found on any of	Low occupational exposure to styrene, benzene, and ethylbenzene did not induce alterations of genotoxicological variables except kinetochore positive micronuclei.

Table 16 - Styrene

Citation	Study methods	Results	Authors' conclusions/comments
	2,340 µg/m^3 (0.08-0.53 ppm); benzene, 73 - 3,540 µg/m^3 (< 0.02-1.11 ppm); toluene, 54-2,960 µg/m^3 (0.01-0.78 ppm); and xylenes, 12-94 µg/m^3 (< 0.01-0.02 ppm).	the cytogenetic variables.	
Sassine MP, Meroler D, Larribe F, Bélanger S. 1996 Mental health deterioration in workers exposed to styrene. Rev Epidemiol Sante Publique. Jan;44(1):14-24.	Styrene exposure in 128 workers occupational exposed (dose not specified). Self-administered questionnaires – Profile of Mood States (POMS), Psychiatric Symptom Index and Well-being Index – were used.	There occurred a significant relationship between post work-shift urinary mandelic acid (biological indicator of styrene exposure) and the scores obtained on the POMS scales of tension-anxiety (Spearman's rank correlation rho = 0.30; p < 0.01), anger-hostility (rho = 0.29; p < 0.01), fatigue-inertie (rho = 0.34; p < 0.01), and confusion-bewilderment (rho = 0.23; p = 0.04), as well as the Psychological Distress Index (rho = 0.30; p < 0.01).	These indicators of mood states do not constitute a diagnosis of mental disease but reveal progressive deterioration of well being associated with neurotoxic exposure in the workplace.
George Cruzan, Janette R. Cushman, Larry S. Andrews, Geoffrey C. Granville, Roland R. Miller, Colin J. Hardy, Derek W. Coombs and Pamela A. Mullins. 1997. Subchronic Inhalation Studies of Styrene in CD Rats and CD-1 Mice. Fundamental and Applied Toxicology Volume 35, Issue 2, February, Pages 152-165	Subchronic inhalation studies with styrene vapor in Charles River (Sprague-Dawley-derived) rats, males and females (10/group) exposed to 0, 200, 500, 1,000, or 1,500 ppm, 6 hr/d, 5d/wk. Also, CRL-CD-1 and B6C3F1 mice, males and females (20/group), exposed to 0, 15, 60, 250, or 500 ppm, 6 hr/d, 5d/wk for 2 wks. Also	Styrene had no effect on survival, hematology, or clinical chemistry. At 1,500 ppm, males weighed 10% less after 13 weeks and males and females at 1,000 and 1,500 ppm consumed more water than controls. Histopathologic changes were confined to the olfactory epithelium of the nasal mucosa. At 250 and 500 ppm, mortality was observed in both CD-1 and B6C3F1 mice; more female mice, but not males, died from exposure to 250 ppm than from 500 ppm. Two females exposed to 200 ppm died during the first week. At 200 ppm, liver toxicity was evident in the decedents and in some	Large variations in the labeling index among animals emphasized the need for large group sizes. For nasal tract effects, a NOAEL was not found in CD-1 mice, but in CD rats, the NOAEL was 200 ppm. For other effects, the NOAEL was 500 ppm in rats and 50 ppm in mice.

Table 16 - Styrene

Citation	Study methods	Results	Authors' conclusions/comments
	CRL-CD-1 mice, males and females (10/group), exposed to 0, 50, 100, 150, or 200 ppm, 6 hr/d, 5d/wk for 13 wks. Also, cell proliferation (BrdU labeling) study in rats (15) and male mice (30) exposed to 0, 50, 100, 150, or 200 ppm, 6 hr/d, 5d/wk for 2, 5, or 13 weeks.	female survivors. Changes were observed in the lungs of mice exposed to 100, 150, or 200 ppm and in the nasal passages of all treatment groups, those exposed to 50 ppm being less affected. No increase in cell proliferation was found in liver of rats or mice or in cells of the bronchiolar or alveolar region of the lung of rats. No increase in labeling index of type II pneumocytes was seen in mouse lungs. At 150 and 200 ppm, an increased labeling index of Clara cells was seen after 2 weeks and in occasional mice after 5 weeks.	
Morgan DL, Mahler JF, Wilson RE, Moorman MP, Price HC Jr, Patrick KR, Richards JH, O'Connor RW 1997. Effects of various pretreatments on the hepatotoxicity of inhaled styrene in the B6C3F1 mouse. Xenobiotica. Apr;27(4):401-11.	Styrene exposure in mice via inhalation – pretreatment with phenobarbital (PB; P450 inducer), SKF 525A (P450 inhibitor), N-acetylcysteine (NAC; GSH precursor), or saline (vehicle control) prior to 0 or 500 ppm for 6 hrs.	Hepatocellular degeneration or necrosis occurred in all groups; these changes were more extensive and severe in mice pretreated with PB. Styrene significantly increased relative liver weights and serum ALT and SDH levels only in mice pretreated with PB. NAC did not prevent GSH depletion or hepatotoxicity. In the fat of SKF 525A-pretreated mice a slight but statistically significant increase in styrene levels was observed, suggesting that metabolism was decreased; the SO/styrene ratio in the fat of PB-pretreated mice showed a slight, but statistically significant, increase indicating a slight increase in styrene metabolism. Neither SKF 525A nor PB caused changes in microsomal enzyme activity *in vitro*.	These results suggested that styrene may be activated by a pathway not totally dependent upon P450 enzyme activity, or more likely that PB and SKF 525A are not specific for the P450 enzymes involved in activation and detoxification of styrene
Susan C.J Sumner, Russell C	Styrene exposure via	Mortality and increased serum alanine	These data indicated that an

Table 16 - Styrene

Citation	Study methods	Results	Authors' conclusions/comments
Cattley, Bahman Asgharian, Derek B Janszen and Timothy R Fennell 1997. Evaluation of the metabolism and hepatotoxicity of styrene in F344 rats, B6C3F1 mice, and CD-I mice following single and repeated inhalation exposures. Chemico-Biological Interactions Volume 106, Issue 1, 29 August, Pages 47-65	inhalation in rats and B6C3F1 and CD mice to 250 ppm for 6 h/d for 1 to 5 days, and liver (mice only) and blood were collected following each day of exposure. Also, rats and mice exposed for 0, 2, or 4 days to styrene (60 pCi/mmol) for 6 h. Urine, feces, and expired air were collected.	aminotransferase (ALT) activity were observed in mice but not in rats. Hepatotoxicity in B6C3F1 mice was characterized by severe centrilobular congestion after one exposure followed by acute centrilobular necrosis. Hepatotoxicity was delayed by 1 day in CD-1 mice, and the increase in ALT and degree of necrosis was less than observed for B6C3F1 mice. Most styrene-derived radioactivity was excreted in urine. The time-course of urinary excretion indicates that rats and CD-1 mice eliminated radioactivity at a faster rate than B6C3F1 mice following a single 250 ppm exposure, consistent with a greater extent of liver injury for B6C3F1 mice. The elimination rate following 3 or 5 days of exposure was similar for rats and both mouse strains. Following three exposures, the total radioactivity eliminated in excreta was elevated over that measured for one exposure for both mouse strains. An increased excretion of metabolites on multiple exposures is consistent with the absence of ongoing acute necrosis following 4 to 5 daily exposures.	induction in styrene metabolism occurs after multiple exposures, resulting in an increased uptake and/or clearance for styrene.
Karakava AE, Karahalil B, Yilmazer M, Aygtin N, Sardas S, Burgaz S 1997. Evaluation of genotoxic potential of styrene in furniture workers using unsaturated polyester resins.	Effects of styrene exposure in 53 furniture workers (polyester resin lamination processing) compared to 41 matched control subjects. Mean air	As a metabolic marker for styrene exposure, mandelic acid + phenylglyoxylic acid was measured in the urine and the mean value was 207 mg/g creatinine. Mean urinary thiother (UT) excretions of exposed workers were 4.43 (± 3.42) mmol SH/mol creatinine; controls were 2.75 (±	Significant effects of work-related exposure were detected in the UT excretion and SCEs analyzed in peripheral blood lymphocytes ($p < 0.05$ and $p < 0.01$, respectively). The MN frequency in lymphocytes from the styrene- exposed group did not

Table 16 - Styrene

Citation	Study methods	Results	Authors' conclusions/comments
Mutat Res. Aug 14;392(3):261-8.	concentration of styrene in the breathing zone of workers was 30.3 ppm.	1.78) mmol SH-/mol creatinine. Mean cell values of SCE frequency in peripheral lymphocytes from the workers and controls were 6.20 (±1.56) and 5.23 (±1.23), respectively. Mean frequencies (%) of MN in the exposed and control groups were 1.98 (±0.50) and 2.09 (±0.35), respectively.	differ from that in the controls ($p > 0.05$). Although data did not demonstrate a dose-response relationship, they did suggest that styrene exposure was evident and that this styrene exposure may contribute to the observed genotoxic damage in furniture workers.
Coccini T, Fenoglio C, Nano R, De Piceis Polver P, Moscato G, Manzo L 1997. Styrene-induced alterations in the respiratory tract of rats treated by inhalation or intraperitoneally. J Toxicol Environ Health. Sep:52(1):63-77	Styrene exposure in rats via inhalation to 300 ppm, 6 h/d, 5 d/wk, for 2 wks. Also, intraperitonieal exposure to 40 or 400 mg/kg styrene for 3 consecutive days.	Electron microscopy analysis showed diffuse cell damage involving the tracheal, bronchiolar, and alveolar epithelium. In the tracheal epithelium, several cell types were affected. Ciliated cells presented vacuolation, detachment of cilia, blebbing of the apical cytoplasm, and compound cilia. Most secretory cells showed scant secretory granules and blebbings. Dense bodies and fibrillary inclusions were seen in intermediate and basal cells. Styrene also caused alterations of cytoplasmic components in type II pneumocytes and bronchiolar cells as well as thickness of the alveolar wall. These abnormalities were accompanied by depletion of glutathione (GSH) in the lung tissue.	Pneumotoxic effects of systemic administration of styrene were dose-dependent and tended to be more severe than those seen in the animals exposed for longer periods to styrene by inhalation. Metabolic activation of styrene and subsequent cell damage induced by the reactive metabolite styrene oxide may be involved in the sequence of events culminating in the toxic insult to the respiratory tract
Cruzan G, Cushman JR, Andrews LS, Granville GC, Johnson KA, Hardy CJ, Coombs DW, Mullins PA, Brown WR. 1998. Chronic toxicity/oncogenicity study of styrene in CD rats by inhalation exposure for 104 weeks. Toxicol Sci. 1998 Dec;46(2):266-81.	Styrene vapor exposure in Charles River CD (Sprague-Dawley-derived) rats, males and females (70/group) to 0, 50, 200, 500, or 1000 ppm, 6 h/d, 5d/week for 104 weeks. Hematologic and clinical	Styrene had no effect on survival in males, but females exposed to 500 or 1000 ppm had a dose-related increase in survival. Levels of styrene in the blood at the end of a 6-hr exposure during week 95 were proportional to exposure concentration. In ≥ 200 ppm exposed animals, levels of styrene oxide in the blood was proportional to styrene exposure	There was no evidence that styrene exposure caused treatment-related increases of any tumor type in males or females or in the number of tumor-bearing rats in the exposed groups compared to controls. In females, there were treatment-related decreases in pituitary adenomas and mammary adenocarcinomas. Based

Table 16 - Styrene

Citation	Study methods	Results	Authors' conclusions/comments
	pathology examinations was conducted at weeks 13, 26, 52, 78, and 104. Nine or 10 per sex per group were necropsied after 52 weeks of exposure and the remaining survivors were necropsied after 104 weeks.	concentration. No changes of toxicologic significance occurred regarding hematology, clinical chemistry, urinalysis, or organ weights. Males exposed to 500 or 1,000 ppm gained less weight than the controls during the first year and maintained the difference during the second year. Females exposed to 200, 500, or 1,000 ppm gained less weight during the first year; those exposed to 500 or 1,000 ppm continued to gain less during months 13-18. Styrene-related non-neoplastic histopathologic changes were confined to the olfactory epithelium of the nasal mucosa.	on an overall evaluation of eight oncogenicity studies, there is clear evidence that styrene does not induce cancer in rats.
Mahler JF, Price HC Jr, O'Connor RW, Wilson RF, Eldridge SR, Moorman MP, Morgan DL 1999. Characterization of hepatocellular resistance and susceptibility to styrene toxicity in B6C3F1 mice. Toxicol Sci. Mar;48(1):123-33	Styrene exposure in male B6C3F1 mice via inhalation to 500 ppm for 6 h/day. Hepatotoxicity was evaluated by microscopic examination, serum liver enzyme levels, and (BrdU)-labeling index (LI). Metabolism was assessed by measurement of blood styrene and styrene oxide.	Both single and repeated exposures resulted in mortality by Day 2; in mice that survived, there was centrilobular (CL) necrosis with elevated BrdU LI at Day 6, and complete restoration of the necrotic parenchyma by Day 15. The BrdU LI in mice given a single exposure had returned to control levels by Day 15. Re-exposure of these mice on Day 15 resulted in additional mortality and hepatocellular necrosis, indicating that regenerated CL cells were again susceptible to the cytolethal effect of styrene following a 14-day recovery. However, in mice repeatedly exposed to styrene for 14 days, the BrdU LI remained significantly increased on Day 15, with preferential labeling of CL hepatocytes with enlarged nuclei (karyomegaly).	These data indicated that resistance to styrene-induced necrosis under conditions of repeated exposure was not due to sustained cell turnover and production of new, metabolically inactive cells, but rather was due to some other, as yet unknown, protective phenotype of the regenerated cells.

Table 16 - Styrene

Citation	Study methods	Results	Authors' conclusions/comments
		If repeated exposures were followed by a 10-day recovery period, CL karyomegaly persisted, but the BrdU LI returned to control level and CL hepatocytes became susceptible again to styrene toxicity as demonstrated by additional mortality and acute necrosis after a challenge exposure.	
Marczynski B, Peel M, Baur X. 2000. New aspects in genotoxic risk assessment of styrene exposure--a working hypothesis. Med Hypotheses. Apr;54(4):619-23.	Review.	Biological monitoring of exposure to styrene is usually carried out by determination of mandelic acid and phenylglyoxylic acid, the two main styrene metabolites in urine. SO binds covalently to human plasma protein and hemoglobin. The ability of SO to induce DNA adducts and DNA strand-breaks has been well documented. Recently *in vitro* results showed that SO may disrupt the pre-existing oxidative status in white blood cells. This disruption would alter the balance between oxidants and antioxidants in cells. Styrene exposure can also result in oxidative DNA damage. A significant increase of 8-hydroxy-2-deoxyguanosine (8-OHdG) has been found in white blood cells of styrene-exposed workers.	According to these findings the authors proposed a new hypothesis for the genotoxic risk assessment of styrene. Depletion of glutathione and an increase in lipid peroxidation, a decrease of high molecular weight DNA fragments after SO exposure compared to hydrogen peroxide exposure, and oxidative DNA damage (increased amounts of 8-OHdG and an increased level of DNA strand-breaks) following styrene or SO exposure are due to oxidative stress which can be a result of the imbalance between oxidants and antioxidants. Formation of protein-, RNA- and DNA-adducts, changes in DNA repair capacity and styrene metabolism following styrene exposure could cause this imbalance between oxidants and antioxidants. Oxidative stress seems to be the basis for genotoxic risk assessment of styrene.
Vettori MV Corradi D, Coccini T, Carta A, Cavazzini S, Manzo L, Mutti A 2000. Styrene-induced	Styrene exposure in female rats (10) via inhalation to 0 and 300	Morphometric analysis showed a loss of TH-IR amacrine cells (6.2/mm^2 vs. 8.7/mm^2 recorded in controls, p = 0.002),	Retinal TH-IR cells were sensitive to styrene exposure, which seems to cause both structural and functional

Table 16 - Styrene

Citation	Study methods	Results	Authors' conclusions/comments
changes in amacrine retinal cells: an experimental study in the rat. Neurotoxicoloov. Aug;21(4):607-14.	ppm for 6 h/day, 5 d/wk, for 12 wks. Whole mounted retinas were used for the morphometry of tyrosine hydroxylase (TH) immunoreactive cells (IR). Dopamine (DA) content and TH activity were measured by HPLC and electrochemical detection and glutathione (GSH) was measured by HPLC tandem mass spectrometry (LC-MS/MS)..	without any peripheral-central variation in cell loss. Dopamine content was also lower in exposed, as compared to control animals (208.64 vs. 267.98 µg/g w.w., p = 0.004). The activity of TH in the whole retina was similar in styrene-exposed and control rats when expressed as a function of the wet weight, whereas it was much higher in styrene-exposed rats (+64%) when expressed as a function of the number of TH-IR amacrine cells (p < 0.001). Finally, retinal GSH was reduced by 30% in exposed as compared to control rats (p = 0.01).	changes, represented by cell loss and DA depletion, respectively. These findings confirm the vulnerability of dopaminergic systems to styrene toxicity, providing some insights on the possible mechanism of loss in chromatic discrimination recorded among workers occupationally-exposed to styrene.
Boogaard PJ, de Kloe KP, Wong BA, Sumner SC, Watson WP, van Sittert NJ 2000. Quantification of DNA adducts formed in liver, lungs, and isolated lung cells of rats and mice exposed to (14)C-styrene by nose-only inhalation Toxicol Sci.Oct;57(2):203-16..	Styrene exposure in mice via nose-only inhalation at 160 ppm and in rats at up to 1,000 ppm for 6 h. Cells were isolated from rat and mouse lung.	Bronchioloalveolar tumors were observed in mice exposed chronically to 160 ppm styrene, whereas no tumors were seen in rats up to concentrations of 1,000 ppm. DNA adduct profiles differed quantitatively and qualitatively in liver, total lung, and enriched lung cell fractions. At 0 and 42 hrs after exposure, the two isomeric N:7-guanine adducts of styrene oxide (HPEG) were present in liver at 3.0 (± 0.2) and 1.9 (±0.3) (rat) and 1.2 (±0.2) and 3.2 (±0.5) (mouse) per 10^8 bases. Several other, unidentified adducts were present at two to three times higher concentrations in mouse, but not in rat liver. In both rat and mouse lung, HPEG was the major adduct at approximately 1 per 10^8 bases at 0 h,	The overall results of this study indicate that DNA adduct formation does not play an important role in styrene tumorigenicity in chronically exposed mice

Table 16 - Styrene

Citation	Study methods	Results	Authors' conclusions/comments
		and these levels halved at 42 h. In both rat Type II and non-Type H cells, HPEG was the major adduct and was about three times higher in Type II cells than in total lung. For mice, DNA adduct levels in Clara cells and non-Clara cells were similar to total lung. The hepatic covalent binding index (CBI) at 0 and 42 h was 0.19 +/- 0.06 and 0.14 +/- 0.03 (rat) and 0.25 +/- 0.11 and 0.44 +/- 0.23 (mouse), respectively. The pulmonary CBIs, based on tissues combined for 0 and 42 h, were 0.17 +/- 0.04 (rat) and 0.24 +/- 0.04 (mouse). Compared with CBIs for other genotoxicants, these values indicate that styrene has only very weak adduct-forming potency.	
Brown NA, Lamb JC, Brown SM, Neal BH. 2000. A review of the developmental and reproductive toxicity of styrene. Redd Toxicol Pharmacol. Dec;32(3):228-47.	Review.	The reproductive and developmental toxicity of styrene has been studied in animals and humans. The animal studies on styrene have diverse study designs and conclusions. Developmental or reproductive toxicity studies have been conducted in rats, mice, rabbits, and hamsters. In most cases, high doses are required to elicit effects, and the effects are not unique to reproduction or development. In a number of the reports, either the experimental designs are limited or the descriptions of the designs and the endpoints measured are insufficient to draw conclusions about the toxicity of styrene. The more complete and better-reported studies showed that styrene does not cause developmental toxicity at dose	Human studies also support the conclusion that styrene is not an endocrine disrupter. Although some study authors have concluded that styrene is either a human or an animal reproductive or developmental toxicant, careful review demonstrates that such conclusions are not justified.

Table 16 - Styrene

Citation	Study methods	Results	Authors' conclusions/comments
		levels that are not maternally toxic. Some neurochemical or neurobehavioral effects have been reported at high exposures. Styrene does not affect fertility or reproductive function. Considerable animal toxicity data on styrene support the conclusion that styrene is neither an endocrine-active substance nor an endocrine disrupter. Human studies often suffer from either inadequate exposure data or exposure to a wide variety of materials, so that attribution of effects to styrene exposure is impossible. Furthermore, investigators often have failed to account for other exposures in the workplace or for other potentially confounding factors in their studies. Menstrual cycle irregularities and congenital abnormalities were initially reported; however, the better and more recent reports do not show that styrene causes developmental or reproductive effects in humans.	
Laffon B, Pásaro E, Méndez J 2001. Genotoxic effects of styrene-7,8-oxide in human white blood cells: comet assay in relation to the induction of sister-chromatid exchanges and micronuclei. Mutat Res, Apr 5;491(1-2):163-72	Styrene oxide treatment of human peripheral white blood cellsat 10-200 µM. Assays included the Comet assay, sister chromatid exchanges (SCE), the cytokinesis-blocked micronucleus (MN) test, and a combination MN fluorescence *in situ* hybridization (FISH)	Results show that SO induced DNA damage, SCE and MN in human leukocytes *in vitro* at concentrations above 50 µM, and that there was a strong relationship between DNA damage, as measured by the comet assay, and cytogenetic damage induced by SO at the doses employed. SO showed preferentially a clastogenic activity and produced a cytostatic effect at high doses, reflected by the significant decrease of the	A good dose-effect relationship was obtained in the three tests performed at the concentration range assayed.

Table 16 - Styrene

Citation	Study methods	Results	Authors' conclusions/comments
	test.	calculated proliferation indices.	
Cruzan G, Cushman JR, Andrews LS, Granville GC, Johnson KA, Bevan C, Hardy CJ, Coombs DW, Mullins PA, Brown WR. 2001. Chronic toxicity/oncogenicity study of styrene in CD-1 mice by inhalation exposure for 104 weeks. J Appl Toxicol. May-Jun;21(3):185-98.	Styrene vapor exposure in male and female Charles River CD-1 mice (70/group) to 0, 20, 40, 80 or 160 ppm, 6 h per day, 5 days per week for 98 weeks (females) or 104 weeks (males). Ten mice of each gender per group were pre-selected for necropsy after 52 and 78 weeks of exposure and the survivors of the remaining 50 of each gender per group were necropsied after 98 or 104 weeks.	Styrene had no effect on survival in males. Two high-dose females died (acute liver toxicity) during the first 2 weeks; the remaining exposed females had a slightly higher survival than control mice. Levels of styrene and styrene oxide (SO) in the blood during week 74 were proportional to exposure concentration, except that at 20 ppm the SO level was below the limit of detection. There were no changes of toxicological significance in hematology, clinical chemistry, urinalysis or organ weights. Mice exposed to 80 or 160 ppm gained slightly less weight than the controls. Styrene-related non-neoplastic histopathological changes were found only in the nasal passages and lungs. In the nasal passages of males and females at all exposure concentrations, the changes included respiratory metaplasia of the olfactory epithelium with changes in the underlying Bowman's gland; the severity increased with styrene concentration and duration of exposure. Loss of olfactory nerve fibers was seen in mice exposed to 40, 80 and 160 ppm. In the lungs, there was decreased eosinophilia of Clara cells in the terminal bronchioles and bronchiolar epithelial hyperplasia extending into alveolar ducts. Increased tumor incidence occurred only in the lung. The incidence of bronchioloalveolar adenomas was significantly increased in males exposed to 40, 80 and 160 ppm and in females	No difference in lung tumors between control and styrene-exposed mice was seen in the intensity or degree of immunostaining, the location of tumors relative to bronchioles or histological type (papillary, solid or mixed). It appears that styrene induced an increase in the number of lung tumors seen spontaneously in CD-1 mice.

Table 16 - Styrene

Citation	Study methods	Results	Authors' conclusions/comments
		exposed to 20, 40 and 160 ppm. The increase was seen only after 24 months. In females exposed to 160 ppm, the incidence of bronchioloalveolar carcinomas after 24 months was significantly greater than in the controls.	
Elizabeth Delzell, Maurizio Macaluso, Nalini Sathiakumar and Robert Matthews 2001. Leukemia and exposure to 1,3-butadiene, styrene and dimethyldithiocarbamate among workers in the synthetic rubber industry. Chemico-Biological Interactions, Volumes 135-136,1 June, Pages 515-534	Cohort study of 1,3-butadiene (BD), styrene (STY) and dimethyldithiocarbamate (DMDTC) exposures in 13,130 synthetic rubber industry workers employed for at least 1 year during 1943-1991.	Leukemia (N = 59) was positively associated with BD ppm-years (RRs of 1.0, 1.2, 2.0 and 3.8, for exposures of 0, >0 to <86.3, 86.3 to <362.2 and 362.2+ ppm-years; only the RR for the highest exposure category was statistically significant), STY ppm-years (RRs of 1.0, 1.2, 2.3 and 3.2, for exposures of 0, >0 to 20.6, 20.6 to <60.4 and 60.4+ ppm-years; only the RR for the highest exposure category was statistically significant) and DMDTC mg-years/cm (RRs of 1.0, 2.3, 4.9 and 2.9, for 0, >0 to <566.6, 566.6 to <1395.1 and 1395.1+ mg-years/cm; the RR for each non-zero exposure category was statistically significant) after adjusting for age and years since hire. After further adjusting each agent-specific set of RRs for the other two agents, a positive but imprecise relation remained for BD and DM DTC but not for STY. The association with BD was stronger for ppm-years due to exposure intensities >100 ppm than for ppm-years due to lower concentrations.	BD and DMDTC, but not STY, were positively associated with leukemia in multivariable analyses. The independent effect of each agent was difficult to evaluate because of correlations with other agents and imprecision.
Green T, Lee R, Toqhill A, Meadowcroft S, Lund V, Foster J. 2001. The toxicity of styrene to the nasal epithelium of mice and	Nasal tissue exposure to styrene in mice at 40 and 160 ppm, 6 h/day for 3 days.	The nasal tissues had a number of degenerative changes including atrophy of the olfactory mucosa and loss of normal cellular organization. Pretreatment of	Metabolism of styrene to its oxide could not be detected in human nasal tissues *in vitro*, but the same tissues did have epoxide hydrolase

Table 16 - Styrene

Citation	Study methods	Results	Authors' conclusions/comments
rats: studies on the mode of action and relevance to humans. Chem Biol Interact. Aug 31;137(2):185-202	Pretreatment of mice with 5-phenyl-1-pentyne.	mice with 5-phenyl-1-pentyne, an inhibitor of both CYP2F2 and CYP2E1, completely prevented the development of a nasal lesion on exposure to styrene establishing that a metabolite of styrene, probably styrene oxide, is responsible for the observed nasal toxicity. Comparisons of the cytochrome P-450 mediated metabolism of styrene to its oxide, and subsequent metabolism of the oxide by epoxide hydrolases and glutathione S-transferases in nasal tissues *in vitro*, have provided an explanation for the increased sensitivity of the mouse to styrene. Whereas cytochrome P-450 metabolism of styrene is similar in rats and mice, the rat is able to metabolize styrene oxide at higher rates than the mouse, thus rapidly detoxifying this electrophilic metabolite.	and glutathione S-transferase activities, and were able to metabolise styrene oxide efficiently, indicating that styrene is unlikely to be toxic to the human nasal epithelium.
Pavel Vodicka, Mikko Koskinen, Ludmila Vodickova, Rudolf S tetina, Petr Smerak, Ivo Barta and Kari Hemminki 2001. DNA adducts, strand breaks and micronuclei in mice exposed to styrene by inhalation. Chemico-Biological interactions, Volume 137, Issue 3, 28 September, Pages 213-227	Styrene exposure in male NMRI mice via inhalation to 750 and 1,500 mg/m^3 for 1, 3, 7 and 21 days (6 h/day, 7 days/week). The formation of SO induced 7-SO-guanines and 1-SO-adenines in DNA was analyzed from lung tissues by two versions of the 32P-post-labelling technique.	In lungs after 21 days of exposure to 1,500 mg/m^3, the level of 7-SO-guanine was 23.0 ± 11.9 adducts/10^8 normal nucleotides, while 1-SO-adenine was detected at the levels of 0.6 ± 0.2 adducts/10^8 normal nucleotides. Both 7-SO-guanines and 1-SO-adenines strongly correlated with exposure parameters, particularly with styrene concentration in blood ($r = 0.875$, $p = 0.0002$ and $r = 0.793$, $p = 0.002$, respectively). In bone marrow of exposed mice, a slight increase of strand breaks can be detected after 7 days of inhalation. A significant increase was revealed in the endonuclease III-sensitive sites after 21 days of inhalation	

Table 16 - Styrene

Citation	Study methods	Results	Authors' conclusions/comments
		in bone marrow. In the liver cells inhalation exposure to both concentrations of styrene did not affect either levels of DNA single-strand breaks or endonuclease III- sensitive sites. The inhalation of 1,500 mg/m^3 of styrene induced significant increase of micronuclei after 7 days of exposure (10.4 ± 2.5/1,000 cells, i.e. twice higher micronuclei frequency than in controls). After 21 days of inhalation no significant difference between the control group and the two exposed groups was observed. Whether the decrease of micronuclei after 21 days of inhalation was due to the inhibition of cell proliferation caused by styrene or due to the natural elimination of chromatide fragments remains to be clarified. (r = 0.721, p = 0.028).	
Tornero-Velez R, Rappaport SM 2001. Physiological modeling of the relative contributions of styrene-7,8-oxide derived from direct inhalation and from styrene metabolism to the systemic dose in humans. Toxicol Sci Dec:64(2):151-61	Occupational styrene and styrene oxide (SO) exposure in 252 workers in the reinforced plastics industry. A physiologically- based pharmacokinetic (PBPK) model was tested against air and blood measurements of styrene and SO.	The highly efficient first-pass hydrolysis of SO via epoxide hydrolase in the liver greatly reduces the systemic availability of SO formed *in situ* from styrene. In contrast, airborne SO, absorbed via inhalation, is distributed to the systemic circulation, thereby avoiding such privileged-access metabolism. The best fit to the model was obtained when the relative systemic availability (the ratio of metabolic SO to absorbed SO per unit exposure) equaled 2.75 x 10^{-4}, indicating that absorbed SO contributed 3,640 times more SO to the blood than an equivalent amount of inhaled styrene.	Since the ratio of airborne styrene to SO rarely exceeds 1,500 in the reinforced plastics industry, this indicates that inhalation of SO presents a greater hazard of cytogenetic damage than inhalation of styrene. The authors concluded that future studies should assess exposures to airborne SO as well as styrene

Table 16 - Styrene

Citation	Study methods	Results	Authors' conclusions/comments
Laffon B, Pásaro E, Méndez J. 2002. Evaluation of genotoxic effects in a group of workers exposed to low levels of styrene. Toxicology. Feb 28;171(2-3):175-86	Occupational styrene exposure in workers in the production of fiberglass-reinforced plastics. Sister-chromatid exchanges (SCE), micronuclei (MN), and DNA damage (evaluated by means of comet assay) were measured in peripheral blood cells from the exposed workers and from a control population.	Average estimated values for styrene exposure were slightly below the threshold limit value (TLV) of 20 ppm recommended by the American Conference of Governmental Industrial Hygienists. Significant increases ($p < or = 0.01$) have been found for SCE and MN frequencies and comet tail length among exposed individuals, as well as significant decreases ($p < or = 0.01$) in the proliferation indices, as compared with the control population. High correlation has been obtained between endpoints evaluated and exposure length, and increased values of SCE and MN frequencies and comet tail length have been found among smokers only in the exposed population.	The high correlation obtained among SCE and MN frequencies and comet tail length, and the increase of these parameters in the exposed group with regard to control group justify the use of these three biomarkers in the evaluation of genotoxic effects in human populations exposed to styrene.
Jonsson F, Johanson G. 2002. Physiologically based modeling of the inhalation kinetics of styrene in humans using a bayesian population approach. Toxicol Aopl Pharmacol. Feb 15;179(1):35-49	A pharmacokinetic model was calibrated to extensive human toxicokinetic data from three previous studies in which 24 volunteers were exposed to 50-386 ppm of styrene at rest and various levels of exercise.	Model fitting was performed in a Bayesian framework using Markov chain Monte Carlo simulation. The uncertainty around the partition coefficients and metabolic parameters for styrene was reduced. The metabolic capacity for styrene in humans was estimated to be 0.92 µmol/kg, with a lognormal standard deviation of 1.66. The estimated Vmax was 40% higher than previously estimated, whereas the population standard deviation was estimated for the first time	
Otteneder M, Lutz U, Lutz WK. 2002. DNA adducts of styrene-7,8-oxide in target and non-target	Liver samples of male and female CD rats at the end of a 2-year	Adducts were above the limit of detection only in the highest dose group (1,000 ppm), with median levels of 9 and 8	The data indicated that species- and organ-specific tumor induction by styrene was not reflected by DNA

Table 16 - Styrene

Citation	Study methods	Results	Authors' conclusions/comments
organs for tumor induction in rat and mouse after repeated inhalation exposure to styrene. Mutat Res. Mar 20;500(1-2):111-6.	exposure were available for DNA adduct analysis, even though tumor incidence was not increased. CD-1 female mice were exposed via inhalation to 0, 40, and 160 ppm styrene, 6h per day, 5 days per week for 2 weeks. Also, female CD rats were exposed via inhalation to 0 and 500 ppm.	adducts per 10^7 nucleotides in males and females, respectively (sum of alpha- and beta-adducts). The result indicated that the rat liver tolerated a relatively high steady-state level of styrene-induced DNA adducts without detectable increase in tumor formation. In none of the lung DNA samples were adducts above a limit of detection of 1 adduct per 10^7 DNA nucleotides.	adduct levels determined in tissue homogenate. The particular susceptibility of the mouse lung might have to be based on other reactive metabolites and DNA adducts, indirect DNA damage and/or cell-type specific toxicity and tumor promotion.
Westblad C Levendis YA, Richter H, Howard JB Carlson J 2002. A study on toxic organic emissions from batch combustion of styrene. chemosphere. Oct;49(4):395-412		This work focuses on emissions of polycyclic aromatic hydrocarbons (PAHs), particulates, as well as carbon monoxide fro styrene monomer. To investigate methods for minimizing such emissions, batch combustion of the styrene monomer was conducted in a two-stage muffle furnace. An additional air mixing chamber was installed between the two stages. Small quantities of the liquid monomer were inserted in the primary furnace which served as a gasifier/burner. The furnace temperature was in the range of 300-1,000 degrees C and diffusion flames were formed under most conditions. Upon mixing with additional air, combustion of unburned gaseous fuel and primary reaction products continued in the secondary furnace (afterburner), which was kept at a constant temperature of	

Table 16 - Styrene

Citation	Study methods	Results	Authors' conclusions/comments
		either 1000 or 800 degrees C. Using this technique, conditions that minimize emissions were explored and theoretical investigations on the fate of pollutants in the secondary furnace were undertaken. Results revealed that combustion of styrene, which is a highly volatile fuel, occurred with the formation of flames that were often non-anchored, unsteady and unstable. Emissions of organic pollutants, soot and CO were more intense than in the case of the polystyrene combustion, studied previously under identical conditions, due to the additional depolymerization/pyrolysis steps therein. The emissions from the secondary furnace exceeded those of the primary furnace, consistent with the fact that a very significant fraction of the fuel conversion occurred in the secondary chamber. Clear trends in the emissions of PAHs and soot, products of incomplete combustion, with the temperature of the primary furnace (gasifier) were observed. Emissions were drastically reduced with lowering the gasifier temperature. While final cumulative emissions of PAHs and soot accounted for more than one-third of the mass of the fuel at high temperatures, their concentrations at the exit of the afterburner were negligible when the primary furnace was operated at 300 degrees C under pyrolytic conditions. In the latter case air was added to the afterburner. Numerical modeling based on a complex reaction network was used	

Table 16 - Styrene

Citation	Study methods	Results	Authors' conclusions/comments
		for the description of the primary furnace as well as of the afterburner. Kinetic analysis showed acetylene and benzene to be key species in the growth of PAHs. Formation of PAHs in the afterburner, found experimentally, was reproduced by the model using a plug-flow assumption.	
Jerry H, Metten M, Gamer AO, Wuttke W. 2002. Effects of 5-day styrene inhalation on serum prolactin and dopamine levels and on hypothalamic and striatal catecholamine concentrations in male rats. Arch Toxicol. Nov;76(11):657-63.	Styrene vapor exposure in male rats via inhalation to 645, 2,150 and 6,450 mg/m_3 for 6 h/d on 5 consecutive days. Serum pituitary hormone prolactin and dopamine levels were measured by radioimmunoassay. Concentrations of catecholamines and their metabolites in the striatum and mediobasal hypothalamus (MBH) were determined by high performance liquid chromatography with electrochemical detection.	Neither in the immediate nor in the recovery group was any statistically significant changes of serum PRL levels observed. Likewise, concentrations of catecholamines and their metabolites in the striatum and MBH remained unaffected. The authors concluded from these data that styrene, even at very high concentrations, had no adverse effects on the neuroendocrine mechanisms regulating PRL release and DA levels in the brain.	With the limitations inherent in any animal model, the authors suggested that the data indicated styrene also has no adverse neuroendocrine effects in humans.
Engelhardt G, Gamer A, Vodicka P, Barta I, Hoffmann HD, Veenstra G 2003. A re-assessment of styrene-induced clastogenicity in mice in a subacute inhalation study. Arch	Styrene exposure in NMRI mice via inhalation to 750 mg/m^3 and 1,500 mg/m^3 for 6h/day for 1, 3, 7, 14 and 21 consecutive	There was no evidence of clastogenicity at any concentration or exposure interval.	

Table 16 - Styrene

Citation	Study methods	Results	Authors' conclusions/comments
Toxicol. Jan;77(1):56-61	days.		
De Piceis Polver P, Fenoglio C, Nano R, Coccini T, Bertone V, Vaccarone R, Gerzeli G 2003. Styrene hepatotoxicity in rats treated by inhalation or intraperitoneally: a structural investigation. Histol Histopathol. Jan;18(1):49-54	Styrene vapor exposure in rats via inhalation to 300 ppm, 6 h/d, 5 d/wk, for 2 wk. Also intraperitoneal exposure to 4, 40, 400 mg/kg for 3 consecutive days.	Using a light microscope, some alterations of liver parenchyma and sinusoid dilation were noticed, more marked in the group treated with the intraperitoneal administration of the chemical. Using an electron microscope, some additional changes were observed (once again, more marked in the latter group of rats): a) an increase in the content of lipids inside hepatocytes, and b) the rise of intracytoplasmic, intercellular and perisinusoidal collagen fibers.	Cell damage and functional disturbance of sinusoids due to perisinusoidal fibrosis were apparent in the liver of both groups of rats exposed to styrene treatment, but these changes were definitely more significant in those subjected to intraperitoneal administration.
Eriksson K, Wiklund L. 2004. Dermal exposure to styrene in the fibreglass reinforced plastics industry. Ann Occup Hyg. Apr;48(3):203-8.	Dermal exposure study in humans using a patch sampling technique. Samplers were fastened at 12 different spots on a sampling overall, each spot representing a body area. One patch was fastened at the front of a cap. A patch fastened to a string worn around the neck assessed the exposure at chest level inside the clothing. Patches were fastened to cotton gloves at sites representing the dorsal side and the palm of the hand to evaluate	The potential body exposure for the participating individuals was between 544 and 17 100 mg/h with a geometric mean (GM) of 3780 mg/h. The legs, arms and outer chest in general had the highest exposures. The left and right hands had mean (GM) exposures of 344 and 433 mg/h, respectively. Styrene was determined for the patch at the inside of the clothing, indicating contamination of the dermal layer.	The charcoal patch can be used to evaluate potential exposure to styrene. The results indicate that the dermal layer of the worker is exposed to styrene. Precautions should be performed to reduce dermal exposure.

Table 16 - Styrene

Citation	Study methods	Results	Authors' conclusions/comments
	exposure on these areas.		
Jerry H, Gamer A, Wuttke W. 2004. Effects of 5-day styrene inhalation on serum LH and testosterone levels and on hypothalamic and striatal amino acid neurotransmitter concentrations in male rats. Inhal Toxicol Apr;16(4):209-15.	Male rats exposed to 0, 150, 500, 1500 ppm for 6 h on 5 consecutive days.	No suppression of LH (leutenizing hormone) or T (thyroid hormone) levels was observed after styrene inhalation. LH levels of the immediate groups with 500 or 1500 ppm exposure were slightly but significantly elevated. Hypothalamic GLU and GABA concentrations remained unchanged. Increased striatal GABA concentrations were determined in recovery groups with 500 or 1500 ppm exposure. Striatal GLU concentrations remained unaffected. Thus, the authors reported to demonstrate slightly increased LH and T levels in styrene-exposed male rats after inhalation of the two higher doses. This effect did not correlate with hypothalamic GLU and GABA concentrations.	
Seeber A, Blaszkewicz M Golka K, Hallier E, Kiesswetter E, Schaper M Van Thriel C. 2004. Neurobehavioral effects of experimental exposures to low levels of styrene. Toxicol Lett. Jun 15;151(1):183-92.	Experiment I: 16 volunteers (8 in the morning, 8 in the afternoon) were exposed to 0.5 and 20 ppm styrene on a constant level for 3h. Experiment II: 24 volunteers (12 in the morning, 12 in the afternoon) were exposed for 4h to 0.5 and 20 ppm as well as to a changing exposure	On simple reaction, choice reaction, attention, acute symptoms, and ratings for well-being, exposure-related performance effects could not be detected.	Analyzing acute symptoms and the state of well-being, the impact of styrene did not reach adverse extents of impaired well-being.

Table 16 - Styrene

Citation	Study methods	Results	Authors' conclusions/comments
	between 0.5 and 40 ppm with a TWA of 14 ppm.		
Cristina Diodovich, Marco Giorgio Bianchi, Gerard Bowe, Francesco Acquati, Roberto Taramelli, Dominique Parent-Massie and Laura Gribaldo 2004. Response of human cord blood cells to styrene exposure: evaluation of its effects on apoptosis and gene expression by genomic technology. Toxicology, Volume 200. Issues 2-3, 5 August, Pages 145-157	Human cord blood cells exposed to 800pM for 24 and 48 h.	At 800 pM, styrene induced an increase in the necrosis of mononuclear cor d blood cells, whereas it did not cause any increase in the apoptotic process. Western blot analysis revealed a modified expression of Bax, BCI-2, c-Jun, c-Fos and Raf-1 proteins in the human cord blood cells after direct exposure to styrene, whereas p53 expression did not change. Macroarray analysis showed that styrene changed cord blood gene expression, inducing up-regulation of monocyte chemotacti c protein 1 (MCP-1), and down-regulation of CC chemokine receptor type 1 (CCR-1) and SLP-76 tyrosine-phosphoprotein.	Styrene changed cord blood gene expression.
Clay, P 2004. Styrene monomer does not induce unscheduled DNA synthesis in the mouse liver following inhalation exposure. Mutagenesis. Nov;19(6):489-92	Exposure in mice via inhalation to styrene monomer (dose unspecified)	The negative response observed in this assay is consistent with the theory that tumours observed in mouse oncogenicity studies are non-genotoxic in origin.	Styrene monomer did not induce unscheduled DNA synthesis in the mouse liver following inhalation exposure
Nestmann ER, Lynch BS, Ratoan F. 2005. Perspectives on the genotoxic risk of styrene. J Toxicol Environ Health B Crit Rev. Mar-Apr;8(2):95-107	Review.	The *in vivo* animal data indicated that styrene is not clastogenic at concentrations (doses) likely encountered by humans under ambient or occupational exposure conditions. DNA binding studies with styrene in rats and mice demonstrated no increased adducts in mice compared to rats or in mouse lung	Results from controlled animal studies involving in vivo exposure to styrene alone do not show clastogenic effects at exposures of up to 1500 $mg/m^3/d$. In any event, these studies show that there is an apparent threshold for styrene-mediated effects.

Table 16 - Styrene

Citation	Study methods	Results	Authors' conclusions/comments
		compared to liver. As a result, DNA adducts in the lungs are unlikely to be the sole explanation of the development of lung tumors in mice exposed to styrene for 2 yr. Some epidemiological studies reported that DNA and/or protein adducts and DNA strand breaks result from occupational exposure to styrene and/or 50. Results of some of these studies, how-ever, are difficult to interpret, given that the statistical significance of reported effects (SCE, CA, and micronucleus formation) was often near or at p values of .05; dose and/or temporal response relationships often were missing; confounding variables could not be excluded; and, concomitant exposures to other industrial chemicals that are potentially genotoxic may also have occurred. These studies suggest that styrene, through metabolism to SO, could be clastogenic in humans at workplace levels in excess of 125 mg/m^3.	
Cruzan G, Faber WD Johnson KA, Roberts LS Hellwiq J, Maurissen J, Beck MJ Radovsky A, Stump DG 2005. Developmental neurotoxicity study of styrene by inhalation in Crl-CD rats. Birth Defects Res B Dev Reprod Toxicol. Jun;74(3):221-32.	Crl:CD (SD)IGS BR rats (25/sex/group) were exposed to 0, 50, 150, and 500 ppm styrene for 6 hr daily for at least 70 consecutive days prior to mating for the FO and F1 generations. Inhalation exposure continued for the FO and F1 females throughout mating and	As expected from previous studies, slight body weight and histopathologic effects on the nasal olfactory epithelium were found in FO and F1 rats exposed to 500 ppm and, to a lesser extent, 150 ppm. There were no indications of adverse effects on reproductive performance in either the FO or F1 generation. There were exposure-related reductions in mean body weights of the F1 and F2 offspring from the mid and high-exposure groups and an overall pattern of slightly delayed development	Taken together, the exposure-related developmental and neuromotor changes identified in F2 pups from dams exposed to 500 ppm occurred in endpoints known to be both age- and weight-sensitive parameters, and were observed in the absence of any other remarkable indicators of neurobehavioral toxicity. Based on the results of this study, an exposure level of 50 ppm was considered to be the NOAEL for growth of F2

Table 16 - Styrene

Citation	Study methods	Results	Authors' conclusions/comments
	through gestation day 20. On lactation days 1 through 4, the FO and F1 females received styrene in virgin olive oil via oral gavage at dose levels of 66, 117, and 300 mg/kg/day (divided into three equal doses, approximately 2 hr apart). Inhalation exposure of the FO and F1 females was re-initiated on lactation day 5 and continued through weaning of the F1 or F2 pups on postnatal day (PND) 21.	evident in the F2 offspring only from the 500-ppm group. This developmental delay included reduced body weight (which continued through day 70) and slightly delayed acquisition of some physical landmarks of development. Styrene exposure of the FO and F1 animals had no effect on survival, the clinical condition or necropsy findings of the F2 animals. Functional observational battery evaluations conducted for all F1 dams during the gestation and lactation periods and for the F2 offspring were unaffected by styrene exposure. Swimming ability as determined by straight channel escape times measured on PND 24 were increased, and reduced grip strength values were evident for both sexes on PND 45 and 60 in the 500-ppm group compared to controls. here were no other parental exposure-related findings in the F2 pre-weaning and post-weaning functional observational battery assessments, the PND 20 and PND 60 auditory startle habituation parameters, in endpoints of learning and memory performance (escape times and errors) in the Biel water maze task at either testing age, or in activity levels measured on PND 61 in the 500-ppm group.	offspring; an exposure level of 500 ppm was considered to be the NOAEL for F2 developmental neurotoxicity.
Cruzan G, Faber WD, Johnson KA, Roberts LS Hellwig J, Carney E, Yarrington JT, Stump DG. 2005. Two generation reproduction study of styrene by	Four groups of male and female Crl:CD(SD)IGS BR rats (25/sex/group) were exposed to 0, 50, 150,	These oral dosages were calculated to provide similar maternal blood peak concentrations as provided by the inhalation exposures. Inhalation exposure of the FO and F1 females was re-initiated	An exposure level of 50 ppm was considered to be the NOAEL for FO and F1 parental systemic toxicity; the NOAEL for FO and F1 reproductive

Table 16 - Styrene

Citation	Study methods	Results	Authors' conclusions/comments
inhalation in Crl-CD rats. Birth Defects Res B Dev Reprod Toxicol. Jun;74(3):211-20.	and 500 ppm styrene for 6 hr daily for at least 70 consecutive days prior to mating for the FO and Fl generations. Inhalation exposure for the FO and F1 females continued throughout mating and gestation through gestation day 20. Inhalation exposure of the FO and F1 females was suspended from gestation day 21 through lactation day 4. On lactation days 1 through 4, the FO and F1 females received styrene in virgin olive oil via oral gavage at dose levels of 66, 117, and 300 mg/kg/day (divided into three equal doses, approximately 2 hr apart).	on lactation day 5. Styrene exposure did not affect survival or clinical observations. Rats in the 150- and 500-ppm groups in both parental generations gained weight more slowly than the controls. There were no indications of adverse effects on reproductive performance in either the FO or F1 generation. Male and female mating and fertility indices, pre-coital intervals, spermatogenic endpoints, reproductive organ weights, lengths of estrous cycle and gestation, live litter size and postnatal survival were similar in all exposure groups. Additionally, ovarian follicle counts and corpora lutea counts for the F1 females in the high-exposure group were similar to the control values. No adverse exposure- related macroscopic pathology was noted at any exposure level in the FO and F1 generations. A previously characterized pattern of degeneration of the olfactory epithelium that lines the dorsal septum and dorsal and medial aspects of the nasal turbinates occurred in the FO and F1 generation animals from the 500-ppm group. In the 500-ppm group, F2 birthweights were reduced compared to the control and F2 offspring from both the 150- and 500-ppm exposure groups gained weight more slowly than the controls	toxicity was 500 ppm or greater.
Pavel Erik Vodicka, Igor Linhart, Jan Novak, Mikko Koskinen, Ludmila Vodickova and Kari Hemminki 2006. 7-Alkylguanine	Urinary excretion of two nucleobase adducts derived from styrene 7,8- oxide (SO), i.e., 7-	Strikingly higher excretion of both isomeric nucleobase adducts in the first day of exposure was recorded, while the daily excretion of nucleobase adducts reached	The total styrene-specific 7-guanine alkylation accounts for about 1.0 x 10-5% of the total styrene uptake, while N1- adenine alkylation

Table 16 - Styrene

Citation	Study methods	Results	Authors' conclusions/comments
adduct levels in urine, lungs and liver of mice exposed to styrene by inhalation. Toxicology and Applied Pharmacology, Volume 210, Issues 1-2, January, Pages 1-8	(2-hydroxy-1-pheny 'ethyl) guanine (N7aG) and 7-(2-hydroxy-2-phenylethyl)guanine (N7 pG), as well as a formation of N7-SO-guanine adduct from the lungs and liver of NMRI male mice exposed via inhalation for 3 weeks	the steady-state level at 4.32 + 1.14 and 6.91 + 1.17 pmol/animal for lower and higher styrene exposure, respectively. 6-SO-guanine DNA adducts in lungs increased with exposure in a linear way (F = 13.7 for linearity and 0.17 for non-linearity, respectively), reaching at the 21st day the level of 23.0 adducts/108 normal nucleotides, i.e., 0.74 fmol/pg DNA of 7-alkylguanine DNA adducts for the concentration of 1,500 mg/m3, while no 7-SO-guanine DNA adducts were detected in the liver after 21 days of inhalation exposure to both of styrene concentrations. A comparison of 7 -alkylguanines excreted in urine with 7-SO-guanines in lungs (after correction for depurination and for missing a-isomers) revealed that persisting 7-SO-guanine DNA adducts in lungs account for about 0.5% of the total alkylation at N7 of guanine.	contributes to this percentage only negligibly.
Tiina Anttinen-Klemetti, Raija Vaaranrinta, Pertti Mutanen and Kimmo Peltonen 2006. Inhalation exposure to 1,3-butadiene and styrene in styrene—butadiene copolymer production. International Journal of Hygiene and Environmental Health, Volume 209, Issue 2, 17 March 2006, Pages 151-158	1,3- butadiene (BD) and styrene exposure in 28 workers from 3 manufacturing plants. Air samples were collected from the breathing zones over 4 months.	For BD, 624 samples were below the limit of quantification (LOQ), 240 samples were between the LOQ and 1 ppm, and 21 samples exceeded the threshold limit value (TLV). For styrene, 336 samples were below the LOQ, 548 samples were between the LOQ and 20 ppm. The TLV was exceeded once. The data gave a comprehensive picture of personal exposure of workers in modern SB latex manufacturing plants.	

Table 16 - Styrene

Citation	Study methods	Results	Authors' conclusions/comments
Godderis L, Aka P, Kirsch-Volders M, Veulemans H. 2007. Comparison of genotoxic potency of styrene 7,8-oxide with gamma radiation and human cancer risk estimation of styrene using the rad-equivalence approach. Mutagenesis. May;22(3):209-15.	Styrene, styrene oxide (SO) and gamma radiation treatment of leukocytes from 20 subjects. SO: 0, 0.1, 0.2, or 0.3 mM (1hr); gamma radiation: 1, 2 or 3 gray (=100, 200, 300 rad). Genotoxicity was evaluated with the cytokinesis-block micronucleus assay.	Comparison of the two slopes of the regression lines between micronuclei and dose revealed a genotoxic potency for styrene 7,8-oxide of 37 rad/mMh, corresponding with a median value derived from mutagenicity studies (1, 37, 208 rad/mMh). At 1 ppm styrene, a blood styrene 7,8-oxide concentration between 0.03×10^{-6} and 0.42×10^{-6} mM is to be expected using data of toxicokinetic models and human exposure studies. With the cancer risk per unit dose of gamma radiation as benchmark, the authors calculated a lifetime risk of acquiring a fatal lympho-hematopoietic cancer of 0.17 in 10^3 workers (between 0.037×10^{-3} and 5.0×10^{-3}) exposed to 20 ppm styrene during 40 years.	
Mikes P, Korinek M Linhart I, Krouzelka J, Frantik E, Vodicková L, Neufussova L. 2009. Excretion of urinary N7 guanine and N3 adenine DNA adducts in mice after inhalation of styrene. Toxicol Lett. Jan 10;184(1):33-7.	Styrene exposure in mice via inhalation to 600 mg/m3 or 1200 mg/m3 for 6h/day for 10 consecutive days. Styrene 7,8-oxide-derived adenine adducts as well as previously identified guanine adducts, 7-(2-hydroxy-1-phenylethyl)guanine (N7alphaG) and 7-(2-hydroxy-2-phenylethyl)guanine (N7betaG) were	The excretion profile during and after a repeated exposure was determined. The excretion was dose dependent. Total N3 adenine adducts (N3alphaA+N3betaA) excreted amounted to nearly 0.8 x 10-5% of the absorbed dose while urinary N7 guanine adducts (N7alphaG+N7betaG) amounted to nearly 1.4x10-5% of the dose.	No accumulation of adducts was observed. Due to rapid depurination from the DNA, the excretion of both N3 adenine and N7 guanine adducts ceased shortly after finishing the exposure. Both N3 adenine and N7 guanine adducts may be used as non-invasive biomarkers of effective dose reflecting only a short time exposure to styrene.

Table 16 - Styrene

Citation	Study methods	Results	Authors' conclusions/comments
	quantified by HPLC-ESI-MS.		
Truchon G, Brochu M, Tardif R. 2009. Effect of physical exertion on the biological monitoring of exposure to various solvents following exposure by inhalation in human volunteers: Ill. Styrene. J Occup Environ HVQ. Aug;6(8):460-7.	4 adult subjects (20-44yrs) exposed via inhalation to 20 ppm involving either aerobic, muscular, or both types of physical exercise for 3 or 7 hr. The target intensities for each 30-min exercise period-interspaced with 15 min at rest were the following: REST, 38 watts; AERO (time-weighted average intensity), 34 watts; AERO/MUSC, 49 watts; AERO/MUSC; AERO, 54 watts for 7 hr; and MUSC, 22 watts for 3 hr.	Compared with exposure at rest, styrene in alveolar air increased by a factor up to 1.7, while the sum of urinary mandelic acid (MA) and phenylglyoxilic acid (PGA) concentrations increased by a factor ranging from 1.2 to 3.5, depending on the exposure scenario. Concentrations of biological indicators of styrene fluctuated with physical exertion and were correlated with the magnitude of the physical activity and pulmonary ventilation. Despite the physical exertion effect, urinary concentrations of styrene metabolites after a single-day exposure remain below the current biological exposure index value recommended by ACGIH; therefore, no additional health risk is expected.	Results showed that work load intensities must be considered in the interpretation of biological monitoring data and in the evaluation of the health risk associated with styrene exposure.

Table 16 - Styrene

Welfare Effects Studies

Citation	Study methods	Results	Authors' conclusions/comments
Cushman JR, Rausina GA Cruzan G Gilbert J, Williams E, Harrass MC Sousa JV, Putt AE, Garvey NA, St Laurent JP, Hoberci JR, Machado MW. 1997. Ecotoxicity hazard assessment of styrene. Ecotoxicol Environ Saf. Jul;37(2):173-80.	Acute toxicity study of styrene in fathead minnows (*Pimephales promelas*), Daphnids (*Daphnia magna*), phipods (*Hyalella azteca*), and freshwater green algae (*Selenastrum capricornutum*), as well as a subacute toxicity study in earthworms (*Eisenia fostida*) exposed to artificial soil, renewed after 7d.	Styrene was moderately toxic to fathead minnows, daphnids, and amphipods. In fathead minnow, LC50 (96 hr) was 10 mg/liter, and the NOEC was 4.0 mg/liter; in daphnids, EC50 (48 hr) was 4.7 mg/liter, and the NOEC was 1.9 mg/liter; in amphipods, LC50 (96 hr) was 9.5 mg/liter, and the NOEC was 4.1 mg/liter. Styrene was highly toxic to green algae: the EC50 (96 hr) was 0.72 mg/liter, and the NOEC was 0.063 mg/liter. These effects were found to be algistatic rather than algicidal. Styrene was slightly toxic to earthworms: the LC50 (14 days) was 120 mg/kg and the NOEC was 44 mg/kg.	There was no indication of a concern for chronic toxicity based on these studies. Styrene's potential impact on aquatic and soil environments was significantly mitigated by its volatility and biodegradability.
Gibbs BF, Mulligan CN 1997. Styrene toxicity: an ecotoxicological assessment. Ecotoxicol Environ Saf.Dec;38(3):181-94.	Review.	In this review, the toxicological effects of styrene on humans, animals, and plants are discussed. Its mode of entry and methods of monitoring its presence are examined. Although its effects on humans and aquatic life have been studied, the data on short- or long-term exposures to plants, birds, and land animals are insufficient to be conclusive. Since exposure to workers can result in memory loss, difficulties in concentration and learning, brain and liver damage, and cancer, development of accurate methods to monitor its exposure is essential. In addition, the review outlines the present state of styrene in the environment and suggests ways to deal with its presence. It	

Table 16 - Styrene

Citation	Study methods	Results	Authors' conclusions/comments
		might appear that the quantities are not sufficient to harm humans, but more data are necessary to evaluate its effect, especially on workers who are regularly exposed to it.	
Tatarazako N, Takao Y, Kishi K, Onikura N, Arizono K, Iquchi T. 2002. Styrene dimers and trimers affect reproduction of daphnid (Ceriodaphnia dubia). Chemosphere. Aug;48(6):597-601.	Styrene dimers and trimers at 0.04-1.7 µg/l on *Ceriodaphnia dubia* bred in polystyrene cups	Styrene dimers and trimers at concentrations of 0.04-1.7 µg/l affected *C. dubia* fertility (25% reduction after seven days).	Styrene dimers and trimers were found to be eluted from the polystyrene cups by hexane and methanol. Data suggested that styrene has the potential to impair crustacean populations in the aquatic environment.

Table 17 – trans-1,3-Dimethylcyclohexane

Welfare Effects Studies

Citation	Study methods	Results	Authors' conclusions/comments
Galindo-Cuspinera V, Lubran MB, Rankin SA. 2002. Comparison of Volatile Compounds in Water- and Oil-Soluble Annatto (Bixa orellana L.) Extracts. J. Agric. Food Chem., 2002, 50 (7), 2010-2015.	A study to identify and compare volatile compounds present in water- and oil-soluble annatto extracts using dynamic headspace-solvent desorption sampling and analyzed using GC-MS.	Of the 107 compounds detected, 56 compounds were tentatively identified and 51 were positively identified. Volatile profile differences exist between water- and oil-soluble extracts, and annatto extracts contain odorants with the potential to influence food aroma.	No specific mention of trans-1,3-dimethylcyclohexane in this study.
Xie G and Barcelona MJ. 2003. Sequential Chemical Oxidation and Aerobic Biodegradation of Equivalent Carbon Number-Based Hydrocarbon Fractions in Jet Fuel. Environ. Sci. Technol., 2003, 37 (20) 4751-4760.	Study of the degradation of various hydrocarbon mixture fractions with or without prechemical oxidation (25 days) by three oxidants (KMnO4, H2O2, MgO2). Sterile and live microcosms were constituted with aquifer samples for aerobic biodegradation (134 days) of JP-4 jet fuel.	The data showed that prechemical oxidations facilitated removal of total petroleum hydrocarbons (TPH) (up to 80%) within shorter times (<50 days) than biological treatment alone. KMnO4 and H2O2 were better oxidants in terms of mass reduction in shorter times yet to some extent inhibited the subsequent microbial activity. MgO2 was a moderate oxidant with less inhibition of microbial activity. The biological processes were much less effective than the prechemical oxidations in transforming aromatic fractions, the more toxic fractions. The favorable substrates (i.e., aliphatic EC 10) for microbial growth were also those most subject to chemical oxidation.	The authors concluded that for remediation of petroleum contaminants, sequential chemical and biological technologies may surpass biological alone and more moderate oxidants such as Mg02 may be better candidates. No specific mention of trans-1,3-dimethyl-cyclohexane in this study.
Townsend GT, Prince RC, Suflita	Biodegradation of	Aquifer microorganisms exhibited previously	The authors reported that these

Table 17 – trans-1,3-Dimethylcyclohexane

Citation	Study methods	Results	Authors' conclusions/comments
JM. 2004. Anaerobic biodegradation of alicyclic constituents of gasoline and natural gas condensate by bacteria from an anoxic aquifer. FEMS Microbiology Ecology, Volume 49, Issue 1, July, 129-135.	alicyclic compounds was studied under methanogenic and sulfate- reducing conditions in samples from a gas condensate-contaminated aquifer amended with whole gasoline.	unrecognized anaerobic alicyclic hydrocarbon metabolism of a broad range of substrates at relatively rapid rates. Simple unsubstituted, methyl-substituted, and ethyl-substituted cyclopentenes, cyclopentanes and cyclohexanes were consumed without a substantial lag in the presence of sulfate, but rather less effectively under methanogenic conditions. Dimethyl-substituted cyclopentanes and cyclohexanes were biodegraded only in the presence of sulfate and a limited isomer-specific biodegradative pattern was seen.	results extended the range of hydrocarbons known to be susceptible to anaerobic decay and helped indicate the patterns of alicyclic hydrocarbon biodegradation that can be expected.
Siddique T, Fedorak PM, MacKinnon MD, Foght JM. 2007. Metabolism of BTEX and Naphtha Compounds to Methane in Oil Sands Tailings. Environ. Sci. Technol., 41 (7), 2350-2356.	BTEX (benzene, toluene, ethylbenzene, and xylenes) and whole naphtha were assessed for biodegradation under methanogenic conditions using MFT from an oil sands tailings settling basin.	Mature fine tailings (MFT) spiked with 0.05-0.1% w/v of BTEX compounds produced up to 2.1 (±0.1) mmol of methane during 36 weeks of incubation. Metabolism of 0.5-1.0% w/v naphtha in MFT yielded up to 5.7 (±0.2) mmol of methane during 46 weeks of incubation. Gas chromatographic analyses showed that BTEX degraded in the sequence: toluene > o-xylene > m- plus p-xylene > ethylbenzene > benzene. Only 15-23% of whole naphtha, mainly n-alkanes (in the sequence: nonane > octane > heptane) and some BTEX compounds (toluene > o-xylene > m-xylene), was metabolized. Other naphtha constituents, such as iso-paraffins and naphthenes, remained unchanged during this period.	The authors concluded that the microbial communities in the MFT can readily utilize certain fractions of unrecovered naphtha in oil sands tailings and support methanogenesis in settling basins.
Prince RC and Suflita JM. 2007. Anaerobic biodegradation of natural gas condensate can be stimulated by the addition of	Study of the biodegradation of a broad range of linear and	Biodegradation was observed when sediment and groundwater samples collected from a gas condensate-contaminated aquifer were incubated under	The authors reported that these findings expanded the range of hydrocarbon molecules known to undergo anaerobic decay and

Table 17 – trans-1,3-Dimethylcyclohexane

Citation	Study methods	Results	Authors' conclusions/comments
gasoline. Biodegradation, Volume 18, Number 4, 515-523.	branched alkanes, parent and alkyl alicyclic hydrocarbons, and benzene and alkyl-substituted benzenes.	methanogenic and especially under sulfate-reducing conditions, even though no exogenous nitrogen or phosphorus was added.	confirms that natural attenuation is an important process at this site.

Table 18 – trans-1,4-Dimethylcyclohexane

Health Effects Studies

Citation	Study methods	Results	Authors' conclusions/comments
M. Karelson; S. Sild; U. Maran 2000. Non-linear QSAR Treatment of Genotoxicity. Molecular Simulation, Volume 24, Issue 4 & 6, Pages 229 - 242	Ames test of heteroaromatic and aromatic amines.	The mutagenic toxicity of the compounds correlated with the molecular descriptors calculated from the molecular structures using quantum-chemical methods. The quantitative models obtained were compared with the results of the linear QSAR treatment. The descriptors appearing in the models revealed the importance of mutagenic interactions of heteroaromatic amines via hydrogen bonding, of effects induced by the solvent, and of the size of compound.	The dependence of molecular descriptors on environmental effects and on molecular conformations was analyzed.
Toshiaki Yoshida and Ichiro Matsunaga 2006. A case study on identification of airborne organic compounds and time courses of their concentrations in the cabin of a new car for private use. Environment International, Volume 32, Issue 1, January, Pages 58-79	162 organic compounds including trans-1,4-dimethylcyclohexane were measured as organic compounds diffusing from the interior materials used in the interior of automobiles.	Trans-1,4-dimethylcyclohexane measured in the interior air samples of cars on the day after delivery was 3.2 μg/m^3.	No toxicological effects were examined in this study.
Toshiaki Yoshida, Ichiro Matsunaga, Kimiko Tomioka, Shinji Kumagai 2006. Interior Air Pollution in Automotive Cabins by Volatile Organic Compounds Diffusing from Interior Materials: I. Survey of 101 Types of Japanese Domestically Produced Cars for Private Use. Indoor and Built	275 organic compounds were sampled in the interior air of 101 different types of Japanese domestically produced private-use cars were	The airborne compounds in the cabins were collected for 24 hr under static conditions with the engine stopped and the windows, doors and vents closed. The sum of the concentrations of 241 compounds excluding formaldehyde was approximately 600 g/rn^3 as a median, ranging from 136 to 3,968 g/rn^3 for the tested cars.	The findings demonstrated that the air in the cabin of these cars was contaminated by high concentrations of e large variety of organic compounds diffusing from the interior materials.

Table 18 – trans-1,4-Dimethylcyclohexane

Citation	Study methods	Results	Authors' conclusions/comments
Environment October vol. 15 no. 5 425-444	examined.		
Toshiaki Yoshida, Ichiro Matsunaga, Kimiko Tomioka, Shinji Kumagai 2006. Interior Air Pollution in Automotive Cabins by Volatile Organic Compounds Diffusing from Interior Materials: II. Influence of Manufacturer, Specifications and Usage Status on Air Pollution, and Estimation of Air Pollution Levels in Initial Phases of Delivery as a New Car. Indoor and Built Environment October, vol. 15 no. 5 445-462	Levels of air pollution in the initial phases of delivery as a new car were estimated using previous data for the time-courses of interior concentrations of organic compounds measured in another new car.	The findings showed greater air pollution in the cabins of luxury cars, with leather seals or leather steering wheels, or high-end catalogue prices. Differences in the specifications contributed more markedly to interior air pollution than differences in manufacturers. Also, usage status, such as everyday ventilation affected the long-term interior air quality. The sum values of interior concentrations of 154 compounds, for which there were time-course data, were estimated to be approximately 1,700 pg/m^3 as a median (max. 11,000 pg/m^3) at 1 month from delivery (interior temperature, 32 ^oC; interior humidity, 46%).	

Table 18 – trans-1,4-Dimethylcyclohexane

Welfare Effects Studies

Citation	Study methods	Results	Authors' conclusions/comments
Guibo Xie and Michael J. Barcelonai 2003. Sequential Chemical Oxidation and Aerobic Biodegradation of Equivalent Carbon Number-Based Hydrocarbon Fractions in Jet Fuel. Environ, Sci, Technol. 2003, 37, 4751-4760	Examination of chemical and biological degradation of 87 individual hydrocarbons grouped into nine equivalent carbon numbers (EC) fractions. With or without prechemical oxidation (25 days) by (KMn04, H202, Mg02). Sterile and live microcosms were constituted with aquifer samples for aerobic biodegradation	The data show that prechemical oxidations facilitated removal of total petroleum hydrocarbons (TPH) (up to 80%) within shorter times (<50 days) than biological alone. KMn04 and H202 were better oxidants in terms of mass reduction in shorter times yet to some extent inhibited the subsequent microbial activity. Mg02 was a moderate oxidant with less inhibition of microbial activity. Selective degradation of the EC fractions was observed for both chemical and biological processes. The biological processes were much less effective than the prechemical oxidations in transforming aromatic fractions, the more toxic fractions. The favorable substrates (i.e., aliphatic EC —10) for microbial growth were also those most subject to chemical oxidation.	Not applicable The results suggest that for remediation of petroleum contaminants, sequential chemical and biological technologies may surpass biological alone and more moderate oxidants such as Mg02 may be better candidates. More work is needed on the optimal dose and residence time for applied oxidants and on the application to engineering design and formulation of cleanup standards.

Attachments – Literature Search Results

Attachment A – 1,2,3-Trimethylbenzene

Health Effects Studies (acute toxicity, subchronic toxicity, chronic toxicity – e.g., including reproductive/developmental toxicity, neurotoxicity, immunotoxicity, organ system effects, mutagenicity/genotoxicity, carcinogenicity)

Title: Gasoline and kerosene components in blood – a forensic analysis.
Author(s): Kimura K Negate T Hara K, Kaqeura M.
Source: Hum Toxicol. 1988 Jul;7(4):299-305.
Abstract: A reliable method to analyse small amounts of fuel components in biological materials, using two simultaneous procedures, head space and solvent extraction methods has been developed. Gas chromatography/mass spectrometry (GC/MS) was used for qualitative and quantitative determinations. The aliphatic hydrocarbons with carbon numbers of 5 to 8 and aromatics such as benzene, toluene and xylenes were detected in laboratory animals, following exposure to gasoline vapour, using the head space method. Aliphatic hydrocarbons with carbon numbers over 9 as well as the aromatics with carbon number 9 group including cumene, mesitylene, pseudocumene and 1,2,3-trimethylbenzene were determined by the solvent extraction method following exposure to kerosene vapour. The lower limits of detection were 0.01 micrograms and 50 pg in gasoline and kerosene components, respectively. The methods were found to be applicable in confirming the cause of human deaths.

Title: Neurotoxic effects of acute and subchronic inhalation exposure to trimethylbenzene isomers (pseudocumene, mesitylene, hemimellitene) in rats.
Author(s): Korsak Z, Rydzynski K.
Source: Int J Occup Med Environ Health. 1996;9(4):341-9.
Abstract: Neurotoxic effects of trimethylbenzene isomers (pseudocumene, mesitylene and hemimellitene) in male rats were investigated in conditions of acute and subchronic inhalation exposure. Rotarod performance and pain sensitivity behaviour were tested in rats exposed to trimethylbenzenes at concentrations of 250-2,000 ppm immediately after termination of a 4- hour exposure. Exposure to each of trimethylbenzene isomers resulted in concentration-dependent disturbances in rotarod performance, and decrease in pain sensitivity in rats. Pseudocumene, mesitylene and hemimellitene EC50 values for rotarod performance behaviour disturbances were 954, 963, 768 ppm and for decreases in pain sensitivity EC50 were 1,115, 1,212, 848, ppm, respectively. In conditions of subchronic inhalation exposure, pseudocumene and hemimellitene at concentrations of 25, 100 and 250 ppm caused concentration-dependent disturbances in rotarod performance behaviour and decrease in pain sensitivity. Neurotoxic effect of hemimellitene was more pronounced than that of pseudocumene and mesitylene. Two weeks after cessation of inhalation exposure to pseudocumene or hemimellitene no recovery in rotarod performance

Attachment A – 1,2,3-Trimethylbenzene

behaviour was observed.

Title: Respiratory irritative effects of trimethylbenzenes: an experimental animal study.
Author(s): Korsak Z, Rvdzviiski K, Jaffe J.
Source: Int J Occup Med Environ Health, 1997;10(3):303-11.
Abstract: Sensory respiratory irritation effects of trimethylbenzene isomers (TMBs) (hemimellitene, mesitylene and pseudocumene) in male Balb/C mice were investigated in conditions of acute exposure and in male Wistar rats in conditions of repeated 90-day inhalation exposure to pseudocumene. The pseudocumene, mesitylene and hemimellitene concentrations depressing the respiratory rate to 50% (RD50) were 578, 519, 541 ppm, respectively. Inhalation exposure to pseudocumene for 90 days increased the total number of cell macrophages, polymorphonuclear leukocytes and lymphocytes number at all three test concentrations compared with the controls. Total protein lactate dehydrogenase (LDH) and acid phosphatase activity in bronchoalveolar lavage (BAL) were significantly increased in all exposed groups. Based on the effects observed in the respiratory tract, the threshold limit value of at least 10 ppm should be considered for the occupational exposure to trimethylbenzene isomers.

Title: Biological monitoring of experimental human exposure to trimethylbenzene.
Author(s): Kostrzewski P, Wiaderna-Brycht A, Czerski B.
Source: Sci Total Environ. 1997 Jun 20;199(1-2):73-81.
Abstract: Trimethylbenzene (TMB) is a component of numerous commercial preparations of organic solvents (Farbasol, Solvesso, Shellsol) used in the chemical, plastics, printing and other industries. TMB is a mixture of three isomers (pseudocumene-1,2,4-TMB; mesitylene-1,3,5-TMB; hemimellitene-1,2,3-TMB). The proportion of individual isomers in the mixture differs. The aim of this study was to obtain toxicokinetic data on the absorption and elimination of trimethylbenzene and its metabolites in biological fluids and to investigate the relationship between the biological indices of exposure and the absorbed dose. Eight-hour inhalation tests were performed in a toxicological chamber. The subjects were eight volunteers aged 20-39 with no history of exposure to TMB. They were exposed to pseudocumene, mesitylene or hemimellitene at concentrations ranging from 5 to 150 mg/m3 air. Exhaled air, capillary blood and urine samples were collected before, during and after the exposure. The determinations of TMB or its metabolites were performed using gas chromatography (HP 5890 II Plus, MSD, FID). Pulmonary ventilation in the volunteers ranged from 0.56 to 1.0 m3/h. The retention of 1,2,4-TMB; 1,3,5-TMB; 1,2,3-TMB in the lungs was 68%, 67% and 71%, respectively. The elimination of TMB from capillary blood occurred in accordance with the open three-compartment model. Urinary excretion of dimethylbenzoic acids (DMBA) proceeded according to the open two-

Attachment A – 1,2,3-Trimethylbenzene

compartment model. Based on the toxicokinetic data, a simulation model of accretion and excretion of DMBA in urine during a 14-day period was developed. The highest rates of metabolite excretion and the highest quantities of DMBA in urine during 24-h intervals were observed on day 5 of exposure. The relationship between the levels of TMB or DMBA in biological material and TMB air concentration or absorbed dose were determined. To select the urine fraction suitable for determining occupational TMB exposure, linear regression analysis was performed. The biological exposure limit (BEL) for TMB has been proposed, with the current maximum allowable concentration (MAC) value of 100 mg/m3 (Polish standard) baseline value.

Title: Behavioural changes following a four-week inhalation exposure to hemimellitene (1,2,3-trimethylbenzene) in rats.

Author(s): Wiaderna D, Gralewicz S, Tomas T.

Source: Int J Occup Med Environ Health. 1998;11(4):319-34.

Abstract: Trimethylbenzene isomers (TMBs): 1,2A-TMB (pseudocumene--PS), 1,2,3-TMB (hemimellitene--HM) and 1,3,5-TMB (mesitylene--MES) are important constituents of solvent mixtures. In the US, the adopted TLV-TWA value for TMBs is 125 mg/m3 or 25 ppm (ACGIH 1996). Recent experiments at our laboratory have revealed an impaired learning of passive and active avoidance responses and a longer persistence of an effect of footshock (increase in latency of the paw-lick response to heat) in rats tested several weeks after a four-week inhalation exposure (6h/day, five days/week) to PS at a concentration of 100 or 250 ppm (15). The concentration-effect relationship appeared to be nonlinear; the effect of 100 ppm HM was more pronounced than that of 250 ppm. In the present experiment we investigated the effects of a repeated four-week (6h/day, 5 days/week) inhalation exposure to HM at concentrations of 0, 25, 100 or 250 ppm on radial-maze performance, open-field activity, passive and active avoidance learning, and on the shock-induced changes in latency of the paw-lick response to heat (hot-plate test). The tests were performed between days 14 and 61 after the last exposure. No significant effects on radial-maze performance and open-field activity were noted in any of the dose groups. In the remaining tests effects of exposure were noted but, similarly as in the case of PS exposure, the concentration-effect relationship was not linear. In rats exposed to HM at 25 or 100 ppm, but not 250 ppm, learning of the passive avoidance, i.e. refraining from performance of a punished response (stepping off an elevated platform) was significantly impaired. Moreover, in rats exposed to 100, but not 250 ppm of HM, acquisition of the two-way active avoidance in the shuttle-box was slower and the footshock-induced increase in latency of the paw-lick response to heat persisted longer than in the unexposed animals. The results suggest that a low-level inhalation exposure to HM, just like low-level exposure to PS, may lead to long-

Attachment A – 1,2,3-Trimethylbenzene

lasting disturbances in the CNS functions. The nonlinear concentration-effect relationship observed in the case of both TMBs requires clarification in further studies.

Title: Genotoxicity evaluation of trimethylbenzenes.
Author(s): Janik-Spiechowicz E Wyszvnska K, Dziubattowska E.
Source: Mutat Res. 1998 Feb 13;412(3):299-305.
Abstract: The three trimethyl isomers of benzene (hemimellitene, 1,2,3-TMB; pseudocumene, 1,2,4-TMB and mesitylene, 1,3,5-TMB) were investigated for different genotoxicity endpoints: in vitro, in the Ames test with Salmonella typhimurium TA97a, TA98, TA100 and TA102 strains in the presence and absence of rat liver S9 metabolic activation; in vivo, in the micronucleus and sister chromatid exchange (SCE) tests with bone marrow cells of Imp:Balb/c mice. Only the isomer of benzene with the methyl-group at position 1, 2, 3 was found to have mutagenic effect on S. typhimurium cells. Increase in bacterial reversions was observed in four conventional strains used in this study, but most clearly in TA97a. The mutagenic responses of 1,2,3-TMB with the Salmonella tester strains were observed in the experiments performed in the absence of enzymatic activation. None of the compounds had an influence on the frequency of micronucleated polychromatic erythrocytes in bone marrow cells of mice. However, all the three compounds were observed to have a cytogenetic potential of increasing the SCE level in these cells. Significant responses in SCE induction, compared with the level of those changes in corresponding solvent-administered controls, were obtained at three test doses of 1,2,3-TMB (730, 1470, 2200 mg/kg) and 1,2,4-TMB (900, 1800, 2700 mg/kg) and at two doses of 1,3,5-TMB (1800, 2700 mg/kg). These data provided a limited evidence for the genotoxic activity of 1,2,3-TMB and inadequate evidence for genotoxic activity of 1,2,4-TMB and 1,3,5-TMB.

Title: A comparison of the long-term behavioral effect after 28 days inhalation exposure to dimethylbenzene (M-xylene) and isomers of trimethylbenzene (pseudocumene, hemimellitene, mesitylene) in rat
Author(s): Wiadema D.; Gralewicz S.; Tomas T.
Source: Toxicology. Letters, Volume 95, Supplement 1, July 1998, pp. 69-69(1).
Abstract: Not available.

Title: Neurotoxicity assessment of selected organic solvents based on spontaneous and evoked cortical and hippocampal activity in rats.
Author(s): Tomas T, Wiaderna D, Swiercz R.
Source: Int J Occup Med Environ Health. 1999;12(1):73-84.
Abstract: In a series of acute experiments on rats the potential of toluene, mesitylene,

Attachment A – 1,2,3-Trimethylbenzene

hemimellitene and pseudocumene to affect the CNS function was assessed following an analysis of spontaneous and evoked hippocampal and cortical activity. The electrophysiological examinations were performed on rats with recording electrodes chronically implanted into selected brain structures. Solvent concentration in peripheral blood was determined by gas chromatography combined with the head space technique in rats with no surgical treatment. The experiments revealed significant quantitative differences between hippocampal and cortical EEG after i.p. injections of equimolar doses of the solvents. A relationship was found between the changes in spontaneous EEG and blood concentration of the solvents. Hemimellitene, with the lowest recorded blood level was found to have the highest potential for inducing the CNS effects.

Title: Synthesis of regioisomeric dimethylbenzyl mercapturic acids anticipated from the metabolism of 1,2,3-trimethylbenzene

Author(s): Yuji Tsujimoto, Tsutomu Noda, Mitsuru Shimizu, Hiroshi Moriwaki and Masanobu Tanaka

Source: Chemosphere, Volume 38, Issue 9, April 1999, Pages 2065-2070.

Abstract: The synthesis of two regioisomeric mercapturic acids, N-acetyl-S-(2,3-dimethylbenzyl)-L-cysteine and N-acetyl-S-(2,6-dimethylbenzyl)-L-cysteine, was undertaken t o investigate the operation of mercapturic acid pathway in the metabolism of 1,2,3-trimethylbenzene. The method applied was based on that we described recently in the synthesis of mercapturic acids derived from m- and p-xylenes.

Title: Identification of the dimethylbenzyl mercapturic acid in urine of rats treated with 1,2,3- trimethylbenzene

Author(s): Yuji Tsujimoto, Tsutomu Noda, Mitsuru Shimizu, Hiroshi Moriwaki and Masanobu Tanaka

Source: Chemosphere, Volume 39, Issue 5, August 1999, Pages 725-730.

Abstract: The structure was investigated of the mercapturic acid excreted in urine of rats after the ip administration of 1,2,3-trimethylbenzene. Of the two regioisomeric mercapturic acids; i.e., N-acetyl-S-(2,3-dimethylbenzyl)-L-cysteine and N-acetyl-S-(2,6-dimethyl-benzyfi-L-cysteine, only the former was isolated by preparative HPLC and identified, by comparison with an authentic specimen. The excretion rate of the mercapturate was estimated to be ~5% of dose, not a substantial metabolic route.

Attachment A – 1,2,3-Trimethylbenzene

Title: Effects of acute exposure to aromatic hydrocarbons C 9 on locomotor activity in rats. Trimethylbenzene isomers.
Author(s): Tomas T, Swiercz R, Wiaderna D, Gralewicz S.
Source: Int J Occup Med Environ Health. 1999;12(4):331-43.
Abstract: This study was performed to find out whether in acute exposure to trimethylbenzene (TMB) isomers the dose effect relationship is linear or biphasic. In experiments performed on rats, the effect of four solvents was studied: three TMB isomers: 1,3,5-TMB (mesitylene), 1,2,3-TMB (hemimellitene), and 1,2,4-TMB (pseudocumene), and toluene, known for its biphasic activity, was used as a reference compound. The solvents were dissolved in olive oil and administered to rats orally at the doses of 0.008, 0.016, and 0.032 mol/kg. Spontaneous locomotor activity was assessed with the open-field test. Solvent concentrations in peripheral blood were determined parallelly by gas chromatography on separate groups of animals. Statistics employed a two-way analysis of variance (ANOVA) and Tukey's test. The results showed that oral administration of toluene at a dose of 0.008 mol/kg induced biphasic changes in the animal locomotor activity. It was found that TMB at applied doses increased slightly the animal locomotor activity, but the magnitude of changes did not indicate their stimulating effect. Contrary to toluene, no time-effect relationship was observed after administration of trimethylbenzene isomers. The mean blood concentrations of solvents were dose-related. The highest concentrations were observed after toluene administration.

Title: Changes in electrocortical arousal following acute trimethylbenzene administration in rats.
Author(s): Tomas T, Lutz P, Wiaderna D.
Source: Int J Occup Med Environ Health. 2000;13(1):67-78.
Abstract: The purpose of this investigation was to compare the neurotoxic potential of trimethylbenzene (TMB) isomers (the solvents) with that of benzene derivatives with a smaller number of methyl groups (toluene). The experiments were performed on WAG/Rij rats with EEG recording electrodes implanted in the fronto-parietal cortex. The solvents, toluene or TMB isomers: 1,3,5-TMB (mesitylene), 1,2,3-TMB (hemimellitene) or 1,2,4-TMB (pseudocumene), were diluted with olive oil and administered intragastrically via gavage at an acute dose of 0.002, 0.008, or 0.032 mol/kg. The electrocortical activity was recorded for 20 min before, and for 60 min after the solvent administration. The electrocorticograms were analysed with respect to the number and duration of the high-voltage spindles (HVS), a form of activity sensitive to the arousal level. In case of each solvent the observed effect--inhibition of the HVS activity--was dose-related. However, the effect produced by TMB isomers was in each case less pronounced than that of toluene. Among TMBs, pseudocumene displayed

Attachment A – 1,2,3-Trimethylbenzene

the least significant effect, and the efficacy of two other TMB isomers was similar.

Title: Subchronic inhalation toxicity of 1,2,3-trimethylbenzene (hemimellitene) in rats.
Author(s): Korsak Z, Stetkiewicz J, Maicherek W, Stetkiewicz I, Jaite J Rvdzynski K.
Source: Int J Occup Med Environ Health. 2000;13(3):223-32.
Abstract: Toxic effects of exposure to 1,2,3-trimethylbenzene (hemimellitene) in the condition of subchronic inhalation experiment were examined. Rats were exposed to vapours of hemimellitene at concentrations of 123 mg/m3, 492 mg/m3 and 1230 mg/m3, 6 h/day, 5 days/week for 3 months. After termination of a 3-month inhalation, animals were necropsied. Blood samples were obtained and selected organs were weighed and prepared for histological examinations. Subchronic inhalation exposure to hemimellitene resulted in an overall, low systemic toxicity. There were no changes in body weight gain and food consumption. At a concentration of 1230 mg/m3, the increase in relative liver weight was observed in male rats. It was accompanied by slight increase in sorbitol dehydrogenase activity. The increase in alkaline phosphatase activity was found in females only. Some disturbances in haematological parameters, characterised by the decrease in red blood cells and slight increase in white blood cells, segmented neutrophils and lymphocytes were observed in rats at high exposure concentration of 1230 mg/m3. The pulmonary lesions as well as the increased number of goblet cells and interstitial lung parenchyma infiltration were noted in male and female rats from the highest exposure groups.

Title: Behavioral Effects Following Subacute Inhalation Exposure to m-Xylene or Trimethylbenzene in the Rat: A Comparative Study
Author(s): Siawomir Gralewicz and Dorota Wiaderna
Source: NeuroToxicology, Volume 22, Issue 1, February 2001, Pages 79-89.
Abstract: Trimethylbenzene (TMB), like xylene (dimethylbenzene), is a significant constituent of some industrial solvent mixtures. In earlier studies, we found that in the rat a subacute low-level inhalation exposure to some of the TMB isomers may result in behavioral alterations detectable weeks after the exposure [Neurotoxicol Teratol 19;1997:327; Int J Occup Med Environ Health 11;1998:319). The purpose of the present study was to compare m-xylene (XYL) and each of the TMB isomers: 1,2,3-TMB (hemimellitene – HM), 1,2,4TMB (pseudocumene – PS), and 1,3,5-TMB (mesitylene – MES) with respect to the ability for inducing behavioral effects in the rat. The rats (10-11 animals per group) were exposed repeatedly for 4 weeks (6 h per day, 5 days per week) to XYL (XYL group), HM (HM group), PS (PS group) or MES (MES group) at 100 ppm, or sham exposed (C group) in 1.3 cu/m dynamic inhalation chambers. Starting 2 weeks after exposure the following forms of rat's behavior were assessed: radial maze performance, spontaneous activity in an open field,

Attachment A – 1,2,3-Trimethylbenzene

learning and retention of passive and active (two-way) avoidance response, and heat-induced paw licking before and after a 2 min footshock (a test for assessment of the stress response). None of the solvent-exposed groups differed considerably from the control one with respect to the radial maze performance. Compared to control rats, the rats of the XYL, PS and MES groups, but not those of HM group, showed a significantly higher spontaneous locomotor activity in the open field, an impaired passive avoidance learning and significantly longer paw-lick latencies 24 h after footshock. Acquisition, but not retention, of the two-way active avoidance response was significantly impaired in all solvent-exposed groups. The XYL group did not differ significantly from PS, MES or HM group in any of the behavioral parameters. The above results show that a short-term exposure to any of the TMB isomers or m-xylene at concentration as low as 100 ppm may induce persistent behavioral alterations in the rat.

Title: Effects of occupational exposure to a mixture of solvents on the inner ear: a field study.

Author(s): Sulkowski WJ, Kowalska S, Matvia W, Guzek W, Wesotowski W, Szimczak W, Kostrzewski P.

Source: Int J Occup Med Environ Health. 2002:15(3):247-56.

Abstract: Some clinical and laboratory studies indicate that industrial solvents such as toluene, styrene, xylene, trichloroethylene and carbon disulfide or their mixtures may affect the inner ear, although the mechanism of this process is still not well understood. The aim of this investigation was to assess the incidence of hearing and vestibular disorders (using modem audiological and vestibular tests) in 61 workers exposed to a mixture of organic solvents at the production of paints and varnishes; the control group included 40 age-matched non-exposed subjects. Environmental and biological monitoring revealed that the most significant exposure can be attributed to the following mixture constituents: ethylbenzene, xylene and trimethylbenzene isomers such as pseudocumene, mesitylene and hemimellitene. Electronystagmographic examinations showed the symptoms of vestibular dysfunction, as well as the decreased duration, amplitude and slow phase angular velocity of induced nystagmus in 47.5% of the subjects exposed versus 5% of controls. This was accompanied by sensorineural high frequency hearing loss, identified by means of pure tone audiometry in 42% of those exposed versus 5% controls, and reduced amplitudes of transiently evoked and distortion-product otoacoustic emissions. The findings closely correspond with the rate of the total exposure to the solvent mixture. A possible mechanism responsible for ototoxicity of solvents is discussed.

Attachment A – 1,2,3-Trimethylbenzene

Title: Case-control study of multiple chemical sensitivity, comparing haematology, biochemistry, vitamins and serum volatile organic compound measures.
Author(s): Baines CJ, McKeown-Evssen GE Riley N Cole DE, Marshall L Loescher B Jazmaji V.
Source: Occup Med (Lond). 2004 Sep;54(6):408-18. Epub 2004 Sep 3.
Abstract: Not available.

Title: Skin analysis to determine causative agent in dermal exposure to petroleum products
Author(s): Yoko Hieda, Yoshio Tsujino and Haruo Takeshita
Source: Journal of Chromatography B, Volume 823, Issue 1, 25 August 2005, Pages 53-59.
Abstract: This study evaluates the usefulness of skin analysis to determine the causative agent in cases of dermal exposure. The study consists of an animal experiment and two human cases. The petroleum components detected at high concentrations in skin samples resembled the composition of those in the corresponding petroleum products. However, the petroleum components in blood were detected at low concentrations and were a different composition. Skin is considered to be an advantageous sample to estimate the petroleum product in clinical and forensic cases of dermal exposure.

Title: Determination of benzenic and halogenated volatile organic compounds in animal-derived food products by one-dimensional and comprehensive two-dimensional gas chromatography-mass spectrometry
Author(s): Jeremy Ratela and Erwan Engel
Source: Journal of Chromatography A, Volume 1216, Issue 45,6 November 2009, Pages 7889-7898.
Abstract: Animal-derived products are particularly vulnerable to contamination by volatile organic compounds (VOCs). These lipophilic substances, which are generated by an increasing number of sources, are easily transferred to the atmosphere, water, soil, and plants. They are ingested by livestock and become trapped in the fat fraction of edible animal tissues. The aim of this work was to determine the occurrence, risk for human health and entryways of benzenic and halogenated VOCs (BHVOCs) in meat products, milks and sea foods using gas chromatography-mass spectrometry (GC-MS) techniques. In the first part, the occurrence and levels of the BHVOCs in animal products were studied. One muscle and three fat tissues were analysed by GC-Quad/MS in 16 lambs. Of 52 BHVOCs identified, 46 were found in the three fat tissues and 29 in all four tissues, confirming that VOCs are widely disseminated in the body. Twenty-six BHVOCs were quantified in fat tissues, and risk for consumer health was assessed for six of these compounds regulated by the US Environmental

Attachment A – 1,2,3-Trimethylbenzene

Protection Agency (EPA). The BHVOC content was found to be consistent with previous reports and was below the maximum contaminant levels set by the EPA. In the second part, the performance of GCxGC-TOF/MS for comprehensively detecting BHVOCs and showing t heir entryways in animal-derived food chains was assessed. Meat, milk and oysters were analysed by GC-Quad/MS and GCxGC-TOF/MS. For all these products, at least a 7-fold increase in the contaminants detected was achieved with the GCxGC-TOF/MS technique. The results showed that the production surroundings, through animal feeding or geographical location, were key determinants of BHVOC composition in the animal products.

Title: Contrasting effects of 4-week inhalation exposure to pseudocumene or hemimellitene on sensitivity to amphetamine and propensity to amphetamine sensitization in the rat

Author(s): Piotr Lutz, Stawomir Gralewicz, Dorota Wiaderna, Radoslaw Swierczi, Zofia Grzelinska, Wanda Majcherek

Source: International Journal of Occupational Medicine and Environmental Health, Volume 23, Number 1 2010.

Abstract: Not available.

Attachment A – 1,2,3-Trimethylbenzene

Welfare Effects Studies (toxicity in aquatic organisms, toxicity in terrestrial species, toxicity to plants/bacteria, effects on visibility/weather/climate, effects on materials, economic effects, effects on personal comfort and well-being)

Title: Environmental fate modeling of chlorodioxins: Determination of physical constants
Author(s): G.R.B. Webster, K.J. Friesen, L.P. Sarna and D.C.G. Muir
Source: Chemosphere, Volume 14, Issues 6-7, 1985, Pages 609-622.
Abstract: Environmental fate modeling of polychlorinated dibenzo-P -dioxins (PCDDs) requires knowledge of a number of fundamental physical parameters of these compounds , viz., octanol-water partition coefficient (K_{ow}), solubility in water (S_w), vapor pressure (p), and Henry's constant (H). Classical methodology for determination of these parameters is often not suitable for use with compounds as hydrophobic as PCDDs, Methods have been developed or refined to enable these properties to be measured.

Title: Photochemical Nitration by Tetranitromethane. Part XXVI. Adduct Formation in the Photochemical Reaction of 1,2,3-Trimethylbenzene: the Formation of „Double' Adducts Including Nitronic Esters
Author(s): Butts, C. P., Eberson, L., Hartshorn, M. P., Robinson, W. T., Timmerman-Vaughan, D. J. and Young, D.A.W.
Source: Acta Chemica Scandinavica. 1996: 50: 29-47.
Abstract: The photolysis of the charge-transfer complex of l,2,3-trimethylbenzene and tetranitromethane gives a complex mixture of products, most of which arise by initial attack of trinitromethanide ion on the unsubstituted ring positions at C4(C6) and C5 of the radical cation of 1,2,3-trimethylbenzene. The products 7-19 are adducts resulting directly or indirectly from the addition of the elements of tetranitromethane to 1,2,3-trimethylbenzene, and the trinitromethyl aromatic compounds 22-25 are formed by eliminations from intermediate adducts. Six adducts are simple „single' adducts, nitro-trinitromethyl adducts 7, 8, 10-12, while nitro cycloadduct 9 is formed by cycloaddition of nitro-trinitromethyl adduct 8. The remaining addition products are "double" adducts, formed by secondary addition reactions initiated by attack of nitrogen dioxide on the buta-l,3-diene system of „single' adducts, and include trinitro-trinitromethyl compounds 13 and 15, the hydroxy-dinitro-trinitromethyl compound 14, and a group of four nitronic esters 16-19 formed by nitro-denitrocyclization of initially formed hydroxy-trinitromethyl and nitro-trinitromethyl 'single' adducts. Minor amounts of other products are formed including two nitrodienones 21 and 22, and the rearrangement product, 4,5,6-trimethyl-2-nitrophenol (28), and the 2,3,4-trimethyl- and 3,4,5 trimethylnitrobenzenes 26 and 27. The modes of formation of the above products are discussed, and X-ray crystal structure determinations are reported for

Attachment A – 1,2,3-Trimethylbenzene

compounds.

Title: Degradation of Trimethylbenzene Isomers by an Enrichment Culture under N(inf2)O-Reducing Conditions
Author(s): A Haner, P Hohener and J Zeyer
Source: Appl. Environ. Microbiol., Mar 1997, 1171-1174, Vol 63, No. 3.
Abstract: A microbial culture enriched from a diesel fuel-contaminated aquifer was able to grow on 1,3,5- trimethylbenzene (1,3,5-TMB) and 1,2,4-TMB under N(inf2)0-reducing conditions, but it did not degrade 1,2,3- TMB. The oxidation of 1,3,5-TMB to CO(inf2) was coupled to the production of biomass and the reduction of N(inf2)O. N(inf2)O was used to avoid toxic effects caused by NO(inf2)(sup-) accumulation during growth with NO(inf3)(sup-) as the electron acceptor. In addition to 1,3,5-TMB and 1,2,4-TMB, the culture degraded toluene, m-xylene, p-xylene, 3-ethyltoluene, and 4-ethyltoluene.

Title: Products of the Gas-Phase Reactions of OH Radicals with p-Xylene and 1,2,3- and 1,2,4-Trimethylbenzene: Effect of NO_2 Concentration
Author(s): Heidi L. Bethe, Roger Atkinson, and Janet Arey
Source: J. Phys. Chem. A, 2000, 104 (39), pp 8922-8929.
Abstract: Products of the gas-phase reactions of the OH radical with p-xylene and 1,2,3- and 1,2,4trimethylbenzene have been measured by gas chromatography in the presence of varying concentrations of NO_2. Our product analyses show that the ring-cleavage products 2,3butanedione (from 1,2,3- and 1,2,4-trimethylbenzene) and 3-hexene-2,5-dione (from p-xylene and 1,2 ,4-trimethylbenzene) exhibit a dependence of their formation yields on the NO_2 concentration, with higher yields from the reactions of the OH-aromatic adducts with O_2 than from their reactions with NO_2. Furthermore, our data show that these ring-cleavage products are primary products of the OH-aromatic adduct reactions. Formation yields extrapolated to zero NO_2 concentration should be applicable to ambient atmospheric conditions (provided that there is sufficient NO that peroxy radicals react dominantly with NO), and are from *p*xylene, p-tolualdehyde, 0.0706 ± 0.0042 (independent of NO_2 concentration), 2,5dimethylphenol, 0.138 ± 0.016 (independent of NO_2 concentration), and 3-hexene-2,5-dione, 0.32 (extrapolated); from 1,2,3-trimethylbenzene, 2,3-butanedione, 0.52 (extrapolated); and from 1,2,4-trimethylbenzene, 2,3-butanedione, 0.10 (extrapolated) and 3-hexene-2, 5-dione, 0.31 (extrapolated). Our formation yields of 3-hexene-2,5-dione from p-xylene and 1,2,4trimethylbenzene are similar to those reported for glyoxal from p-xylene and of methylglyoxal from 1,2,4-trimethylbenzene and therefore suggest that these are coproducts, as expected from reaction schemes presented here.

Title: Monocyclic Aromatic Hydrocarbons in the Ocean

Attachment A – 1,2,3-Trimethylbenzene

Author(s): Neff JM.
Source: Bioaccumulation in Marine Organisms: Effect of Contaminants from Oil Well Produced Water. Amsterdam; Boston: Elsevier Ltd., 2002, pages 225-240.
Abstract: Not available.

Title: Visualization experiments of biodegradation in porous media and calculation of the biodegradation rate
Author(s): Vayenas DV, Michalopoulou E, Constantinides GN, Pavlou, Payatakes AC
Source: Advances in Water Resources Volume 25, Issue 2, February 2002, Pages 203-219.
Abstract: Biodegradation in porous media is studied with carefully controlled and well-characterized experiments in model porous media constructed of etched glass. Porous media of this type allow visual observation of the phenomena that take place at pore scale. An aqueous solution of five organic pollutants (toluene, phenol, o-cresol, naphthalene and 1,2,3-trimethylbenzene) was used as a model NAPL (representing creosote). The bacteria used were Pseudomonas fluorescens, which are indigenous (even predominant) in many contaminated soils. The maximum aqueous concentrations of the specific organic substances, below which biodegradation becomes possible, were determined as a function of temperature from toxicity experiments. Visualization experiments were made under various flow velocities and organic loadings to study the morphology and thickness of the biofilm as a function of the pore size and the distance from the entrance, and the efficiency of biodegradation. The efficiency of biodegradation decreased as the aqueous concentration of NAPL at the inlet increased and/or as the flow velocity increased. The thickness of biofilm. decreased as the distance from the inlet increased and/or the pore diameter decreased. A quasi-steady-state theoretical model of biodegradation was used to calculate the values of the mesoscopic biochemical rates and to predict the profile of NAPL concentration in the porous medium and the thickness of biofilm in pores. The agreement between experimental data and model predictions is quite satisfactory.

Title: Assessing the aquatic hazard of commercial hydrocarbon resins
Author(s): R.W. Woods, D.J. Letinskia, E.J. Febbo, C.L. Dzamba, M.J. Connelly and T.F. Parkerton
Source: Ecotoxicology and Environmental Safety Volume 66, Issue 2, February 2007, Pages 159-168.
Abstract: Hydrocarbon resins are used to modify polymer products to achieve desired functional properties for a diverse range of products. These complex hydrocarbon-based mixtures are typically poorly soluble in water. However, resins may leach lower-molecular-weight monomers or impurities upon contact with water, thus posing a potential hazard to the aquatic environment. The

Attachment A – 1,2,3-Trimethylbenzene

bioavailability and toxicity of leachable constituents of four solid and three liquid resins were evaluated by analyzing water-accommodated fractions prepared with each resin, using biomimetic solid phase microextraction (SPME) techniques. Liquid resins exhibited concentrations of bioavailable constituents that were sufficiently elevated to cause acute toxicity to the aquatic organism *Daphnia magna.* All solid resins exhibited lower bioavailable concentrations of leachable constituents that were unlikely to pose an aquatic toxicity concern. Since observed toxicity of both resin types was generally consistent with bioavailable concentrations determined using SPME fiber measurements, it is concluded t hat this approach provides a convenient in vitro screening tool that can help reduce the use of animal testing in environmental hazard assessment of complex hydrocarbon-based substances.

Title: Effect of the water- soluble fraction of petroleum on microsomal lipid metabolism of *Macrobrachium borellii* (Arthropoda: Crustacea)

Author(s): S. Lavarias, F. Garcia, R.J. Pollero and H. Heras

Source: Aquatic Toxicology, Volume 82, Issue 4, 31 May 2007, Pages 265-271.

Abstract: The effect of the water-soluble fraction of crude oil (WSF) on lipid metabolism was studied at critical metabolic points, namely fatty acid activation, enzymes of triacylglycerol and phospholipid synthesis, and membrane (lipid packing) properties in the freshwater prawn *Macrobrachium borellii.* To determine the effect of the contaminant, adults and embryos at different stages of development were exposed to a sublethal concentration of WSF for 7 days. After exposure, microsomal palmitoyl-CoA synthetase (ACS) showed a two-fold increase in adult midgut gland. Embryo's ACS activity was also affected, the increment being correlated with the developing stage. Endoplasmic reticulum acylglycerol synthesis was also increased by WSF exposure in adults and stage 5 embryos, but not at earlier stages of development. Triacylglycerol synthesis was particularly increased (18.5%) in adult midgut gland. The microsomal membrane properties were studied by fluorescent steady-state anisotropy, using the rotational behavior of the fluorescent probe 1,6-diphenyl-1,3,5-hexatriene (DPH). Microsomes from midgut gland of WSF-exposed prawn showed no differences in fluidity. Nevertheless, microsomes incubated with WSF *in vitro* increased their fluidity in a temperature- and WSF concentration- dependent fashion. Both, aliphatic and aromatic hydrocarbons individually tested elicited an increase in membrane fluidity at 10 mg/l, but at 4 mg/l only nC10-C16 aliphatics did. *In vivo* results indicate that WSF increased the activity of microsomal enzymes that are critical in lipid metabolism, though this change was not due to direct alterations in membrane fluidity, suggesting a synthesis induction, or an enzyme- regulatory mechanism. Nevertheless, hydrocarbons elicited membrane fluidity alterations in *in vitro* experiments at concentrations that could be found in the environment

Attachment A – 1,2,3-Trimethylbenzene

after an oil spill.

Attachment B – 1,3-Dimethylbenzene

Health Effects Studies (acute toxicity, subchronic toxicity, chronic toxicity – e.g., including reproductive/developmental toxicity, neurotoxicity, immunotoxicity, organ system effects, mutagenicity/genotoxicity, carcinogenicity)

Title: Indoor air pollution due to organic gases and vapours of solvents in building materials
Author(s): Lars Molhave
Source: Environment International, Volume 8, Issues 1-6, 1982, Pages 117-127
Abstract: The emission of organic gases and vapours of solvent type from 42 commonly used building materials was measured under standard atmospheric conditions. An average of 22 compounds was found in the air around each building material, and the total concentration of gases and vapours was from 0.01 to 1410 mg/m^3. The average arithmetic emission rate was 9.5 mg/m^2 h, and 52 different compounds were identified. A mathematical model was established for t he indoor air concentrations of pollutants originating from building materials. The model was tested on three model rooms constructed from the materials investigated. The calculated total air concentrations of gases and vapours in the three rooms ranged from 1.6 to 23.6 mg/m^3, and the number of compounds in the air from 23 to 32. These concentrations and num ber of compounds did not differ from those found in actual rooms similar in size and construction to the model rooms. The risks of health effects due to the compounds identi fled from the building materials were investigated, and criteria for future air quality standards are discussed. It is concluded that the possibility of negative health effects cannot be neglected, especially not for the more sensitive minority of the general population.

Title: Diethylbenzene-induced sensorimotor neuropathy in rats.
Author(s): Gacmaire F, Marienac B, de Ceaurriz J.
Source: J Appl Toxicol. 1990 Apr;10(2):105-12.
Abstract: The commercial isomer mixture of diethylbenzene (DEB mixture), 1,2-diethylbenzene (1,2-DEB), 1,3-diethylbenzene (1,3- DEB) and 1,4-diethylbenzene (1,4-DEB) were administered orally to male Sprague-Dawley rats. The experimental rats and the appropriate controls were examined electrophysiologically for motor and sensory conduction velocities (MCV and SCV), and for the amplitude of the sensory action potential (ASAP) of the tail nerve, at weekly or bi-weekly intervals. Oral administration of DEB mixture (750 or 500 mg kg-1, once daily, 5 days per week for 10 weeks) and 1,2-DEB (100 mg kg-1, once daily, 4 days per week for 8 weeks) produced a time-dependent decrease in MCV, SCV and ASAP. Rats treated with DEB mixture and 1,2-DEB exhibited a blue discoloration of tissues and urine. No changes in MCV, SCV and ASAP developed in rats administered orally with 1,3-DEP and 1,4-DEB (500 mg

Attachment B – 1,3-Dimethylbenzene

kg-1, once daily, 5 days per week for 8 weeks). The results indicate that 1,2-DEB is the isomer responsible for neurotoxicity and suggest that a metabolic pathway giving rise to coloured compounds is involved in the neurotoxicity of DEB.

Title: Dangerous and cancer- causing properties of products and chemicals in the oil refining and petrochemical industry: VIII. Health effects of motor fuels: Carcinogenicity of gasoline—Scientific update

Author(s): Myron A. Mehlman

Source: Environmental Research, Volume 59, Issue 1, October 1992, Pages 238-249

Abstract: Summary

1. Significant increases in tumors of kidney, liver, and other tissues and organs following exposure to gasoline provide sufficient evidence of carcinogenicity.
2. Benzene, a significant component of gasoline, has been established without question as a human carcinogen by IARC, EPA, and WHO.
3. 1,3-Butadiene, a component of gasoline, is a powerful carcinogen in both animals and humans.
4. Sufficient evidence for the carcinogenicity of alkyl benzenes, very significant component s of gasoline, has also been established.
5. Human epidemiologic studies show important increases in cancers of the kidney, stomach, brain, pancreas, prostate, lung, and skin as well as hematopoietic and lymphatic leukemias as a result of exposure to gasoline, its components, and its vapors.
6. Stage 2 controls are being implemented to reduce exposure of the human population to gasoline vapors.

Title: Air toxics in ambient air of Delhi

Author(s): Anjali Srivastava, A.E. Joseph, S. Patil, A. More, R.C. Dixit and M. Prakash

Source: Atmospheric Environment, Volume 39, Issue 1, January 2005, Pages 59-71

Abstract: Volatile organic compounds (VOCs) are major group of air pollutants which play critical role in atmospheric chemistry. It contributes to toxic oxidants which are harmful t o ecosystem human health and atmosphere. Data on levels of VOCs in developing countries is lacking. In India information at target VOCs as defined in USEPA compendium method TO-14 is almost totally lacking. The present work deals with estimation of target VOCs at 15 locations in five categories namely residential, industrial, commercial, traffic intersections and petrol refueling stations in Delhi, the capital of India. The monitoring was carried out during peak hours in morning and evening each month for a year in 2001. Ambient air was adsor bed on adsorbent tubes, thermally desorbed and analyzed on GC—MS. The results show t hat levels of VOCs are high and stress the need for regular monitoring programme of VOCs in urban environment.

Attachment B – 1,3-Dimethylbenzene

Welfare Effects Studies (toxicity in aquatic organisms, toxicity in terrestrial species, toxicity to plants/bacteria, effects on visibility/weather/climate, effects on materials, economic effects, effects on personal comfort and well-being)

Title: The composition and concentration of hydrocarbons in the range of C2 to C18 emitted from motor vehicles
Author(s): Barbara Zielinska and Kochy K. Fung
Source: Science of The Total Environment, Volumes 146-147, 23 May 1994, Pages 281-288
Abstract: Sampling was carried out in the Caldecott Tunnel, located in the San Francisco (California) area. Three daily samples were collected, using stainless steel canisters and Tenax-TA solid adsorbent cartridges, over 2 days in June 1991. The samples were analyzed using high resolution gas chromatographic separation and Fourier transform infrared/mass spectrometric detection (GC/IRD/MSD) or flame ionization detection (FID) of individual hydrocarbons. The comparison of hydrocarbon concentrations found in the Tenax and canister samples and the assessment of the contribution of semivolatile hydrocarbons (C10—C18 range) to total non-methane hydrocarbons (C2—C10), as measured by the canister method, is presented.

Title: Forensic Environmental Geochemistry: differentiation of fuel- types, their sources and release time
Author(s): Isaac R. Kaplan, Yakov Galperin, Shan-Tan Lu and Ru-Po Lee
Source: Organic Geochemistry, Volume 27, Issues 5-6, 15 November 1997, Pages 289-299, 301-317
Abstract: During the evolution of organic and petroleum geochemistry, attention has focused mainly on investigation of either the gaseous or high molecular-weight hydrocarbons. Characterization of novel and environment- specific compounds has enriched our understanding of paleoenvironments, fossil biota and the alteration processes leading to the formation of energy resources. The "fingerprinting" methods developed for reconstructing geologic events can also be used with some modification for characterizing current processes affecting fugitive crude oil and its refined products that have impacted the environment and become an ecologic threat. In order to identify the source of the escaped hydrocarbon products, it is often essential to determine (a) what fuel types the hydrocarbons represent, (b) when the release(s) occurred and (c) how much of each fuel is mixed in the plume. These requirements can be accomplished by the combination of specialized analytical procedures used in standard contamination characterization with methodology developed in organic geochemistry, a combination we refer to as Forensic Environmental Geochemistry.

Attachment B – 1,3-Dimethylbenzene

Title: Attractiveness of 79 compounds and mixtures to wild Ceratitis capitata (Diptera: Tephritidae) in field trials.

Author(s): Casana-Giner V, Gandia-Balaquer A, Hernànder-Alamos MM, Menqod-Puerta C, Garrido-Vivas A, Primo-Millo J, Primo-Yufera E.

Source: J Econ Entomol. 2001 Aug; 94(4): 898-904.

Abstract: Seventy-nine volatile compounds chosen from those emitted by Mediterranean fruit fly males, fruits and food baits, were tested for their attractiveness to females of Ceratitis capitata (Wiedemann) in field trials with a wild population. To correct for density fluctuations, the results were presented as a percentage of males caught in the same type of traps baited with trimedlure, a known male attractant. The mixtures of corn steep liquor with ammonia compounds and amines were the most effective attractants for females. Other compounds exhibiting C. capitata attraction were a mixture ethyl acetate+acetic acid+ethanol for both sexes, and p-cymene, 1,3-diethylbenzene and borneol for females.

Title: A case study on identification of airborne organic compounds and time courses of their concentrations in the cabin of a new car for private use

Author(s): Toshiaki Yoshida and Ichiro Matsunaga

Source: Environment International Volume 32, Issue 1, January 2006, Pages 58-79

Abstract: The cabin of an automobile can be considered to be a part of the living environment because many people spend long periods of time during business, shopping, recreation or travel activities. However, little is known about the interior air contamination due to organic compounds diff using from the interior materials used in the interior of automobiles. In the present study, the compounds in the interior air of a new car were identified, and the time courses of their concentrations were examined for over 3 years after the delivery (July, 1999). A total of 162 organic compounds, involving many aliphatic hydrocarbons and aromatic hydrocarbons, were identified. High concentrations of n-nonane (458 pg/m^3 on the day following delivery), n-decane (1301 pg/m^3), n-undecane (1616 pg/m^3), n-dodecane (716 pg/m^3), n-tridecane (320 pg/m^3), 1-hexadecene (768 pg/m^3), ethylbenzene (361 pg/m^3), xylene (4003 pg/m^3) and 2,2'-azobis (isobuty ronitrile) (429 pg/m^3) were detected, and the sum of the concentrations determined for all compounds excluding formaldehyde (TVOC) was approximately 14 mg/m^3 on the day after the delivery. The concentrations of most compounds decreased with time, but increased with a rise of the interior temperature. The TVOC concentration in the next summer (July, 2000) was approximately one-tenth of the initial concentration. During the 3-year study period, the TVOC concentrations in summer exceeded the indoor guideline value (300 pg/m^3) proposed by Seifert (1995) [Seifert B. Volatile organic compounds . In: Maroni M, Seifert B, Lindvall T, editors. Indoor air quality. A comprehensive reference book. Air quality monographs, vol. 3. Netherlands: Elsevier Science;

Attachment B – 1,3-Dimethylbenzene

1995. p. 819-21]. The interior temperature and day s lapsed after delivery were the main factors affecting the interior concentrations of most compounds according to multiple linear regression analysis. The results of this study offer useful fundamental data for investigations on air pollution in automotive cabins due to the organic compounds diffusing from the interior materials.

Title: Emissions of unregulated pollutants from European gasoline and diesel passenger cars

Author(s): Isabelle Caplain, Fabrice Cazier, Habiba Nouali, Agnes Mercier, Jean-Claude Dechaux, Valerie Nollet, Robert Joumard, Jean-Marc Andre and Robert Vidon

Source: Atmospheric Environment, Volume 40, Issue 31, October 2006, Pages 5954-5966

Abstract: Within the framework of the European Artemis project, the emissions of unregulated compounds were measured on new technology passenger cars. A sample of passenger cars was tested on a chassis dynamometer with constant volume sampling (CVS). The measurement of unregulated compounds comprised about 100 different volatile organic compounds (VOC) in the C_2—C_6 and C_7—C_{16} range and carbonyl compounds. The sampling of these compounds was made using sorbent tubes followed by analysis with liquid and gas phase's chromatography. The influence of cold and warm starting conditions on the VOC composition was determined. The emission factors were determined and compared for both gasoline and diesel vehicles. The influence of the technology was evaluated and a first approach to understand the effect of recent technology on the ozone formation was developed.

Title: 11 - Biodegradation of oil hydrocarbons and its implications for source identification

Author(s): Roger C. Prince and Clifford C. Walters

Source: Oil Spill Environmental Forensics Fingerprinting and Source Identification 2007, Pages 349-379

Abstract: Hydrocarbons have been part of the biosphere from its inception, produced initially by prebiotic processes, and subsequently both by living organisms and during the generation of fossil fuels. As highly reduced forms of carbon, hydrocarbons provide a rich source of energy and carbon to those organisms, typically microorganisms, which are able to consume them. Indeed, almost all hydrocarbons are readily degraded under appropriate conditions. There is, nevertheless, a clear preference for the catabolism of some molecules before others; hence, the composition of a fuel or crude oil changes as biodegradation proceeds.

Attachment B – 1,3-Dimethylbenzene

Title: Chapter 23 - An Overview of In-Situ Burning
Author(s): Merv Fingas
Source: Oil Spill Science and Technology 2011, Pages 737-903
Abstract: In-situ burning is recognized as a viable alternative for cleaning up oil spills on land and water. When performed under the right conditions, in-situ burning can rapidly reduce the volume of spilled oil and eliminate the need to collect, store, transport, and dispose of recovered oil. In-situ burning can shorten t he response time to an oil spill, thus reducing the chances that the oil will spread on the water surface and thereby aiding in environmental protection. Such rapid removal of oil can also prevent the oil from reaching shorelines, which are difficult to clean. What remains after an in- situ burn are burn by-products such as carbon dioxide, water, some smoke particulate, and unburned oil (residue). Sufficient information is now available to predict levels of these emissions and calculate safe distances downwind of the fire. This chapter contains a compilation of information about in-situ burning of oil spills and includes the scientific aspects of the burning process and its effects, examples from the extensive research into in-situ burns, and practical information about the procedures to be followed and equipment required for carrying out an in-situ burn.

Ignition may be a concern as heavier oils may require a primer such as kerosene or diesel fuel for ignition. Heavy oils require longer heating times and a hotter flame to ignite than lighter oils. If not enough vapors are produced, the fire either will not start or will be quickly extinguished. The amount of vapors produced is dependent on the amount of heat radiated back to the oil. If the oil slick is too thin, some of this heat is conducted to the water layer below it. It is uncertain whether oil that is completely emulsified with water can be ignited, although oil containing some emulsion can be ignited and burned. Containment of the oil on water may be necessary to carry out in-situ burning as the oil must be thick enough to quantitatively burn. Recent studies have shown that thickness is not critical as was once thought. The oil burn rate is largely a function of oil type. Once burning, the heat radiated back to the slick and the insulation are usually sufficient to allow combustion down to about 0.5 to 1 mm of oil. If greater amounts of fuel are vaporized than can be burned, more soot is produced as a result of incomplete combustion, and fuel droplets are released downwind. The residue from burning oil is largely unburned oil with some lighter or more volatile products removed. When the fire ceases, unburned oil is left that is simply too thin to sustain combustion. In addition to unburned oil, oil is also present that has been subjected to high heat and is thus weathered. Finally, heavier particles are re -precipitated from the smoke plum e into the fire and thus become part of the residue. Highly efficient burns of some types of heavy crude oil may result in oil residue that sinks in seawater after cooling.

The emissions of burning are of concern and are covered in this chapter. These

Attachment B – 1,3-Dimethylbenzene

emissions include the smoke plume, particulate matter precipitating from the smoke plume, combustion gases, unburned hydrocarbons, organic compounds produced during the burning process, and the residue left at the burning pool site. Soot particles, though consisting largely of carbon particles, contain a variety of absorbed and adsorbed chemicals. The following is a brief summary of each type of emission.

Particulate Matter/Soot-All burns, especially those of diesel fuel, produce an abundance of particulate matter. Particulate matter at ground level is a health concern close to the fire and under the plume. Particulate matter is distributed exponentially downwind from the fire.

Polyaromatic Hydrocarbons (PAHs-Oils contain significant quantities of polyaromatic hydrocarbons that are largely destroyed in combustion. The PAH concentrations in the smoke, both in the plume and the particulate precipitation at ground level, are much less than in the starting oil. This includes the concentration of multiringed PAHs. There is a slight increase in the concentration of multiringed PAHs in the burn residue. When considering the mass balance of the burn, however, most of the five- and six-ringed PAHs are destroyed by the fire.

Volatile Organic Compounds (VOCs)-Many VOCs are emitted by fires, but in lesser quantity than when the oil is not burning. VOCs are not typically a concern, but can rise almost to health levels of concern very close to the fire.

Organic Compounds -No exotic or highly toxic compounds are generated as a result of the combustion process. Organic macromolecules are in lesser concentration in the smoke and downwind than they are in the oil itself. Dioxins and dibenzofurans have not been measured as emissions of oil fires to date.

Carbonyls-Carbonyls such as aldehydes and ketones are created by oil fires, but do not exceed health concern levels even very close to fires.

Gases-Combustion gases such as car bon dioxide, carbon monoxide, and sulfur dioxide are produced by oil fires but are significantly below any health concern level.

Overall, emissions are now understood to the extent that emission levels and safe distances can be or fires of various sizes and types. This chapter provides equations for predicting concentrations of emissions for the various groups and for more than 150 specific compounds, as well as tables of results.

The particulate concentrations from in-situ oil fires should be monitored in some circumstances.

Title: Which emission sources are responsible for the volatile organic compounds in the atmosphere of Pearl River Delta?

Author(s): H. Guo, H.R. Cheng, Z.H. Ling, P.K.K. Louiec and G.A. Ayokod

Source: Journal of Hazardous Materials Volume 188, Issues 1-3, 15 April 2011,

Attachment B – 1,3-Dimethylbenzene

Pages 116-124

Abstract: A field measurement study of volatile organic compounds (VOCs) was simultaneously carried out in October—December 2007 at an inland Pearl River Delta (PRD) site and a Hong Kong urban site. A receptor model i.e. positive matrix factorization (PM F) was applied to the data for the apportionment of pollution sources in the region. Five and six sources were identified in Hong Kong and the inland PRD region, respectively. The major sources identified in the region were vehicular emissions, solvent use and biomass burning, whereas extra sources found in inland PRD included liquefied petroleum gas and gasoline evaporation. In Hong Kong, the vehicular emissions made the most significant contribution to ambient VOCs (48 ± 4%), followed by solvent use (43 ± 2%) and biomass burning (9 ± 2%). In inland PRD, the largest contributor to ambient VOCs was solvent use (46 ± 1%), and vehicular emissions contributed 26 ± 1% to ambient VOCs. The percentage contribution of vehicular emission in Hong Kong in 2007 is close to that obtained in 2001-2003, whereas in inland PRD the contribution of solvent use to ambient VOCs in 2007 was at the upper range of the results obtained in previous studies and twice the 2006 PRD emission inventory. The findings advance our knowledge of ozone precursors in the PRD region.

Title: 11 - Biodegradation of oil hydrocarbons and its implications for source identification

Author(s): Roger C. Prince and Clifford C. Walters

Source: Oil Spill Environmental Forensics Fingerprinting and Source Identification 2007, Pages 349-379

Abstract: Hydrocarbons have been part of the biosphere from its inception, produced initially by prebiotic processes, and subsequently both by living organisms and during the generation of fossil fuels. As highly reduced forms of carbon, hydrocarbons provide a rich source of energy and carbon to those organisms, typically microorganisms, which are able to consume them. Indeed, almost all hydrocarbons are readily degraded under appropriate conditions. There is, nevertheless, a clear preference for the catabolism of some molecules before others; hence, the composition of a fuel or crude oil changes as biodegradation proceeds.

Attachment C – 1-Methyl-1-ethyl-cyclopentane

Welfare Effects Studies (toxicity in aquatic organisms, toxicity in terrestrial species, toxicity to plants/bacteria, effects on visibility/weather/climate, effects on materials, economic effects, effects on personal comfort and well-being)

Title: Characterization and compositional study of lighter fractions from coal-derived liquids

Author(s): P. Kumar, R.K. Kuchhal, B. Kumar, H.C. Chandolaa, L. Dixit and P.L. Gupta

Source: Fuel, Volume 66, Issue 8, August 1987, Pages 1036-1045

Abstract: Synfuel fractions boiling in the range IBP-150 ° C and 150-250 °C have been characterized. Individual component and hydrocarbon type distributions have been carried out quantitatively employing high resolution capillary gas chromatography and mass spectrometric techniques independently. More than 180 compounds including 60 olefins have been identified and quantified in the IBP-150 °C fraction only. A comparative study of hydrocarbon structures present in synfuel and crude oil fractions has been m ade and revealed that the cyclic character of coal-derived oils is due to predominance of by droaromatics and cycloolefins rather than naphthenes alone. Moreover, for most of the homologous series of cyclic and aromatic structures, parent compounds are relatively more abundant in coal-derived oil than in natural crude petroleum and cracked petroleum fractions. The fractions have been evaluated for their suitability as gasoline/kerosene blending components in view of their estimated octane number and smoke point respectively.

Attachment D – 2,2-Dimethylhexane

Welfare Effects Studies (toxicity in aquatic organisms, toxicity in terrestrial species, toxicity to plants/bacteria, effects on visibility/weather/climate, effects on materials, economic effects, effects on personal comfort and well-being)

Title: Theoretical modeling of biodegradation and biotransformation of hydrocarbons in subsurface environments

Author(s): M. Yavuz, Corapcioglu and M. Akhter Hossein

Source: Journal of Theoretical Biology, Volume 142, Issue 4, 22 February 1990, Pages 503-516

Abstract: Hydrocarbons such as TCE, PCE, TCA, gasoline and kerosene which are widely used in the industry, enter soils and groundwater from chemical waste disposal sites and from accidents. These types of substances are the most commonly encountered groundwater contaminants nationwide. Biotransformation of dissolved chlorinated hydrocarbons can provide complete mineralization to harmless end products such as CO_2. It is the objective of this work to investigate the biodegradation and biotransformation, and transport of hydrocarbons in groundwater. This will be achieved first by defining and identifying relevant physical and biological processes which contribute to the fate of hydrocarbon contaminants in unsaturated/saturated soils, and providing a conceptual framework for incorporating these processes into a mathematical formulation. The conservation principles ex pressed in terms of quantifications of the physical, chemical and microbial processes described above lead to a system governing the phenomenon which consists of nonlinear partial differential equations. Microbial transformation conducted by both anaerobic and aerobic bacteria are considered.

Title: A *Mycobacterium* Strain with Extended Capacities for Degradation of Gasoline Hydrocarbons

Author(s): Floriane Solano-Serena, Remy Marchal, Serge Casaregola, Christelle Vasnier, Jean-Michel Lebeault, and Jean-Paul Vandecasteele

Source: Applied and Environmental Microbiology, June 2000, p. 2392-2399, Vol. 66, No. 6

Abstract: A bacterial strain (strain IFP 2173) was selected from a gasoline-polluted aquifer on the basis of its capacity to use 2,2,4-trimethylpentane (isooctane) as a sole carbon and energy source. This isolate, the first isolate with this capacity to be characterized, was identified by 165 ribosomal DNA analysis, and 100% sequence identity with a reference strain of *MycObacterium austroafricanurn* was found. *Mycobacterium* sp. strain IFP 2173 used an unusually wide spectrum of hydrocarbons as growth substrates, including n-alkanes and multimethyl-substituted isoalkanes with chains ranging from 5 to 16 carbon atoms long, as well as substituted monoaromatic hydrocarbons. It also attacked ethers, such as

Attachment D – 2,2-Dimethylhexane

methyl C.-butyl ether. During growth on gasoline, it degraded 86% of the substrate. Our results indicated that strain IFP 2173 was capable of degrading 3-methyl groups, possibly by a carboxylation and deacetylation mechanism. Evidence that it attacked the quaternary carbon atom structure by an as-yet-undefined mechanism during growth on 2,2,4-trimethylpentane and 2,2-dimethylpentane was also obtained.

Title: Non-methane hydrocarbons and carbonyls in the Lower Fraser Valley during PACIFIC 2001

Author(s): D. Wang, J.D. Fuentes, D. Travers, T. Dann and T. Connolly

Source: Atmospheric Environment Volume 39, Issue 29, September 2005, Pages 5261-5272

Abstract: During August 2001, a field investigation was undertaken to determine the hydrocarbon and carbonyl loadings in the Lower Fraser Valley of British Columbia, Canada. Intensive ambient air sampling was carried out at sites representing urban and rural locales to identify and quantify atmospheric levels of non-methane hydrocarbon and carbonyl species. Results indicated that in the urban atmosphere (3-h) non-methane hydrocarbon levels exceeded 20 parts per billion (ppbv). Rural sites had lower levels of non-methane hydrocarbons. The dominant anthropogenic hydrocarbon species were propane, but ane, 2-methylbutane and toluene. The most common biogenic hydrocarbons included isoprene, a-pinene, 13- pinene and limonene. On average, biogenic hydrocarbons represented approx imately 23% of the total hydrocarbon reactivity estimated for the airshed in the Lower Fraser Valley. Based on the hydroxyl radical-reactivity scale, limonene was determined to be the most photochemically relevant species at the urban site in the Lower Fraser Valley of British Columbia. Formaldehyde, acetaldehyde, and acetone were the dominant carbonyl compounds found in the Lower Fraser Valley. Three-hour averaged carbon yls levels reached 10 ppbv (3-h) and resulted from both direct emissions and hydrocarbon photooxidation. Atmospheric conditions strongly modulated the abundance of hydrocarbon and carbonyl compounds.

Title: Can topological indices be used to predict gas-phase rate coefficients of importance to tropospheric chemistry? Free radical abstraction reactions of alkanes

Author(s): Max R. McGillen, Carl J. Percivala, Teresa Raventos-Duran, Gabriela Sanchez-Reyna and Dudley E. Shallcross

Source: Atmospheric Environment, Volume 40, Issue 14, May 2006, Pages 2488-2500

Attachment D – 2,2-Dimethylhexane

Abstract: As tropospheric chemical models become ever more complex, reliable rate coefficient data for gas-phase hydrogen abstraction reactions involving trace hydrocarbons becomes increasingly necessary. There are hundreds of non-methane hydrocarbons (NMHCs) of potential importance to tropospheric chemistry, many of which have not been subjected to experimental inquiry. This study—the first of its kind to investigate hydrocarbons ranging from C_2 to C_{10} using topological indices—aims to provide a reliable and accessible method of estimating rate coefficients for these species. Rate coefficients of free radical abstraction reactions of NMHCs were correlated with the Randid and Balaban topological indices for radical moieties OH, CI and NO_3. These correlations were compared with those of an established frontier orbit al approach based on calculated ionization potentials (IPs).
The Randid index was found to correlate better than IP for each of the radicals studied, and correlated particularly effectively for the CI radical. The Balaban index did not correlate for branched alkanes except for NO_3. Where only unbranched alkanes were considered, the Balaban index proved most reliable, demonstrating a clear linear relationship. Topological indices present an accurate and diverse method for estimating free radical abstraction rate coefficients that does not require the computing power, specialist software packages, or complex mathematics inherent in ab initio calculations. A further advantage of using topological indices is that they are calculated in an unambiguous manner. More reliable tools for estimating rate coefficients have direct implications for improving models, and may also provide a direction for future laboratory work, either by highlighting particularly reactive species or identifying potentially spurious rate coefficient data.

Title: Pyrolysis and combustion of waste lubricant oil from diesel cars: Decomposition and pollutants

Author(s): M.J. Fuentes, R. Font, M.F. Gomez-Rico and I. Martin-Gullon

Source: Journal of Analytical and Applied Pyrolysis, Volume 79, Issues 1-2, May 2007, Pages 215-226

Abstract: Pyrolysis and combustion of waste lubricant oil were studied due to its important potential value as a fuel. A thermogravimetric analysis in nitrogen atmosphere conditions and with different proportions of nitrogen:oxygen (N_2, N_2:0_2 mixtures at 4:1 and 9:1 ratios), different weights (1, 2.5 and 5 mg) and different heating rates (5, 10 and 15 °C min^{-1}) were carried out to study the thermal decomposition. Both pyrolysis and combusti on can be represented in two processes: in the first process, a volatilization of the oil takes place (this depends on the initial mass and the heating rate) and in the second process there is a decomposition, which is a different process in combustion from the process in pyrolysis.
The identification of the volatile and semivolatile compounds from pyrolysis and

Attachment D – 2,2-Dimethylhexane

combustion of used diesel oil was carried out in a tubular reactor at 500 and 850 °C. On the one hand, the semivolatile PAH compounds (naphthalene, phenanthrene, anthracene, etc.) were detected as much in pyrolysis as in combustion (850 °C), but the most highly carcinogenic is benzo(a)pyrene which was only found in pyrolysis at 850 °C. On the other hand, the levels of PCDD/Fs and dioxin-like PCBs were estimated after a fuel-rich combustion process at around 113 pg WHO-TEQ g^{-1} burnt oil (corresponding to 80 pg I-TEQ g^{-1}) and 3 pg WHO-TEQ g^{-1} burnt oil, respectively. The PCDD/Fs concentration in the raw sample was estimated at around 7-16 pg WHO-TEQ g^{-1} used oil (or 6-13 pg I-TEQ g^{-1}) and PCBs were not detected.

Title: Biodegradability of alkylates as a sole carbon source in the presence of ethanol or BTEX

Author(s): Jaiho Cho, Maher M. Zein, Makram T. Suidan and Albert D. Venosa

Source: Chemosphere, Volume 68, Issue 2, June 2007, Pages 266-273

Abstract: The biodegradability of alky late compounds in serum bottles was investigated in the presence and absence of ethanol or benzene, toluene, ethylbenzene, and p-xylene (BTEX). The biomass was acclimated to three different alkylates, 2,3-dimethylpentane, 2,4- dimethylpentane and 2,2,4-trimethylpentane in porous pot reactors. The alkylates were completely mineralized in all three sets of experiments. They degraded more slowly in the presence of BTEX than in their absence because BTEX inhibited the microbial utilization of alkylates. However, in the presence of ethanol, their slower biodegradation was not related to inhibition by the ethanol. Throughout the experiments alkylates, ethanol, and BTEX concentrations did not change in the sterile controls.

Attachment E – 2,4,4-Trimethyl-2-pentene

Health Effects Studies (acute toxicity, subchronic toxicity, chronic toxicity – e.g., including reproductive/developmental toxicity, neurotoxicity, immunotoxicity, organ system effects, mutagenicity/genotoxicity, carcinogenicity)

Title: Pollutant Emissions from Gasoline Combustion. 1. Dependence on Fuel Structural Functionalities

Author(s): Hongzhi R. Zhang, Eric G. Eddings, and Adel F. Sarofim

Source: Environ. Sci. Technol. 2008, 42, 5615-5621

Abstract: To study the formation of air pollutants and soot precursors (e.g., acetylene, 1,3-butadiene, benzene, and higher aromatics) from aliphatic and aromatic fractions of gasoline fuels, the Utah Surrogate Mechanisms is extended to include submechanisms of gasoline surrogate compounds using a set of mechanism generation techniques. The mechanism yields very good predictions of species concentrations in premixed flames of *n*-heptane, isooctane, benzene, cyclohexane, olefins, oxygenates, and gasoline using a 23-component surrogate formulation. The 1,3-butadiene emission comes mainly from minor fuel fractions of olefins and cyclohexane. The benzene formation potential of gasoline components shows the following trends as functions of (i) chemical class: n-paraffins < isoparaffins < olefins < naphthalenes < alkylbenzenes < cycloparaffins < toluene; (ii) carbon number: n-butane < n-pentane < n-hexane; and (iii) branching: n-hexane < 2,2,4-trimethylpentane < 2,2,3,3-tetramethylbutane. In contrast, fuel structure is not the main factor in determining acetylene formation. Therefore, matching the benzene formation potential of the surrogate fuel to that produced by the real fuel should have priority when selecting candidate surrogate components for combustion simulations.

Attachment F – 2-Butyne

Health Effects Studies (acute toxicity, subchronic toxicity, chronic toxicity – e.g., including reproductive/developmental toxicity, neurotoxicity, immunotoxicity, organ system effects, mutagenicity/genotoxicity, carcinogenicity)

Title: A chemical study of 2-butyne-1,4-diol.
Author(s): d'Amboise M, Mathieu D, Piron DL.
Source: Talenta. 1988 Oct;35(10):763-8.
Abstract: Commercial grade 2-butyne-1,4-diol has been used in electroplating for several years. In laboratory experiments, its presence in the electrolyte increases the current efficiency of zinc electro-winning. Its chemical behaviour in solution is not well known. The present paper indicates that the brownish technical grade 2-butyne-1,4-diol contains the monomer, the dimer and some trimer. Pure monomeric 2-butyne-1,4-diol is a white solid obtained by evaporation of the technical grade product. The monomer is slowly transformed into (timer and possibly into a trimer when dissolved in water. Various analytical techniques were used in the study of this system. Factor analysis with column cross-validation was applied to chromatographic data to help in the resolution of the system.

Title: Short-Term Toxicity of Nine Industrial Chemicals
Author(s): Komsta E, Secours VE, Chu 1, Valli VE, Morris R, Harrison J, Baranowski E, Villeneuve DC
Source: Bulletin of Environmental Contamination and Toxicology, Vol. 43, No. 1, pages 87-94, 7 references 1989
Abstract: Nine Industrial chemicals were screened in a short term study using male and female Sprague-Dawley-rats to determine whether additional studies were needed. The chemicals tested included tri(butoxyethyl)phosphate (78513), dimethylol-urea (140954), 2-butyne-1,4-diol (1106S6), triallyl-s-triazine-trione, cyclohexanone-oxime (100641), p-toluene-sulfonhydrazide, 2- nitroaniline (88744), propargyl-alcohol (107197), and 5-methyl-1H-benzotriazole. The assay consisted of a 14 day oral dosing regime using doses of 1, 10, and 100 mg/kg, except for two chemicals. These two included propargyl-alcohol administered at 0.1, 1.0 or 10mg/kg, and p-toluene-sulfonhydrazide administered at 0.5, 5.0 or 50mg/kg. Of these nine chemicals, only 2- butyne-1,4-diol, cyclohexanone-oxime, p-toluene-sulfonhydrazide, and triallyl-s-triazine-trione demonstrated toxic properties. Triallyi-s-triazine-trione was the most toxic and caused severe body weight changes and histopathological changes including elevated bilirubin and cholesterol levels and decreases In calcium, phosphorus and potassium levels. The authors conclude that additional studies should be made on these four compounds to determine potential long term effects.

Attachment F – 2-Butyne

Title: Assessment of long-term exposures to toxic substances in air.
Author(s): Rappaport SM.
Source: Ann Occup Hyg., 1991 Feb;35(1):61-121.
Abstract: Because airborne exposure varies greatly over time and between individual workers, occupational hygienists should adopt sampling strategies which recognize the inherent statistical nature of assessing exposure. This analysis indicates that the traditional practice of testing 'compliance' with occupational exposure limits (OELs) should be discarded. Rather, it is argued that acceptable exposure should be defined with reference to the exposure distribution. Regarding the many statistical issues which come into play, it is concluded that hygienists should continue to apply the log-normal model for summarizing and testing data. However, sampling designs should move away from methods which are biased (e.g. sampling only the worst case) and which rely upon job title and observation as the primary means of assigning workers into groups. Since exposure data often lack independence (e.g. owing to the autocorrelation of serial measurements) and there exist large differences in exposure between workers in the same job group, random sampling designs should be adopted. It is also shown that the relationship between the mean of a log-normal distribution and exposures in the right tail allows one to evaluate simultaneously the mean exposure and the maximum frequency with which exposures exceed the OEL. Investigation of the biological concepts relies heavily upon a conceptual model which depicts the exposure-response continuum as a sequence of time series related to exposure, burden, damage and risk. Analysis of the linkages between these processes identifies two kinetic conditions which are necessary if variability of exposure is to affect appreciably the individual's risk of chronic disease. First, the variation of exposure from interval to interval must be efficiently translated into burden and damage (no damping), and second, during periods of intense exposure the relationship between burden and damage must be non-linear (curving upwards), On the basis of current knowledge it appears that relatively few chronic toxicants satisfy both these conditions. Even for those substances which cause damage only when a threshold is exceeded, a statistical argument suggests that the maximum risk can still be related to the mean exposure received over time. It is concluded that the risk of chronic disease generally depends upon the mean exposure received by the individual worker over time. Thus, the sampling strategy must allow the distribution of Individual mean exposures to be characterized across the population at risk. It follows from this paradigm for assessing exposures that relatively little effort should be devoted to the evaluation of short-term 'peak' exposures since such transients are unlikely to exert undue influence on long-term effects (ABSTRACT TRUNCATED AT 400 WORDS)

Attachment F – 2-Butyne

Title: Acute Toxicity of 2-Butyne-1,4-diol in Laboratory Animals
Author(s): Jedrychowski RA, Czajkowska T, Stetkiewicz J, Stetkiewicz I
Source: Journal of Applied Toxicology, Vol. 12, No. 2, pages 113-115, 1992.
Abstract: The acute toxicity of 2-butyne-1,4-dlol (110656) (BYD) was studied using Wistar-Imp:DAK-rats, White-Vienna-rabbits (WV), White-New-Zealand-rabbits (WNZ), and Hartley-albino-guinea-pigs. Rats were administered 10% aqueous BYD in doses of 100, 150, 180, 200, or 250mg/kg body weight. Daily observations for signs of toxicity and mortality over a 14 day period, an detailed necropsy with histopathological study of the internal organs, were carried out. Female rats were tested for acute dermal toxicity of 100% or 40% aqueous BYD applied to clipped sides and dorsum. WV rabbits were tested for dermal irritation by 100%, 40% and 20% BYD, at 1, 24, 48, and 72 hours (hr) after application. Short term dermal irritation was tested for using 40% BYD painted behind the ears for 10 consecutive days. Ocular irritation was tested for by placing 100mg BYD solution in the conjunctival sac of one eye in WNZ rabbits; the other eye served as control. The ocular irritation Index was computed by averaging Draize scores at 1, 24, 48, and 72hr, and after 7 days. Allergic contact dermatitis was tested for using guinea-pigs, with 2% BYD injected intradermally, and 20% BYD applied topically; the challenge test was with 5% and 20% BYD. Oral median lethal dose (LD50) values were 132mg/kg for male and 176 mg/kg for female rats. Death occurred within 48hr of oral dosing. Gross pathologies included diarrhea and congestion of internal organs, while histopathological changes included perivascular edema, bronchopneumonia, hyperemia, liver necrosis, and nephrosis. Liver degeneration progressed after 48hr from focal to diffuse and panlobular necrosis, accompanied by fatty changes. After 14 days, slight lymphocytic infiltrations, and mitotic cells were seen. The renal cortex showed sloughing of tubular epithelium after 48hr, and after 14 days, some regeneration was evident. Dermal application of solid BYD was not lethal, but 40% BYD killed eight of 16 rats within 48hr. Diffuse necrosis of liver and kidneys was seen. By 14 days after application, the most prominent feature was extensive vacuolation of the liver parenchyma. Dermal irritation, and allergic contact dermatitis tests were negative. Marked lacrimation and slightly closed lids were observed in all rabbits after 1hr of ocular application. After 24 and 48hr, minimal conjunctival erythema was evident. The authors conclude that BYD is a moderately toxic substance. In acute poisonings it is hepatotoxic and nephrotoxic, but it has no apparent skin sensitizing potential,

Attachment F – 2-Butyne

Title: Subacute Oral Toxicity of 2-Butyne-1,4-diol in Rats
Author(s): Jedrychowski RA, Czajkowska T, Gorny R, Stetkiewicz J, I Stetkiewicz
Source: Journal of Applied Toxicology, Vol. 12, No. 2, pages 117-122, 9 references, 1992.
Abstract: The subacute oral toxicity of 2-butyne-1,4-diol (110656) (BYD), was ascertained using Wistar-Imp:DAK-rats. Rats of both sexes were dosed once daily for 2B consecutive days with BYD at doses of 1mg/kg (low dose), 10mg/kg (medium dose) and 50mg/kg (high dose). Animals were observed daily for signs of toxicity, and necropsied at the end of the test period. Blood was drawn for hematological studies, just prior to necropsy. In the high dose group, three of eight males and three of eight females died. Histopathology showed congested internal organs, pulmonary edema, and necrosis of kidneys and liver. Body weight gain for males in this group was less than in controls, while females did not show any differences. Hematological differences in high dose females included a decrease in erythrocyte (RBC) count, hematocrit value, and hemoglobin concentration. Medium doses diminished ROC count in females, while high doses increased reticulocyte and leukocyte counts In both sexes. Serum chemistry parameters of high dose animals showed significant Increases in sorbitol-dehydrogenase In both sexes, higher total protein concentration in females, and elevated glucose in males. Hepatic lesions were seen on microscopic examination of medium and high dose animals. Splenic changes in animals from these two groups reflected disturbances In peripheral blood. The authors conclude that prolonged exposure to BYD can cause serious health problems.

Title: Toxicology of 2-Butyne-1,4-diol [110-65-6], Review of Literature
Author(s): Tyce, R. PhD.
Source: Integrated Laboratory Systems, Research Triangle Park, North Carolina. January 14, 1997.
Abstract: Not available.

Title: Customer exposure to gasoline vapors during refueling at service stations.
Author(s): Hakkola MA, Saarinen LH.
Source: Appl Occup Environ Hyg. 2000 Sep;15(9):677-80.
Abstract: Gasoline is a volatile complex mixture of hydrocarbon compounds that is easily vaporized during handling under normal conditions. Modern reformulated gasoline also contains oxygenates to enhance octane number and reduce ambient pollution. This study measured the difference in the exposure of customers to gasoline and oxygenate vapors during refueling in service stations with and without vapor recovery systems. Field measurements were carried out at two self- service stations. One was equipped with Stage I and the other with Stage II vapor recovery systems. At Stage I stations there is vapor recovery only

Attachment F – 2-Butyne

during delivery from road tanker, and at Stage II stations additional vapor recovery during refueling. The exposure of 20 customers was measured at both stations by collecting air samples from their breathing zone into charcoal tubes during refueling with 95-octane-reformulated gasoline. Each sample represented two consecutive refuelings. The samples were analyzed in the laboratory by gas chromatography using mass-selective detection for vapor components. The Raid vapor pressure of gasoline was 70 kPa and an oxygen content 2 wt%. Oxygenated gasoline contained 7 percent methyl tert-butyl ether (MtBE) and 5 percent methyl tert-amyl ether (MtAE). The geometric mean concentrations of hydrocarbons (C3-C11) in the customers' breathing zone was 85 mg/m3 (range 2.5-531 mg/m3) at the Stage I service station and 18 mg/m3 (range < 0.2-129 mg/m3) at the Stage II service station. The geometric mean of the exposure of customers to MtBE during refueling at the Stage I service station was 15.3 mg/m3 (range 1.8-74 mg/m3), and at the Stage II service station 3.4 mg/m3 (range 0.2-16 mg/m3). The differences in exposure were statistically significant ($p < 0.05$). The mean refueling times were 57 seconds (range 23-207) at the Stage I and 66 seconds (range 18-120) at the Stage II station. The measurements were done on consecutive days at the various service stations. The temperature ranged from 10 to 17 degrees C, and wind velocity was 2-4 m/s. The climatic conditions were very similar on the measurement days. Based on this study it was found that the Stage II vapor recovery system reduces gasoline emission considerably. The exposure level of customers at the Stage II station during refueling was circa 20-25 percent of the exposure at the Stage I service station when conditions were equal and no other confounding factors such as leaks or spills were present.

Title: Misinterpretation and misuse of exposure limits.
Author(s): Hewett P.
Source: Appl Occup Environ Hyg. 2001 Feb;16(2):251-6.
Abstract: Users of occupational exposure limits (OELs) often fail to distinguish between the complementary processes of risk assessment and exposure (risk) management. The former refers to those activities that lead to the selection of a reasonably protective exposure limit and often includes an analysis of exposure databases and an evaluation of group-based risk. The latter focuses on individual risk, and refers to those actions required of employers to ensure that each employee is unlikely to incur harm to health. This presentation focuses on how this failure to distinguish leads to misinterpretation and misuse of OELs. A typical OEL definition consists of at least three components: a concentration, an averaging time, and a target (usually the individual worker). OELs are occasionally improperly applied, resulting in a reduction of the expected level of protection. For example, sampling strategies proposed by the American Industrial Hygiene

Attachment F – 2-Butyne

Association (AIHA) and Comite European de Normalisation (CEN) permit workers to be aggregated into exposure groups. Under certain circumstances this practice can leave some workers unevaluated and unprotected. Protection is also reduced when the averaging time is extended from a single shift to multiple shifts. Frequently, OELs are misinterpreted as upper limits to exposures averaged over weeks, months, or even years, rather than a single shift. Much of this confusion can be traced to the desire of some to reconcile research (epidemiology) sampling strategies with compliance sampling strategies. But the two have fundamentally different goals and objectives. Others are simply attracted to alternative OEL interpretations that permit frequent overexposures (i.e., measurements that exceed the OEL), thus making compliance easier. Given the current limitations of industrial hygiene and occupational epidemiology, and the general unwillingness of employers to routinely collect exposure data, OELs should continue to be defined as upper limits for single shift exposures. The current OEL model, which permits the use of proximate risk management goals to realize long-range objectives, should be retained, There are, however, valid reasons for augmenting this model to include criteria for evaluating compliance with long-range objectives. The augmented OEL model would be applicable to future new and revised OELs. The author suggests that OEL setting organizations consider harmonizing definitions and statistical interpretations for both existing and new OELs, thus minimizing future misinterpretation and misuse.

Title: Inhibition of bovine plasma amine oxidase by 1,4-diamino-2-butenes and -2-butynes

Author(s): Heung-Bae Jeon, Younghee Lee, Chunhua Qiao, He Huang and Lawrence M. Sayre

Source: Bioorganic & Medicinal Chemistry Volume 11, Issue 21, 15 October 2003, Pages 4631-4641

Abstract: Bovine plasma amine oxidase (BPAO) was previously shown to be irreversibly inhibited by propargylamine and 2-chloroallylamine. 1,4- Diamine versions of these two compounds are here shown to be highly potent inactivators, with IC_{50} values near 20 pM. Mono-N-alkylation or N,N-dialkylation greatly lowered the inactivation potency in every case, whereas the mono-N-acyl derivatives were also weaker inhibitors and enzyme activity was recoverable. The finding that the bis-primary amines 1,4- diamino-2-butyne (a known potent inhibitor of diamine oxidases) and Z-2-chloro-1,4-diamino2-butene are potent inactivators of BPAO is suggestive of unexpected similarities between plasma amine oxidase and the diamine oxidases and implies that it may be unwise to attempt to develop selective inhibitors of diamine oxidase using a diamine construct.

Attachment F – 2-Butyne

Title: Reassessment of occupational exposure limits.
Author(s): Stouten H, Bouwman C, Wardenbach P.
Source: Am J Ind Med, 2008 Jun;51(6):407-18.
Abstract: BACKGROUND: Although the Netherlands currently has its own procedure for evaluating chemical compounds and setting occupational exposure limits (OELs), most of these limits were originally adopted in the 1970s from threshold limit values (TLVs) set by the American Conference of Governmental Industrial Hygienists (ACGIH). However, beginning in the late 1980s, criticism about non-scientific considerations being used to set TLV's suggested that TLVs might not offer sufficient health protection to workers. This situation prompted the Dutch Ministry of Social Affairs and Employment to request that the Health Council of the Netherlands reassess the health protection of MAC values that were contained in the 1994 Dutch MAC list.
METHODS: Criteria documents were prepared for 161 compounds. They were evaluated by a committee of the Health Council of the Netherlands consisting of international experts who reassessed the toxicological hazards of these substances and recommended, whenever possible, health-based OELs. The results of the reassessment by the Health Council were compared with the MAC values of the 1994 Dutch MAC list, ACGIH TLVs, and existing German OELs.
RESULTS: The toxicological database met the committee's criteria for a health-based OEL for only about 40% of the compounds.
CONCLUSIONS: Many older MAC values were either too high or not scientifically supported and therefore not health- based.

Title: Background, approaches and recent trends for setting health-based occupational exposure limits: a minireview.
Author(s): Nielsen GD, Ovrebø S.
Source: Regal Toxicol Pharmacol, 2008 Aug;51(3):253-60, Epub 2008 Apr 14.
Abstract: The setting of occupational exposure limits (OELs) are founded in occupational medicine and the predictive toxicological testing, resulting in exposure-response relationships. For compounds where a No-Observed-Adverse-Effect-Level (NOAEL) can be established, health-based OELs are set by dividing the NOAEL of the critical effect by an overall uncertainty factor. Possibly, the approach may also be used for carcinogens if the mechanism is epigenetic or the genetic effect is secondary to effect from reactions with proteins such as topoisomerase inhibitors, and mitotic and meiotic spindle poisons. Additionally, the NOAEL approach may also be used for compounds with weak genotoxic effect, playing no or only a minor role in the development of tumours, No health-based OEL can be set for direct-acting genotoxic compounds where the life-time risks may be estimated from the low-dose linear non-threshold extrapolation, allowing a politically based exposure level to be set. OELs are set by several agencies in

Attachment F – 2-Butyne

the US and Europe, but also in-house in major chemical and pharmaceutical companies. The benchmark dose approach may in the future be used where it has advantage over the NOAEL approach. Also, more attention should be devoted to sensitive groups, toxicological mechanisms and interactions as most workplace exposures are mixtures.

Title: Setting limit values for chemical substances in the workplace: DNEL(INH) setting according to REACH principles following the example of 2-butyne-1,4-diol

Author(s): [Article in Polish] Kupczewska-Dobecka M, Swiercz R.

Source: Med Pr. 2009;60(5):347-57.

Abstract: Background: Derived No Effect Level (DNEL(inh)) has been set for occupational exposure to but-2-yno-1,4-diol according to REACH principles. Maximum allowable concentration (MAC) and DNEL(inh) have been compared. Material and Methods: Experimental data from two inhalation studies on rats and three oral studies have been used to calculate DNEL. Results: Estimated DNEL(inh) values show significant differences and fall within the range of 0.33-0.02 mg/11)3, depending on the chosen experiment and critical effect. It seems that a 30-day inhalation study best reflects the penetration of xenobiotic to the human organism. This experiment has been used to set MAC value of but-2-yno-1,4-diol of 0,25 mg/m^3 and it is close to local DNEL(inh) – 0.10 mg/m^3. Both values have been estimated on the same starting point NOAEC(loc) – 0.5 mg/m^3, but different assessment factors have been applied. Conclusions: A general feeling is that because of the differences in methodologies for calculating DNELs versus those used for calculating health-based OELs, the DNEL will tend to be lower than any corresponding health-based OEL for that chemical. This indicates that the OEL does not provide the appropriate level of protection required by REACH. The calculation leads to a new value (DNEL) that requires different risk management measures and operational conditions.

Attachment F – 2-Butyne

Welfare Effects Studies (toxicity in aquatic organisms, toxicity in terrestrial species, toxicity to plants/bacteria, effects on visibility/weather/climate, effects on materials, economic effects, effects on personal comfort and well-being)

Title: Molecular mode of interaction of plant amine oxidase with the mechanism-based inhibitor 2-butyne-1,4-diamine

Author(s): Ivo Frêbort, Marek Sebela, lb Svendsen, Shun Hirota, Masaaki Endo, Osamu Yamauchi, Andrea Bellelli, Karel Lemr, Pavel Pec

Source: European Journal of Biochemistry, Vol 267; Issue 5; pages 1423-1433, March 2000

Abstract: 2-Butyne-1,4-diamine (DABI) is a mechanism-based inhibitor of copper-containing plant amine oxidases; the number of turnovers that leads to enzyme inactivation is 20. The product of DABI oxidation is a very reactive aminoallene that reacts with an essential nucleophilic group at the enzyme active site, forming a covalently bound pyrrole and producing an inactive enzyme. The inactivated enzyme shows a new absorption maximum at 295 nm and gives coloured derivatives with p-dimethylaminobenzaldehyde and *p*-dimethylaminocinnamaldehyde that are spectrally similar to the products of pyrrole treated with the above reagents. Resonance Raman spectra of the *p*-dimethylaminobenzaldehyde adduct of pyrrole and the inactivated enzyme show very high degree of similarity, supporting the idea that the product of inactivation is indeed a bound pyrrole. The bound pyrrole is formed already in the anaerobic step of the reaction, while the topa semiquinone radical is not affected, as shown by the EPR and stopped-flow absorption measurements. Peptides containing the DABI binding site were obtained by proteolysis of inactivated enzyme, isolated by HPLC and analysed by amino acid sequencing and MS. The crystal structure of the amine oxidase from pea has been determined; inhibition is caused mainly by the highly reactive DABI product, 4-amino-2-butynal, binding to a nucleophilic residue at the entrance to the substrate channel. As other DABI labeled peptides were also found and no free DABI product was detected by MS after complete inhibition of the enzyme, it is likely that the DABI product binds also to other solvent exposed nucleophilic residues on the enzyme surface.

Title: Copper/topa quinine-containing amine oxidases — Recent research developments

Author(s): Marek Sebela, Ivo Frebort, Marek Petrivalsky and Pavel Pec

Source: Studies in Natural Products Chemistry Volume 26, Part 7, 2002, Pages 1259-1299 Bioactive Natural Products

Abstract: Amine oxidases (EC 1.4.3.6) that contain copper/topa quinone cofactor belong to a new protein group of quinoproteins emerging in recent years. This review brings together information on the general properties of the enzymes and their

Attachment F – 2-Butyne

Physiological functions. In plants, these enzymes are involved in processes of development and senescence, they reduce the concentration of toxic amines produced during exposure to stress conditions, provide hydrogen peroxide for wall stiffening and lignification and precursor compounds for biosynthesis of some alkaloids. Major attention is currently being paid to the structure of the active site of the enzymes that contains copper ions and a post translationally modified tyrosyl residue, topa quinone. Three-dimensional structures recently obtained for several amine oxidases by X-ray diffraction analysis of the respective crystals provide important structural information about the unique protein folding of the native enzyme and molecular arrangement of the active site. Biogenesis of the quinone cofactor is another issue that is addressed frequently at present. Differences between copper/topa quinone-containing amine oxidases and flavoprotein polyamine oxidases are outlined. Finally, future research directions on amine oxidases and the possibilities of their practical application are discussed.

Title: UV-absorption spectra of the radical transients generated from the 193-nm photolysis of allene, propyne, and 2-butyne.

Author(s): Fahr A, Laufer AH.

Source: J Phys Chem A. 2005 Mar 24;109(11):2534.9.

Abstract: The 193-nm photochemistry of allene (H2C=C=CH2), propyne (H3C-C[triple bond]CH), and 2-butyne (1-13C-C[triple bond]C-CH3) has been examined, and the UV spectral region between 220 and 350 nm has been surveyed for UV-absorption detection of transient species generated from the photolysis of these molecules. Time-resolved UV-absorption spectroscopy was used for detection of transient absorption. Gas chromatographic/mass spectroscopic (GC/MS) analysis of the photolyzed samples were employed for identification of the final photodissociation products, An emphasis of the study has been on the examination of possibilities of formation of different C31-13 isomeric radicals, that is, propargyl (H2CCCH) or propynyl (H3CCC), from the 193-nm photolysis of these molecules. Survey of the UV spectral region, following the 193-nm photolysis of dilute mixtures of allene/He resulted in detection of a strong absorption band around 230 nm and a weaker band in the 320-nm region with a relative intensity of about 8:1. The time-resolved absorption traces after the photolysis event show an instantaneous rise, followed by a simple decay. The spectral features, observed in this work, following 193nm photolysis of allene are in good agreement with the previously reported spectrum of H20CCH radical in the 240- and 320-nm regions and are believed to originate primarily from propargyl radicals. In comparison, the spectra obtained from the 193-nm photolysis of dilute mixtures of HCCCH3/He and CH3CCCH3/He were nearly identical, consisting of two relatively broad bands centered at about 240- and

Attachment F – 2-Butyne

320-nm regions with a relative intensity of about 2:1, respectively. In addition, the time-resolved absorption traces after photolysis of propyne and 2-butyne samples, both in the 240 and 320 nm regions, indicated an instant rise followed by an additional slower absorption rise. The distinct differences between the results of allene with those of propyne and 2-butyne suggest the observed absorption features following 193-nm photolysis of these molecules are likely to be composite with contributions from a number of transient species other than propargyl radicals. Propyne and 2-butyne are structurally similar. The methyl (CH3) and propynyl (CH3C[triple bond]C) radicals are likely to be among the photodissociation products of 2-butyne, and similarly, propynyl is likely to be a photodissociation product of propyne. GC/MS product analysis of photolyzed 2-butyne/He mixtures indicates the formation of C2H6 (formed from the combination of CH3 radicals), and a number of C6H6 and C4H6 isomers formed from self- and cross reactions of C3H3 and CH3 radicals, including 1,5-hexadiyne and 2,4-hexadyine, that are potential products of combination reactions of propargyl as well as propynyl radicals.

Title: The gas-phase reaction between silylene and 2-butyne: kinetics, isotope studies, pressure dependence studies and quantum chemical calculations.

Author(s): Becerra R, Cannady JP, Dormer G, Walsh R.

Source: Pill's Chem Chem, Phys. 2009 Jul 14;11(26):5381-44. Epub 2009 Apr 17.

Abstract: Time-resolved kinetic studies of the reactions of silylene, SiH(2), and dideutero-silylene, SiD(2), generated by laser flash photolysis of phenylsilane and phenylsilane-d(3), respectively, have been carried out to obtain rate coefficients for their bimolecular reactions with 2-butyne, CH(3)C[triple bond, length as m-dash]CCI-1(3). The reactions were studied in the gas phase over the pressure range 1-100 Torr in SF(6) bath gas at five temperatures in the range 294-612 K. The second-order rate coefficients, obtained by extrapolation to the high pressure limits at each temperature, fitted the Arrhenius equations where the error limits are single standard deviations: log(k(H)(Infinity)/cm(3) molecule(-1) s(-1) = (-9.67 +/- 0.04) + (1.71 +/0.33) kJ mol(1)/RTIn101og(k(D)(Infinity)/cm(3) molecule(-1) s(-1) = (-9.65 +/- 0.01) + (1.92 +/- 0.13) kJ moll-1)/RTIn10. Additionally, pressure-dependent rate coefficients for the reaction of SiH(2) with 2-butyne in the presence of He (1-100 Torr) were obtained at 301, 429 and 613 K. Quantum chemical (ab initio) calculations of the SiC(4)H(8) reaction system at the G3 level support the formation of 2,3-dimethylsilirene [cyclo-S11^{-} 1(2)C(CH(3))double bond, length as m-dash](CH(3))-1 as the sole end product. However, reversible formation of 2,3-dimethylvinylsilylene [CH(3)CH[double bond, length as m-dash]C (CH(3))SiH] is also an important process. The calculations also indicate the probable involvement of several other intermediates, and possible products. RRKM calculations are in reasonable

Attachment F – 2-Butyne

agreement with the pressure dependences at an enthalpy value for 2,3-dimethylsilirene fairly close to that suggested by the ab initio calculations. The experimental isotope effects deviate significantly from those predicted by RRKM theory. The differences can be explained by an isotopic scrambling mechanism, involving H-D exchange between the hydrogens of the methyl groups and the D-atoms in the ring in 2,3-dimethylsilirene-1,1-d(2). A detailed mechanism involving several intermediate species, which is consistent with the G3 energy surface, is proposed to account for this.

Title: Reaction dynamics of the phenyl radical (C_6H_5) with 1-butyne ($HCCC_2H_5$) and 2-butyne (CH_3CCCH_3)

Author(s): Ralf I. Kaiser,' Fangtong Zhang, Xibin Gu, Vadim V. Kislov and Alexander M. Mebei

Source: Chemical Physics Letters, Volume 461, Issues 1-3, 19 October 2009, Pages 46-53

Abstract: The reactions of the phenyl radical (C_6H_5) with 1-butyne ($HCCC_2H_5$) and 2-butyne ($CH_3CCCI\text{-}1_3$) were studied in a crossed molecular beam machine to shed light on the formation of $C_{10}H_{10}$ isomers in combustion flames. Combining these data with electronic structure calculations, we find that both reactions involve indirect scattering dynamics and are initiated by additions of the phenyl radical with its radical center to the acetylenic carbon atoms. The reaction intermediates fragmented through the ejection of hydrogen atoms yielding 1-phenyl-3-methylallene and 1- phenyl-1-butyne (both from 1-butyne) as well as 1-phenyl-1-methylallene (from 2-butyne).

Title: Intrinsic acidity and electrophilicity of gaseous propargyl/allenyl carbocations.

Author(s): Lalli PM, Corilo YE, Abdelnur PV, Ebertin MN, Laali KK.

Source: Org Biomol Chem, 2010 Jun 7;8(11):2580-5, Epub 2010 Mar 29,

Abstract: The ion/molecule chemistry of four representative propagyl/allenyl cations 1-4 of the general formula R(1)C1-1(+)-C[triple bond]C-R (a) <--> R(1)CH=C=C(+)-R (b), that is, the reactive C(3)H(3)(+) ions of m/z 39 from EI of propargyl chloride (11(2) C(+)-C[triple bond]C-H, 1 a), isomeric C(4)H(5)(+) ions of m/z 53 from EI of 3-butyne-2-ol (2a, 1-1(2)C(+)-C[triple bond]C-CH (3)) and 2-butyne-1-ol (CH(3)-CH(+)-C[triple bond]C-H, 3a), and Ph-C(3)H(2)(+) ions of m/z 115 from 3-phenyl-2-propyn-1- ol (H(2)C(+)-C[triple bond]C-Ph, 4a) was studied via pentaquadrupole mass spectrometry. With pyridine, proton transfer was observed as the predominant process for 1 and the sole reaction channel for the isomeric 2 and 3, whereas 4 reacted preferentially by adduct formation. These outcomes were rationalized using DFT calculations from isodesmic proton transfer reactions. Similar reaction tendencies were observed with acetonitrile and acrylonitrile, with adduct formation appearing again as a minor pathway for

Attachment F – 2-Butyne

1, 2 and 4, and as a major reaction channel for 4. With 1,3-dioxolane, hydride abstraction was a dominant reaction, with proton transfer and adduct formation competing as side reactions. With 2,2-dimethy1-1,3- dioxolane, an interplay of reactions including methyl anion abstraction, proton transfer, hydride abstraction and adduct formation were observed depending on the ion structure, with 4 reacting again mainly by adduct formation. Proton transfer was also observed as a dominant process in reactions with ethanol for 1, 2 and 3, with 4 being nearly unreactive whereas no adduct formation was observed for any of the carbocations studied. Limited reactivity was exhibited by these ions in cycloaddition reaction with isoprene.

Attachment G – 2-Methylhexane

Health Effects Studies (acute toxicity, subchronic toxicity, chronic toxicity – e.g., including reproductive/developmental toxicity, neurotoxicity, immunotoxicity, organ system effects, mutagenicity/genotoxicity, carcinogenicity)

Title: Involuntary bioaccumulation of environmental pollutants in nonsmoking heterogeneous human population
Author(s): Boguslaw K., Krotoszynski, Hugh J. O'Neill
Source: Journal of Environmental Science and Health, Part A: Toxic/Hazardous Substances and Environmental Engineering, Volume 17, Issue 6, 1982, Pages 855 - 883
Abstract: Chemical bioaccumulation in nonsmoking heterogeneous human population was determined by a noninvasive expired air technique. Ubiquitous and nonubiquitous environmental pollutants were isolated and identified and the respective distribution and inhalation hazards were evaluated. Attempts were made to isolate and identify the state of health related expired air constituents.

Title: Predicting toxicity through a computer automated structure evaluation program.
Author(s): G Klopman
Source: Environ Health Perspect. 1985 September; 61: 269-274.
Abstract: The computer automated structure evaluation program (CASE) has been extended to perform automatic quantitative structure-activity relationships (QSAR). Applications include the carcinogenicity of polycyclic aromatic hydrocarbons and of N-nitrosamines. Agreement with experiment is satisfactory.

Title: The photo-oxidation of automobile emissions: measurements of the transformation products and their mutagenic activity
Author(s): Tadeusz E Kleindienst, David F Smith, Edward E Hudgens, Richard F Snow, Erica Perry, Larry D Claxton, Joseph J Bufalini, Francis M Black and Larry T Cupitt
Source: Atmospheric Environment, Part A. General Topics, Volume 26, Issue 16, November 1992, Pages 3039-3053
Abstract: Dilute mixtures of automobile emissions (comprising 50% exhaust and 50% surrogate evaporative emissions) were irradiated in a 22.7 m^3 smog chamber and tested for mutagenic activity by using a variant of the Ames test. The exhaust was taken from a single vehicle, a 1977 Ford Mustang equipped with a catalytic converter. Irradiated and nonirradiated gas-phase emissions were used in exposures of the bacteria, *Salmonella typhimurium,* strains TA100 and TA98. A single set of vehicular operating conditions was used to perform multiple exposures. The mutagenic activities of extracts from the particulate phase were also measured with the standard plate incorporation assay. (In most experiments only direct-acting mutagenic compounds wer e measured.) The gas-phase data

Attachment G – 2-Methylhexane

for TA100 and TA98 showed increased activity for the irradiated emissions when compared to the nonirradiated mixture, which exhibited negligible activity with respect to the control values. The particulate phase for both the irradiated and nonirradiated mixtures showed negligible activity when results were compared to the control values for both strains. However, the experimental conditions limited the amount of extractable mass which could be collected in the particulate phase. The measured activities from the gas phase and particulate phase were converted to the number of revertants per cubic meter of effluent (i.e. the *mutagenic density)* to compare the contributions of each of these phases to the total mutagenic activity for each strain. Under the experimental conditions of this study, the mutagenic density of the gas-phase component of the irradiated mixture contributed approximately two orders of magnitude more of the total TA100 activity than did the particulate phase. For TA98 the gas-phase component contributed approximately one order of magnitude more. However, caution must be exercised in extrapolating these results to urban atmospheres heavily impacted by automotive emissions, because the bacterial mutagenicity assay was used as a screening method, and additional assays using mammalian systems have not yet been conducted. In addition, only limited number of conditions were able to be tested. The significance and limitations of the results are discussed.

Title: Increased sister chromatid exchange frequencies observed in a cohort of inhabitants of a village located at the boundary of an industrial dumping ground: phase I.

Author(s): C Laurent, T Lakhanisky, P Jadot, I Joris, M Ottogali, C Planard, D Bazzoni, J M Foidart and Y Ros

Source: Cancer Epidemiol Biomarkers Prev July 1993 2; 355

Abstract: Since the mid-eighties, a sand pit located at the boundary of Mellery, a small village in Belgium, has been used as a dumping ground for industrial waste. After a particularly dry summer, many people complained of very foul smells coming from the dumping ground. An analysis of the environmental atmosphere detected alkanes and chlorinated saturated and unsaturated hydrocarbons in various concentrations. Consequently, the Belgian Ministry of the Environment requested additional measurements from the dumping site and the surrounding regions. Given the hazards and possible ill health effects associated with simultaneous exposure to low levels of many chemicals, biomarkers of personal exposure were measured in a representative group of people living in this village. The cytogenetic consequences of daily exposure to a mixture of genotoxicants were measured by the Sister Chromatid Exchange assay. The study included a group of 51 environmentally exposed persons (including 11 children) and 52 controls. A significant increase in Sister Chromatid Exchange frequency was detected

Attachment G – 2-Methylhexane

among the inhabitants of the village compared to that of the control group, especially among the children living in Mellery as compared to the matched control children.

Title: Molecular Similarity and Risk Assessment: Analog Selection and Property Estimation Using Graph Invariants
Author(s): S. C. Basak; G. D. Grunwald
Source: SAR and QSAR in Environmental Research, Volume 2, Issue 4, 1994, Pages 289 - 307
Abstract: Four molecular similarity measures have been used to select the nearest neighbor of chemicals in two data sets of 139 hydrocarbons and 15 nitrosamines, respectively. The similarity methods are based on calculated graph invariants which include atom pairs, connectivity indices and information theoretic topological indices. The property of the selected nearest neighbor by each method was taken as the estimate of the property under investigation. The results show that for these data sets, all four methods give reasonable estimates of the properties studied.

Title: A nested case-control study of kidney cancer among refinery/petrochemical workers.
Author(s): J F Gamble, E D Pearlman, and M J Nicolich
Source: Environ Health Perspect. 1996 June; 104(6): 642-650.
Abstract: A nested case-control study was designed to evaluate whether a nearly twofold excess of kidney cancer among workers at a refinery/petrochemical plant was associated with cumulative exposure to C2-05 saturated, C2-05 unsaturated, C6-C10 aliphatic saturated, C6-C10 aliphatic unsaturated, and C6-C10 aromatic process streams. Nonoccupational risk factors were body mass index (BMI), blood pressure (both measured at about age 28), and smoking. There was no significant association with cumulative exposure or tenure as estimated by conditional logistic regression and adjusted for nonoccupational risk factors. Categorical analysis showed increased odds ratios only in the second (low) and fourth (high) quartiles compared to the first quartile reference group of lowest exposed workers, and a three-quarter-fold increased odds ratio for > 32 years' tenure compared to the < 25-year reference group. The number of cases was small with wide confidence intervals around estimate of risk, so the possibility of an exposure- response trend cannot be ruled out. Multivariate analysis identified overweight (high BMI; $p < 0.01$) as the most important risk factor in this data set, followed by tenure and increased blood pressure. There was a weak association with current smoking, but not with pack-years smoked. The risk of kidney cancer for a nonsmoker with normal blood pressure but 25% overweight was increased about 2.6-fold (95% CI = 1.2-5.4). The risk of kidney cancer for a nonsmoker of

Attachment G – 2-Methylhexane

normal weight with high blood pressure (e.g., 150/110), was increased about 4.5 (95% CI, 0.8-26).

Title: Prioritizing testing of organic compounds detected as gas phase air pollutants: structure-activity study for human contact allergens.
Author(s): R Johnson, 0 T Macina, C Graham, H S Rosenkranz, G R Cass, and M H Karol
Source: Environ Health Perspect. 1997 September; 105(9): 986-992
Abstract: Organic compounds that are used or generated anthropogenically in large quantities in cities can be identified through their presence in the urban atmosphere and in air pollutant source emissions. Compounds identified by this method were screened to evaluate their potential to act as contact allergens. The CASE and MULTICASE computer programs, which are based on the detection of structure-activity relationships (SAR), were used to evaluate this potential. These relationships first are determined by comparing chemical structures to biological activity within a learning set comprised of 458 compounds, each of which had been tested experimentally in human trials for its sensitization potential. Using the information contained in this learning set, CASE and MULTICASE predicted the activity of 238 compounds found in the atmosphere for their ability to act as contact allergens. The analysis finds that 21 of 238 compounds are predicted to be active contact allergens (probability >0.5), with potencies ranging from mild to very strong. The compounds come from chemical classes that include chlorinated aromatics and chlorinated hydrocarbons, N-containing compounds, phenols, alkenes, and an S-containing compound. Using the measured airborne concentrations or emission rates of these compounds as an indication of the extent of their use, together with their predicted potencies, provides an efficient method to prioritize the experimental assessment of contact sensitization of untested organic compounds that can be detected as air pollutants.

Title: Chronic inhalation carcinogenicity study of commercial hexane solvent in F-344 rats and B6C3F1 mice.
Author(s): W Daughtrey, P Newton, R Rhoden, C Kirwin, L Haddock, J Duffy, T Keenan, W Richter and M Nicolich
Source: Toxicol. Sci. (1999) 48 (1): 21-29
Abstract: The carcinogenic and chronic toxicity potential of commercial hexane solvent was evaluated in F-344 rats and B6C3F1 mice (50/sex/concentration/species) exposed by inhalation for 6 h/day, 5 days/week for 2 years. Target hexane vapor concentrations were 0, 900, 3000, and 9000 ppm. There were no significant differences in survivorship between control and hexane-exposed groups, and clinical observations were generally unremarkable. Small, but statistically significant decreases in body weight gain were seen in rats of both sexes in the mid- and high-exposure groups and in high-expsoure female mice. The only

Attachment G – 2-Methylhexane

noteworthy histopathological finding in rats was epithelial cell hyperplasia in the nasoturbinates and larynx of exposed groups. This response was judged to be indicative of upper respiratory tract tissue irritation. No significant differences in tumor incidence between control and hexane-exposed rats were found, In mice, uterine tissue from the high-exposure females exhibited a significant decrease in the severity of cystic endometrial hyperplasia compared to controls. An increase in the combined incidence of hepatocellular adenomas and carcinomas was observed in high-exposure female mice. The incidence of liver tumors was not increased in the mid- or low-exposure female mice or in male mice exposed to hexane. An increased incidence of pituitary adenomas was observed In female, but not male mice. This finding was not believed to have been treatment-related because the incidence in the control group was unusually low, and the incidence in exposed groups was not dose-related and was within the historical control range. No other neoplastic changes judged to be treatment-related were observed in tissues from male or female mice. In conclusion, chronic exposure to commercial hexane solvent at concentrations up to 9000 ppm was not carcinogenic to F-344 rats or to male B6C3F1 mice, but did result in an increased incidence of liver tumors in female mice.

Title: Non-Linear QSAR Treatment of Genotoxicity
Author(s): M. Karelson; S. Sild; U. Maran
Source: Molecular Simulation, Volume 24, Issue 4 & 6, 2000, Pages 229-242
Abstract: The nonlinear QSAR approach using the Chebyshev polynomial expansion and neural networks has been applied for the prediction of genotoxicity of compounds. The mutagenic toxicity of heteroaromatic and aromatic amines, measured by the Ames test, was correlated with the molecular descriptors calculated from the molecular structures using quantum-chemical methods. The quantitative models obtained were compared with the results of the linear QSAR treatment. The descriptors appearing in the models reveal the importance of mutagenic interactions of heteroaromatic amines *via* hydrogen bonding, of effects induced by the solvent, and of the size of compound. The dependence of molecular descriptors on environmental effects and on molecular conformations was analysed.

Title: Exhaled methyl nitrate as a noninvasive marker of hyperglycemia in type 1 diabetes
Author(s): B. J. Novak, D. R. Blake, S. Meinardi, F. S. Rowland, A. Pontello, D. M. Cooper, and P. R. Galassetti
Source: Proc Natl Acad Sci U S A. 2007 October 2; 104(40): 15613-15618
Abstract: Recent technical advances allow detection of several hundred volatile organic compounds (VOCs) in human exhaled air, many of which reflect unidentified

Attachment G – 2-Methylhexane

endogenous pathways. Our group has previously estimated plasma glucose levels in healthy adults during a standard oral glucose tolerance test via exhaled VOC analysis. As a result of the metabolic characteristics of hyperglycemia in the diabetic (low insulin and increased free fatty acids and ketones), we hypothesized that different exhaled VOC profiles may be present in children with type 1 diabetes mellitus (T1 DM) during spontaneous hyperglycemia. Exhaled methyl nitrate strongly correlated specifically with the acute, spontaneous hyperglycemia of T1 DM children. Eighteen experiments were conducted among 10 T1 DM children. Plasma glucose and exhaled gases were monitored during either constant euglycemia *(n* = 5) or initial hyperglycemia with gradual correction *(n* = 13); all subjects received i.v. insulin and glucose as needed. Gas analysis was performed on 1.9-liter breath samples via gas chromatography using electron capture, flame ionization, and mass selective detection. Among the ~100 measured exhaled gases, the kinetic profile of exhaled methyl nitrate, commonly present in room air in the range of 5-10 parts per trillion, was most strongly statistically correlated with that of plasma glucose *(P* = 0.003-0.001). Indeed, the kinetic profiles of the two variables paralleled each other in 16 of 18 experiments, including repeat subjects who at different times displayed either euglycemia or hyperglycemia.

Title: Toxicity assessment of volatile organic compounds and polycyclic aromatic hydrocarbons in motorcycle exhaust

Author(s): Chang-Tang Chang and Bor-Yann Chen

Source: Journal of Hazardous Materials, Volume 153. Issue 3, 30 May 2008, Pages 1262-1269

Abstract: This study investigates the toxicity of various pollutant species from motorcycle exhaust via dose—response analysis and margin of safety using *Escherichia coil* DH5a. The toxicity evaluation of the major components of motorcycle exhaust volatile organic compounds (V005), collected with impinger, and polycyclic aromatic hydrocarbons (PAHs), collected with filter and XAD-2, is essential to determine emission standards for motorcycles. The toxicity of benzene (B), toluene (T), ethyl benzene (E) and xylene (X) was selected for comparison as standard V OCs emitted from motorcycles. In addition, three types of reformulated gasoline (high oxygenate and high benzene content (No. 1), low oxygen and high benzene (No. 2), and low oxygen and low benzene (No. 3) were prepared t o reveal combined toxicity of individual compositions. Motorcycle exhaust is significantly more toxic than BTEX due to the highly toxic VOCs generated from incomplete combustion. Overall toxicity evaluation showed that the toxicity, indicated as EC_{50}, was approximately as follows: PAHs > two-stroke engines > four- stroke engines > BTEX.

Attachment G – 2-Methylhexane

Title: An integrated QSAR-PBPK modeling approach for predicting the inhalation toxicokinetics of mixtures of volatile organic chemicals in the rat

Author(s): K. Price; K. Krishnan

Source: SAR and QSAR in Environmental Research, Volume 22, Issue 1 & 2, 2011, Pages 107-128

Abstract: The objective of this study was to predict the inhalation toxicokinetics of chemicals in mixtures using an integrated QSAR-PBPK modelling approach. The approach involved: (1) the determination of partition coefficients as well as Vmax and K_m based solely on chemical structure for 53 volatile organic compounds, according to the group contribution approach; and (2) using the QSAR-driven coefficients as input in interaction-based PBPK models in the rat to predict the pharmacokinetics of chemicals in mixtures of up to 10 components (benzene, toluene, m-xylene, *o*-xylene, p-xylene, ethylbenzene, dichloromethane, trichloroethylene, tetrachloroethylene, and styrene). QSAR-estimated values of Vmax varied compared with experimental results by a factor of three for 43 out of 53 studied volatile organic compounds (VOCs). K_m values were within a factor of three compared with experimental values for 43 out of 53 VOCs. Cross-validation performed as a ratio of predicted residual sum of squares and sum of squares of the response value indicates a value of 0.108 for Vmax and 0.208 for K_m. The integration of QSARs for partition coefficients, Vmax and K_m, as well as setting the K_m equal to *K* (metabolic inhibition constant) within the mixture PBPK model allowed to generate simulations of the inhalation pharmacokinetics of benzene, toluene, m-xylene, o-xylene, *p*-xylene, ethylbenzene, dichloromethane, trichloroethylene, tetrachloroethylene and styrene in various mixtures. Overall, the present study indicates the potential usefulness of the QSAR-PBPK modelling approach to provide first-cut evaluations of the kinetics of chemicals in mixtures of increasing complexity, on the basis of chemical structure.

Attachment G – 2-Methylhexane

Welfare Effects Studies (toxicity in aquatic organisms, toxicity in terrestrial species, toxicity to plants/bacteria, effects on visibility/weather/climate, effects on materials, economic effects, effects on personal comfort and well-being)

Title: Reported emissions of organic gases are not consistent with observations
Author(s): Ronald C. Henry, Clifford H. Spiegelman, John F. Collins, and EunSug Park
Source: Proc Natl Acad Sci U S A. 1997 June 24; 94(13): 6596-6599.
Abstract: Regulatory agencies and photochemical models of ozone rely on self-reported industrial emission rates of organic gases. Incorrect self-reported emissions can severely impact on air quality models and regulatory decisions. We compared self-reported emissions of organic gases in Houston, Texas, to measurements at a receptor site near the Houston ship channel, a major petrochemical complex. We analyzed hourly observations of total nonmethane organic carbon and 54 hydrocarbon compounds from C-2 to C-9 for the period June through November, 1993. We were able to demonstrate severe inconsistencies between reported emissions and major sources as derived from the data using a multivariate receptor model. The composition and the location of the sources as deduced from the data are not consistent with the reported industrial emissions. On the other hand, our observationally based methods did correctly identify the location and composition of a relatively small nearby chemical plant. This paper provides strong empirical evidence that regulatory agencies and photochemical models are making predictions based on inaccurate industrial emissions.

Title: A *Mycobacterium* Strain with Extended Capacities for Degradation of Gasoline Hydrocarbons
Author(s): Floriane Solano-Serena, Remy Marchal, Serge Casaregola, Christelle Vasnier, Jean-Michel Lebeault, and Jean-Paul Vandecasteele
Source: Appl Environ Microbiol. 2000 June; 66(6): 2392-2399
Abstract: A bacterial strain (strain IFP 2173) was selected from a gasoline-polluted aquifer on the basis of its capacity to use 2,2,4- trimethylpentane (isooctane) as a sole carbon and energy source. This isolate, the first isolate with this capacity to be characterized, was identified by 16S ribosomal DNA analysis, and 100% sequence identity with a reference strain of *Mycobacterium austroafricanum* was found. *Mycobacterium* sp. strain IFP 2173 used an unusually wide spectrum of hydrocarbons as growth substrates, including *n*-alkanes and multimethyl-substituted isoalkanes with chains ranging from 5 to 16 carbon atoms long, as well as substituted monoaromatic hydrocarbons. It also attacked ethers, such as methyl t-butyl ether. During growth on gasoline, it degraded 86% of the substrate. Our results indicated that strain IFP 2173 was capable of degrading 3-methyl groups, possibly by a carboxylation and deacetylation mechanism. Evidence that it attacked the quaternary carbon atom structure by an as-yet-undefined

Attachment G – 2-Methylhexane

mechanism during growth on 2,2,4-trimethylpentane and 2,2-dimethylpentane was also obtained.

Title: Air toxics in ambient air of Delhi
Author(s): Anjali Srivastava, A.E. Joseph, S. Patil, A. More, R.C. Dixit and M. Prakash
Source: Atmospheric Environment, Volume 39, Issue 1, January 2005, Pages 59-71
Abstract: Volatile organic compounds (VOCs) are major group of air pollutants which play critical role in atmospheric chemistry. It contributes to toxic oxidants which are harmful t o ecosystem human health and atmosphere. Data on levels of VOCs in developing countries is lacking. In India information at target VOCs as defined in USEPA compendium method TO-14 is almost totally lacking. The present work deals wit h estimation of target VOCs at 15 locations in five categories namely residential, industrial, commercial, traffic intersections and petrol refueling stations in Delhi, the capital of India. The monitoring was carried out during peak hours in morning and evening each month for a year in 2001. Ambient air was adsorbed on adsorbent tubes, thermally desorbed and analyzed on GC—MS. The results show t hat levels of VOCs are high and stress the need for regular monitoring programme of VOCs in urban environment.

Title: Occupational Exposure to Volatile Organic Compounds and Aldehydes in the U.S. Trucking Industry
Author(s): M. E. Davis, A. P. Blicharz, J. E. Hart, F. Laden, E. Garshick, and T. J. Smith
Source: Environ Sci Technol. 2007 October 15; 41(20): 7152-7158.
Abstract: Diesel exhaust is a complex chemical mixture that has been linked to lung cancer mortality in a number of epidemiologic studies. However, the dose—response relationship remains largely undefined, and the specific components responsible for carcinogenicity have not been identified. Although previous focus has been on the particulate phase, diesel exhaust includes a vapor phase of numerous volatile organic compounds (VOCs) and aldehydes that are either known or suspected carcinogens, such as 1,3- butadiene, benzene, and formaldehyde. However, there are relatively few studies that quantify exposure to VOCs and aldehydes in diesel-heavy and other exhaust-related microenvironments. As part of a nationwide assessment of exposure to diesel exhaust in the trucking industry, we collected measurements of VOCs and aldehydes at 15 different U.S. trucking terminals and in city truck drivers (with 6 repeat site visits), observing average shift concentrations in truck cabs and at multiple background and work area locations within each terminal. In this paper, we characterize occupational exposure to 18 different VOCs and aldehydes, as well as relationships with particulate mass (elemental carbon in PM < 1 *p* m and PM2.5) across locations to determine source characteristics. Our results show that occupational exposure to VOCs and aldehydes varies significantly across the different sampling

Attachment G – 2-Methylhexane

locations within each terminal, with significantly higher exposures noted in the work environments over background levels ($p < 0.01$). A structural equation model performed well in predicting terminal exposures to VOCs and aldehydes as a function of job, background levels, weather conditions, proximity to a major road, and geographic location (R^2 = 0.2-0.4 work area; R^2 = 0.5-0.9 background).

Title: Assessing photochemical ozone formation in the Pearl River Delta with a photochemical trajectory

Author(s): H.R. Cheng, H. Guo,,S.M. Saunders, S.H.M. Lam, F. Jiang, X.M. Wang, I.J. Simpson, D.R. Blake, P.K.K. Louie and T.J. Wang

Source: Atmospheric Environment, Volume 44, Issue 34, November 2010, Pages 4199-4208

Abstract: A photochemical trajectory model (PTM), coupled with the Master Chemical Mechanism (MCM) describing the degradation of 139 volatile organic compounds (VOCs) in the troposphere, was developed and used for the first time to simulate the formation of photochemical pollutants at Wangqingsha (WQS), Guangzhou during photochemical pollution episodes between 12 and 17 November, 2007. The simulated diurnal variations and mixing ratios of ozone were in good agreement with observed data (R^2 = 0.80, $P < 0.05$), indicating that the photochemical trajectory model — an integration of boundary layer trajectories, precursor emissions and chemical processing — provides a reasonable description of ozone formation in the Pearl River Delta (PRD) region. Calculated photochemical ozone creation potential (POCP) indices for the region indicated that alkanes and oxygenated organic compounds had relatively low reactivity, while alkenes and aromatics presented high reactivity, as seen in other airsheds in Europe. Analysis of the emission inventory found that the sum of 60 of the 139 VOC species accounted for 92% of the total POCP-weighted emission. The 60 VOC species include C_2—C_6 alkenes, C_6—C_8 aromatics, biogenic VOCs, and so on. The results indicated that regional scale ozone formation in the PRD region can be mainly attributed to a relatively small number of VOC species, namely isoprene, ethene, m-xylene, and toluene, etc. A further investigation of the relative contribution of the main emission source categories to ozone formation suggested that mobile sources were the largest contributor to regional 0_3 formation (40%), followed by biogenic sources (29%), VOC product-related sources (23%), industry (6%), biomass burning (1%), and power plants (1%). The findings obtained in this study would advance our knowledge of air quality in the PRD region, and provide useful information to local government on effective control of photochemical smog in the region.

Attachment G – 2-Methylhexane

Title: A Three Year Study on 14 VOCs at One Site in Rome: Levels, Seasonal Variations, Indoor/Outdoor Ratio and Temporal Trends

Author(s): Sergio Fuselli, Marco De Felice, Roberta Morlino, and Luigi Turrio-Baldassarri

Source: Int J Environ Res Public Health. 2010 October; 7(10): 3792-3803

Abstract: Fourteen volatile organic compounds (VOCs)—twelve hydrocarbons and two organochlorine compounds— were monitored both outdoors and indoors for three years at one site in Rome. Results showed that 118 out of 168 indoor seasonal mean values were higher than the corresponding outdoor concentrations. The most relevant source of outdoor hydrocarbons was automotive exhaust emissions. Due to the enforcement of various measures to protect health and the environment, outdoor levels of monoaromatic hydrocarbons decreased about ten fold over 15 years, and aliphatic hydrocarbons also decreased. With the decrease in these outdoor concentrations, indoor air sources are likely to be more relevant for indoor air exposures. Winter outdoor values for monoaromatic hydrocarbons were generally markedly higher than the summer ones. The gradual replacement of the current fleet of circulating cars with new cars complying with EURO 5 standards, further reducing hydrocarbon emissions, may possibly lead to an increase in the observed indoor/outdoor ratios. It is indeed more difficult to remove indoor sources, some of which are still unknown.

Attachment H – 3,4-Dimethyl-1-pentene

Welfare Effects Studies (toxicity in aquatic organisms, toxicity in terrestrial species, toxicity to plants/bacteria, effects on visibility/weather/climate, effects on materials, economic effects, effects on personal comfort and well-being)

Title: Analyses of volatile organic compounds concentrations and variation trends in the air of Changchun, the northeast of China

Author(s): Chunming Liu, Zili Xu, Yaoguo Du and Haich en Guo

Source: Atmospheric Environment, Volume 34, Issue 26,2 August 2000, Pages 4459-4466

Abstract: The concentrations of volatile organic compounds (VOCs) were monitored from September 1997 to July 1998 at five different function zones in Changchun, the northeast of China. The VOCs levels by the roadside (the center zone of the main street) and In the downtown area were higher than those in other three areas, with the lowest concentration observed at rural site. A seasonal variation of VOCs in all sampling sites was observed, with the maximum in winter and the minimum in spring. The diurnal variation of the total mean VOC concentrations showed two peaks related with traffic density for the A, B and D sites, and a single peak for the industrial area.

Attachment I – 3-Methyl-1-pentene

Health Effects Studies (acute toxicity, subchronic toxicity, chronic toxicity – e.g., including reproductive/developmental toxicity, neurotoxicity, immunotoxicity, organ system effects, mutagenicity/genotoxicity, carcinogenicity)

Title: Continuous hourly analysis of C2-C10 non-methane hydrocarbon compounds in urban air by GC-FID
Author(s): Charles T. Farmer, Peter J. Milne, Daniel D. Riemer, Rod G. Zika
Source: Environ. Sci. Technol., 1994, 28 (2), pp 238-245
Abstract: None provided.

Title: Use of PI Index in Computer-Aided Designing of Bioactive Compounds
Author(s): Khadikar, Padmakar V.; Diudea, Mircea V.; Singh, Jyoti; John, Peter E.; Shrivastva, Anjalee; Singh, Shalini; Karmarkar, Sneha; Lakhwani, Meenakshi; Thakur, Purnima
Source: Current Bioactive Compounds, Volume 2, Number 1, March 2006, pp. 19-56(38)
Abstract: In this review we critically examine PI (Padmakar-Ivan) index of organic compounds acting as drugs and discussed its applications in Computer-Aided designing of bioactive compounds with special reference to designing of carbonic anhydrase inhibitors, lipophilicity, toxicity, tadpole narcosis, bio-concentration factor, diuretic activity and carcinogenic activity of aromatic hydrocarbons and heterocycles etc.

Title: Chemical speciation and anthropogenic sources of ambient volatile organic compounds (VOCs) during summer in Beijing, 2004
Author(s): Sihua Lu, Ying Liu, Min Shao and Shan Huang
Source: Frontiers of Environmental Science & Engineering in China, 2007, Volume 1, Number 2, 147-152
Abstract: Volatile organic compounds (VOCs) were measured at six sites in Beijing in August, 2004. Up to 148 VOC species, including C_3 to C_{12} alkanes, C_3 to C_{11} alkenes, C_6 to C_{12} aromatics, and halogenated hydrocarbons, were quantified. Although the concentrations differed at the sites, the chemical compositions were similar, except for the Tongzhou site where aromatics were significantly high in the air. Based on the source profiles measured from previous studies, the source apportionment of ambient VOCs was preformed by deploying the chemical mass balance (CMB) model. The results show that urban VOCs are predominant from mobile source emissions, which contribute more than 50% of the VOCs (in mass concentrations) to ambient air at most sites. Other important sources are gasoline evaporation, painting, and solvents. The exception is at the Tongzhou site where vehicle exhaust, painting, and solvents have about equal contribution. around 35% of the ambient VOC concentration, As the receptor model is not valid for deriving the sources of reactive species, such as isoprene and 1,3-

Attachment I – 3-Methyl-1-pentene

butadiene, other methodologies need to he further explored.

Title: Pollutant Emissions from Gasoline Combustion. 1. Dependence on Fuel Structural Functionalities

Author(s): Hongzhi R. Zhange, Eric G. Eddings and Adel F. Sarofim

Source: Environ. Sci. Technol., 2008, 42 (15), pp 5615-5621

Abstract: To study the formation of air pollutants and soot precursors (e.g., acetylene, 1,3-butadiene, benzene, and higher aromatics) from aliphatic and aromatic fractions of gasoline fuels, the Utah Surrogate Mechanisms is extended to include submechanisms of gasoline surrogate compounds using a set of mechanism generation techniques. The mechanism yields very good predictions of species concentrations in premixed flames of n-heptane, isooctane, benzene, cyclohexane, olefins, oxygenates, and gasoline using a 23-component surrogate formulation. The 1,3-butadiene emission comes mainly from minor fuel fractions of olefins and cyclohexane. The benzene formation potential of gasoline components shows the following trends as functions of (i) chemical class: n-paraffins < isoparaffins < olefins < naphthalenes < alkylbenzenes < cycloparaffins toluene; (ii) carbon number: n-butane < n-pentane n- hexane; and (iii) branching: n-hexane < isohexane < 2,2,4-trimethylpentane < 2,2,3,3-tetramethylbutane. In contrast, fuel structure is not the main factor in determining acetylene formation. Therefore, matching the benzene formation potential of the surrogate fuel to that produced by the real fuel should have priority when selecting candidate surrogate components for combustion simulations.

Attachment I – 3-Methyl-1-pentene

Welfare Effects Studies (toxicity in aquatic organisms, toxicity in terrestrial species, toxicity to plants/bacteria, effects on visibility/weather/climate, effects on materials, economic effects, effects on personal comfort and well-being)

Title: Formic acid and acetic acid: Emissions, atmospheric formation and dry deposition at two southern California locations

Author(s): Daniel Grosjeana

Source: Atmospheric Environment, Part A. General Topics, Volume 26, Issue 18, December 1992, Pages 3279-3286

Abstract: Emission rates, in situ formation rates and removal rates by dry deposition are estim ated for formic acid (HCOOH, C1) and acetic acid (CH3COOH, C2), which are the most abundant acids in southern California air and together account for much of the airborne acidity and are the leading contributors to acid dry deposition. Using data for eight unreactive tracers, direct emission rates during the fall 1987 are estimated to be 5.6 and 12.8 metric tons d^{-1} for C1 and C2, respectively, at a coastal source-dominated site. These emissions rates increase to 9.6(C1) and 20.4 (C2) metric tons d^{-1} during the summer. *In situ* formation in the atmosphere via the ozone- olefin reaction is an important source for both acids. This reaction produces an estimated 25.0 and 10.1 metric tons d^{-1} of C1 and C2, respectively, during the day and 34.5 (C1) and 4.3 (C2) metric tons d^{-1} at night. More acetic acid than formic acid is emitted by direct sources, with C2/C1 emission rate ratios of 2.1-2.3. The reverse is true of in situ formation, with C1/C2 production rate ratios of 2.5 (day) and 8.0 (night). Dry deposition removal rates depend on season (fall > summer) and location (inland > coastal) and are 22-52 metric tons c1-1 for Cl, and 32-83 metric tons d^{-1} for C2. Source (emissions + in situ formation) and sink (dry deposition) terms are of the same magnitude in all six cases studied and balance each other well in three of these cases. Uncertainties in emission, in situ production and removal rates are discussed and reflect uncertaintes in olefin and unreactive tracer emission rates, yields of organic acids from the Criegee biradical (oz one-olefin reaction), and dry deposition velocity, respectively.

Title: Isolation and Characterization of Anaerobic Ethylbenzene Dehydrogenase, a Novel Mo-Fe-S Enzyme

Author(s): Hope A. Johnson, Dale A. Pelletier, and Alfred M. Spormann

Source: J Bacterial. 2001 August; 183(15): 4536-4542

Abstract: The first step in anaerobic ethylbenzene mineralization in denitrifying Azoarcus sp. strain EB1 is the oxidation of ethylbenzene to (S)-(-)-1-phenylethanol. Ethylbenzene dehydrogenase, which catalyzes this reaction, is a unique enzyme in that it mediates the stereoselective hydroxylation of an aromatic hydrocarbon in the absence of molecular oxygen. We purified ethylbenzene dehydrogenase to

Attachment I – 3-Methyl-1-pentene

apparent homogeneity and showed that the enzyme is a heterotrimer (a(3y) with subunit masses of 100 kDa (a), 35 kDa ((3), and 25 kDa (y). Purified ethylbenzene dehydrogenase contains approximately 0.5 mol of molybdenum, 16 mol of iron, and 15 mol of acid-labile sulfur per mol of holoenzyme, as well as a molydopterin cofactor. In addition to ethylbenzene, purified ethylbenzene dehydrogenase was found to oxidize 4-fluoro-ethylbenzene and the nonaromatic hydrocarbons 3-methyl-2-pentene and ethylidenecyclohexane. Sequencing of the encoding genes revealed that ebdA encodes the a subunit, a 974-amino-acid polypeptide containing a molybdopterin-binding domain. The ebdB gene encodes the 6 subunit, a 352-amino-acid polypeptide with several 4Fe-4S binding domains. The ebdC gene encodes the y subunit, a 214-amino-acid polypeptide that is a potential membrane anchor subunit. Sequence analysis and biochemical data suggest that ethylbenzene dehydrogenase is a novel member of the dimethyl sulfoxide reductase family of molybdopterin-containing enzymes.

Title: 11 - Biodegradation of oil hydrocarbons and its implications for source identification
Author(s): Roger C. Prince and Clifford C. Walters
Source: Oil Spill Environmental Forensics Fingerprinting and Source Identification 2007, Pages 349-379
Abstract: Hydrocarbons have been part of the biosphere from its inception, produced initially by prebiotic processes, and subsequently both by living organisms and during t he generation of fossil fuels. As highly reduced forms of carbon, hydrocarbons provide a rich source of energy and carbon to those organisms, typically microorganisms, which are able to consume them. Indeed, almost all hydrocarbons are readily degraded under appropriate conditions. There is, nevertheless, a clear preference for the catabolism of some molecules before others; hence, the composition of a fuel or crude oil changes as biodegradation proceeds.

Title: Study of the Alarming Volatile Characteristics of Tessaratoma papillose Using SPME-GC-MS
Author(s): Zhang, Zhuo-Min; Wu, Wen-Wei; Li, Gong-Ke
Source: Journal of Chromatoarashic Science, Volume 47, Number 4, April 2009, pp. 291-296(6)
Abstract: The stinkbug's volatile compositions would alter very much before and after stinkbugs were disturbed or irritated, which caused the alarming effect. An efficient headspace solid-phase microextraction sampling method was established to study the alarming volatile characteristics and potential alarming volatiles of stinkbugs (Tessaratoma papillose) followed by gas chromatography-mass spectrometry detection. The number of volatiles identified was 16 and 22

Attachment I – 3-Methyl-1-pentene

before and after stinkbug irritation, respectively. Long-chain alkanes, alkenes, and alcohols consisted of the main volatile compositions of Tessaratoma papillose. When stinkbugs were disturbed, the typical unsaturated volatiles were released, especially including a series of tridecane derivatives. In comparison with the volatile compounds of lichi leaf and flower (plants the stinkbug eats), it could be seen that most stinkbug alarming volatiles were synthesized by the insects themselves, and that they do not originate from their food. The different statistical alarming volatile characteristics of Tessaratoma papillose before and after irritation were interpreted by principal component analysis in the original Chromatography Data Processing System. However, temperature and light did not affect the alarming volatile characteristics. The variety of the stinkbug alarming volatile characteristics before and after irritation was specified by common model strategy. Tridecane, [E]-2-hexenal, dodecane, [E]-2-hexen-1-ol acetate, and 2,3-dimethyl-1-pentene contributed most to the various alarming volatile characteristics before and after irritation, which might be the potential alarming volatiles. It is hoped that this work will provide useful information for insect control.

Attachment J – cis-1,2-Dimethylcyclohexane

Health Effects Studies (acute toxicity, subchronic toxicity, chronic toxicity – e.g., including reproductive/developmental toxicity, neurotoxicity, immunotoxicity, organ system effects, mutagenicity/genotoxicity, carcinogenicity)

Title: Non-Linear QSAR Treatment of Genotoxicity
Author(s): M. Karelson; S. Sild; U. Maran
Source: Molecular Simulation, Volume 24, Issue 4 & 6, 2000, Pages 229-242
Abstract: The nonlinear QSAR approach using the Chebyshev polynomial expansion and neural networks has been applied for the prediction of genotoxicity of compounds. The mutagenic toxicity of heteroaromatic and aromatic amines, measured by the Ames test, was correlated with the molecular descriptors calculated from the molecular structures using quantum-chemical methods. The quantitative models obtained were compared with the results of the linear QSAR treatment. The descriptors appearing in the models reveal the importance of mutagenic interactions of heteroaromatic amines via hydrogen bonding, of effects induced by the solvent, and of the size of compound. The dependence of molecular descriptors on environmental effects and on molecular conformations was analysed.

Title: Interior Air Pollution in Automotive Cabins by Volatile Organic Compounds Diffusing from Interior Materials: I. Survey of 101 Types of Japanese Domestically Produced Cars for Private Use
Author(s): Toshiaki Yoshida, Ichiro Matsunaga, Kimiko Tomioka, Shinji Kumagai
Source: Indoor and Built Environment, Vol. 15(5): 425-444, Oct. 2006
Abstract: The types and concentrations of organic compounds in the interior air of 101 different types of Japanese domestically produced private-use cars were examined. All the vehicles had been registered in the summer season as new cars and were less than 3 years old. The airborne compounds in the cabins were collected for 24h under static condition with the engine stopped and the windows, doors and vents closed. A total of 275 organic compounds, including many aliphatic hydrocarbons and aromatic hydrocarbons, were identified, and 242 of them could be quantitated for each cabin. The sum of the concentrations of 241 compounds excluding formaldehyde was approximately 600 g m^{-3} as a median, ranging from 136 to 3968 g m^{-3} for the tested cars. The findings demonstrated that the air in the cabin of these cars was contaminated by high concentrations of a large variety of organic compound diffusing from the interior materials.

Attachment J – cis-1,2-Dimethylcyclohexane

Title: Interior Air Pollution in Automotive Cabins by Volatile Organic Compounds Diffusing from Interior Materials: II. Influence of Manufacturer, Specifications and Usage Status on Air Pollution, and Estimation of Air Pollution Levels in Initial Phases of Delivery as a New Car

Author(s): Toshiaki Yoshida, Ichiro Matsunaga, Kimiko Tomioka, Shinji Kumagai

Source: Indoor and Built Environment, Vol. 15(5): 445-462, Oct. 2006

Abstract: Air pollution in the cabins of 101 Japanese cars due to organic compounds diffusing from the interior materials has been described in our previous report. In the present study, the influence of the manufacturer, specifications and usage status of these cars on the interior air pollution was evaluated by covariance analysis. Also, the levels of air pollution in the initial phases of delivery as a new car were estimated using previous data for the time-courses of interior concentrations of organic compounds measured in another new car. The findings showed greater air pollution in the cabins of luxury cars, with leather seats or leather steering wheels, or high-end catalogue prices. Differences in the specifications contributed more markedly to interior air pollution than differences in manufacturers. Also, usage status, such as everyday ventilation affected the long- term interior air quality. The sum values of interior concentrations of 154 compounds, for which there were time-course data, were estimated to be approximately 1700 μg m^{-3} as a median (max. 11,000 μg m^{-3}) at 1 month from delivery (interior temperature. 32 C; interior humidity, 45%).

Attachment J – cis-1,2-Dimethylcyclohexane

Welfare Effects Studies (toxicity in aquatic organisms, toxicity in terrestrial species, toxicity to plants/bacteria, effects on visibility/weather/climate, effects on materials, economic effects, effects on personal comfort and well-being)

Title: Acute toxicities to larval rainbow trout of representative compounds detected in Great Lakes fish
Author(s): Carol Cotant Edsall
Source: Bull. Environ. Contam. Toxicol. (1991) 46:173-176
Abstract: Contribution 752, of the National Fisheries Research Center-Great Lakes, U.S. Fish and Wildlife Service, 1451 Green Road, Ann Arbor, MI 48105

Title: Honey Bees: Estimating the Environmental Impact of Chemicals
Author(s): edited by James Devillers, Minh-Ha-Pham-Delegue
Source: London and New York: CRC Press.
Abstract: Honey Bees: Estimating the Environmental Impact of Chemicals is an updated account of the different strategies for assessing the ecotoxicity of xenobiotics against these social insects, which play a key role in both ecology and agriculture. In addition to the classical acute laboratory test,semi-field cage tests and full field funnel tests, new tests based mainly on behavioral responses are for the first time clearly described. Information on the direct and indirect effects on honey bees of radionuclides, heavy metals, pesticides, semi-volatile organic compounds and genetically modified plants is also presented.

Attachment K – cis-2-Octene

Health Effects Studies (acute toxicity, subchronic toxicity, chronic toxicity – e.g., including reproductive/developmental toxicity, neurotoxicity, immunotoxicity, organ system effects, mutagenicity/genotoxicity, carcinogenicity)

Title: Hydrocarbon Gases Emitted from Vehicles on the Road. 1. A Qualitative Gas Chromatography/Mass Spectrometry Survey

Author(s): Christine V. Hampton, William R. Pierson, T. Michael Harvey, William S. Updegrove, and Richard S. Marano

Source: Environ. Sci. Technol. 1982, 16, 287-298

Abstract: The gas-phase hydrocarbons ≥C_5 generated by motor vehicles in highway operation were surveyed in the Allegheny Mountain Tunnel of the Pennsylvania Turnpike in 1979. The samples were collected on Tenax GC polymer adsorbent and analyzed by glass-capillary gas chromatography/mass spectrometry. Approximately 400 vehicle-generated compounds were detected. Of these, over 300 were either completely or partially identified. In the various tunnel samples the same peaks are always present, with relative intensities varying according to the traffic composition. The compounds identified fall largely into homologous series of normal and branched alkanes, alkenes, and various alkyl series based on cyclopentane, cyclohexane, benzene, styrene, indan, naphthalene, and decalin. Compounds not associated with homologous series include indene, divinylbenzene, phenylacetylene, benzaldehyde, phenol, and a few halocarbons. Results from a preliminary experiment in 1977 at the Tuscarora Mountain Tunnel are also reported.

Title: In situ organic aerosol formation during a smog episode: Estimated production and chemical functionality

Author(s): Daniel Grosjean

Source: Atmospheric Environment, Part A. General Topics, Volume 26, Issue 6, April 1992, Pages 953-963

Abstract: Estimates are presented of the amount and chemical functionality of the organic aerosol formed in situ in the atmosphere during a smog episode. Input parameters used in these estimates include speciated volatile organic carbon (VOC) emission rates, their rates of reaction, products including condensible species, and the nature and yields of these condensible species. The functional groups predicted to form and the estimated amount produced during a typical smog episode are carbonyls, aliphatic nitrates, aliphatic carboxylic acids, phenols and nitro aromatics (670, 240, 1360, 3120 and 3120 kg day^{-1}, respectively, using southern California V OC emission data as an example of application). Similarities and differences between calculated organic aerosol composition and experimental observations (two data sets obtained using different methods) are

Attachment K – cis-2-Octene

discussed in terms of organic aerosol formation pathways and sampling artifact for the following functional groups: aliphatic carboxylic acids, carbonyls, aliphatic nitrates, phenols, aromatic acids, nitro aromatics, amides and esters.

Title: A fresh look at meat flavor
Author(s): C.R. Calkins and J.M. Hodgen
Source: Meat Science, Volume 77, Issue 1, September 2007, Pages 63-80
Abstract: Hundreds of compounds contribute to the flavor and aroma of meat. Complex interactions between various compounds influence the perception of meat flavor. Inherent flavor of a meat product can be influenced by oxidation, lipid content, feeding/diet, myoglobin, and pH. Diet plays an important role in both ruminants and nonruminants. New research reveals important relationships in flavor among multiple muscles within a single animal carcass. This animal effect includes the presence of off- flavors. Diets high in polyunsaturated fatty acids may be contributing to the appearance of off-flavors in beef. Compounds associated with liver-like off-flavor notes in beef have been identified in raw tissue.

Attachment L – Ethanol

Health Effects Studies (acute toxicity, subchronic toxicity, chronic toxicity – e.g., including reproductive/developmental toxicity, neurotoxicity, immunotoxicity, organ system effects, mutagenicity/genotoxicity, carcinogenicity)

Title: Alpha- and beta-adrenergic receptors in rat myocardium membranes; rater prolonged ethanol inhalation
Author(s): D. Sabourault, Francoise Bauche, Y. Giudicelli, J. Nordmann and R. Nordmann
Source: Cellular and Molecular Life Sciences, 1981, Volume 37, Number 3, 227-229
Abstract: After 3 weeks of continuous ethanol intoxication by inhalation, the maximal number and affinity of the α- and β-receptors of rat heart were unchanged. These data indicate that adrenergic receptor disturbances are not involved in the mechanism of chronic ethanol-induced triglyceride deposition in the heart.

Title: Changes in the rat hepatic mixed function oxidase system associated with chronic ethanol vapor inhalation
Author(s): Edward T. Morgan, Michael Devine and Paul Skett
Source: Biochemical Pharmacology, Volume 30, Issue 6, 15 March 1981, Pages 595-600
Abstract: Chronic ethanol vapor inhalation by rats increased hepatic microsomal aniline hydroxylase activity, increasing the turnover number and decreasing the K_m. Activity of ethanol-induced microsomes toward other substrates was also examined. The increase in aniline hydroxylase activity as a result of ethanol treatment is attributed to an increase in a form of cytochrome P-450 with a high specific activity toward aniline. Since the ethanol effect on aniline hydroxylation had disappeared 24 hr after treatment was discontinued, a high rate of turnover of this enzyme was deduced. Dimethylsulfoxide (56 mM) produced a reverse type I spectral change in ethanol-induced, but not in control, microsomes. This was interpreted as being due to a change in the spin state of the cytochrome P-450 in these microsomes. Acetone added to the incubation produced an increased rate of aniline hydroxylation by microsomes from control and ethanol-induced rats. The difference between the rate of aniline hydroxylation by control microsomes and the rate by ethanol-induced microsomes was, however, abolished at higher acetone concentrations.

Title: Sex dependent effect of chronic ethanol consumption in rats on hepatic microsome mediated mutagenicity of benzo[a]pyrene
Author(s): H.K. Seitz, A.J. Garro and CS. Lieber
Source: Cancer Letters, Volume 13, Issue 2, July 1981, Pages 97-102
Abstract: The effect of chronic ethanol consumption by rats on hepatic m icrosomal metabolism of the procarcinogen benz o[a]pyrene (B[a]P) was investigated both with respect to induction of microsomal arylhydrocarbon hydroxylase (AHH) activity and activation of B[a]P to a mutagen. In female rats, chronic ethanol

Attachment L – Ethanol

ingestion produced a 42% increase in AHH activity (P < 0.01), as measured in isolated microsomes, and also resulted in a significantly enhanced capa city (P < 0.01) of these microsomes to activate B[a]P to a mutagen detectable in the Ames bacterial mutagenesis assay. Hepatic m icrosomes from male rats on the other hand did not exhibit any significant differences, either in AHH activity or in their capacity to activate B[a]P to a mutagen after chronic ethanol feeding.

Title: Lung function changes by ethanol inhalation.
Author(s): Zuskin E, Bouhuys A, Sarid M.
Source: Clin Allergy. 1981 May;11(3):243-8.
Abstract: The acute effect of ethanol inhalation on ventilatory capacity was studied in a group of six healthy volunteers and the results were compared to those obtained after the inhalation of a saline solution. There was a significant decrease in flow rates on partial expiratory flow-volume (PEFV) curves up to 90 min after ethanol inhalation. FEV1 values did not change significantly, either after ethanol or saline aerosol. Pretreatment with atropine did not prevent the acute reductions in flow rates in comparison with the reductions without atropine. Pretreatment with disodium cromoglycate (DSCG) considerably diminished the acute reductions of flow rates caused by ethanol inhalation. These results suggest that ethanol in some persons may act, at least partly, through releasing mediators with bronchoconstrictive action.

Title: Effect of ethanol on vinyl chloride carcinogenesis.
Author(s): Radike MJ, Stemmer KL, Bingham E.
Source: Environ Health Perspect. 1981 Oct;41:59-62.
Abstract: Four treatment groups (80 male Sprague-Dawley rats/group) were used in a 2 X 2 factorial design: inhalation of 600 ppm vinyl chloride (VC) 4 hr/day, 5 days/week for 1 year; VC and ingestion of 5% ethanol in water (v/v); filtered air and ethanol; filtered air. Ingestion of ethanol was begun 4 weeks prior to inhalation of VC and continued for life or termination of the study at two and one-half years from the first VC exposure, In this model system, ethanol potentiated the carcinogenic response to VC in the liver and produced an excess of neoplasms in animals receiving ethanol alone. Inhalation of VC induced angiosarcoma of the liver in 23% of the exposed animals; ethanol in addition to VC inhalation increased the incidence to 50%. Concomitant administration of VC and ethanol also produced an excess of hepatocellular carcinoma and lymphosarcoma. Ethanol with or without VC had a strong tumorigenic effect on the endocrine system. These results indicate that ethanol is a cocarcinogen in relation to the carcinogen VC.

Title: Comparison of unconditioned reflex and conditioned avoidance tests in rats

Attachment L – Ethanol

exposed by inhalation to carbon monoxide, 1,1,1-trichloroethane, toluene or ethanol.

Author(s): Mullin LS, Krivanek ND.

Source: Neurotoxicology. 1982 Jul:3(1):126-37.

Abstract: The sensitivity of unconditioned reflex and conditioned avoidance tests in evaluating behavioral toxicity was compared. Male rats were exposed by inhalation up to four hours to 0, 200, 400, 800 or 1600 ppm carbon monoxide (CO); 0, 1500, 3000, 6000 or 12,000 ppm 1,1,1-trichloroethane; 0, 800, 1600, 3200, or 6400 ppm toluene; or 0, 4000, 8000, 16,000 or 32,000 ppm ethanol. Animals were tested for behavioral changes at one-half, one, two and four hours during exposure and eighteen hours after exposure ended. In unconditioned reflex testing the presence or absence of specific unconditioned reflexes (such as corneal, placing, grasping and righting reflexes) and simple behavior patterns including locomotor activity and coordination were observed. The conditioned reflex task consisted of shock avoidance by lever press following simultaneous light and sound stimuli. Rats began to fail unconditioned reflex tests at 800 ppm CO, 3000 ppm trichloroethane, 800 ppm toluene and 8000 ppm ethanol. Decrements in conditioned avoidance were observed at 800 ppm CO, 6000 ppm trichloroethane, 3200 ppm toluene and 8000 ppm ethanol. Neither test was consistently more sensitive than the other in detecting behavioral changes. For both methods, the concentrations at which changes were detected in rats were two to tenfold higher than those reported for human effects.

Title: The activity of 2-(2',4'-diaminophenoxy)ethanol in 3 genetic toxicity bioassays.

Author(s): Brusick DJ Jaoannath DR, Matheson D.

Source: Mutat Res. 1982 Dec;102(4) 361-72

Abstract: 2-(2',4'-Diaminophenoxy)-ethanol, a hair-dye ingredient was evaluated for genetic activity in vitro using urine collected from mice in an Ames test and in vivo using the mouse dominant-lethal assay and the mouse spot test for somatic mutation detection. All 3 studies were conducted using dermal application of the dye material to shaved skin. The applied dose levels ranged from 15 to 1500 mg/kg body weight. The results of these 3 studies were considered to be negative although urine analysis and spot-test data showed non-significant dose-related increases.

Title: Adaptive effects of dietary ethanol in the pig: changes in plasma high-density lipoproteins and fecal steroid excretion and mutagenicity.

Author(s): Topping DL, Weller RA, Nader CJ, Calvert GD, Iliman RJ.

Source: Am J Clin Nutr. 1982 Aug;36(2):245-50.

Abstract: Six young mature male pigs were maintained on a high fat, low fiber "Western" type diet. Substitution of ethanol for sucrose raised plasma total cholesterol, an

Attachment L – Ethanol

increase that was solely due to a rise in high-density lipoproteins. Plasma triacylglycerols and apo-B concentrations were unchanged and although apo-Al rose with ethanol, this was not statistically significant. Ethanol did not alter total fecal steroids but both bile acids and the ratio of bile acids/neutral sterols were increased. In fecal extracts from these animals, mutagenic activity in the Ames bacterial test was also raised. The data are discussed in relation to the relationships between dietary ethanol and coronary heart disease and colorectal cancer.

Title: The mutagenic effect in bacteriophage T4D of a hair dye, 1,4 diaminoanthraquinone, and of two solvents, dimethylsulfoxide and ethanol.
Author(s): Kvelland I.
Source: Hereditas 1983,99(2).209-13.
Abstract: Not available.

Title: Influence of ethanol and methanol gasoline blends on the mutagenicity of particulate and exhaust extracts
Author(s): Charles R. Clark, John S. Dutcher, Roger 0. McClellan, Ted M. Naman and Donald E. Seizinger
Source: Archives of Environmental Contamination and Toxicology, 1983, Volume 12, Number 3, 311-317
Abstract: The Salmonella mutagenicity test was used to evaluate the influence of alcohol fuel extenders on the genetic toxicity of particulate exhaust extracts. Four spark-ignition engine equipped vehicles were operated on gasoline alone, 10% blends of ethanol or methanol in gasoline, and a commercially available "gasohol.' The tests were conducted on a chassis dynomometer and the particulate exhaust was collected on high volume filters after dilution in a tunnel. The vehicles used were a 1980 Chevrolet Citation, a 1980 Mercury Monarch, a 1981 Ford Escort and a 1981 Oldsmobile Cutlass. Dichloromethane extracts of the exhaust particles from all tests were mutagenic inSalmonella typhimurium strains TA 100 and TA 98. The extracts were less mutagenic in the nitroreductase deficient strains TA 98NR and TA 98DNPR suggesting that nitro substituted polycyclic aromatic hydrocarbons may be responsible for part of the mutagenicity. In all the alcohol blended fuel tests, the mass of particle associated organics emitted from the exhaust was lower than that observed during the control tests using gasoline alone. Thus, in most cases, estimates of the emission of mutagenic combustion products from the exhaust were lower in the alcohol blend tests.

Attachment L – Ethanol

Title: Influence of ethanol and methanol gasoline blends on the mutagenicity of particulate exhaust extracts.
Author(s): Clark CR, Dutcher JS, McClellan RO, Naman TM, Seizinger DE.
Source: Arch Environ Contam Toxicol. 1983 May;12(3):311-7.
Abstract: Not available.

Title: [Embryotoxic and mutagenic activity of ethanol and acetaldehyde in intra-amniotic exposure]. [Article in Russian]
Author(s): Bariliak IR, Kozachuk Slu
Source: Tsitol Genet. 1983 Sep-Oct;17(5):57-60.
Abstract: The administration of acetaldehyde on the 13th day of pregnancy results in a considerable embryolethal effect, appearance of developmental abnormalities in rat embryos and chromosomal aberrations in fetal cells. Ethanol is found to have no teratogenic and mutagenic activity.

Title: Liver superoxide dismutases and catalase during ethanol inhalation and withdrawal.
Author(s): Ribiere C, Sinaceur J Nordmann J Nordmann R.
Source: Pharmacol Biochem Behay. 1983;18 Suppl 1:263-6.
Abstract: Manganese superoxide dismutase (Mn-SOD) studied during ethanol vapor inhalation shows no changes during the inhalation period (4 days) and a transient increase 12 hours after ethanol withdrawal. A significant decrease in cytosolic Cu-Zn-SOD is found at the end of the inhalation period and was sustained during 48 hours following ethanol withdrawal. It is suggested that this decrease in Cu-Zn-SOD activity might be related to an inactivation of the enzyme linked to the increase in hydroxyl radical production related to ethanol metabolism. Cytosolic catalase is reduced at the end of the ethanol inhalation period. This decrease could be related to an enhanced superoxide radical concentration linked to the reduced Cu -Zn-SOD activity.

Title: Metabolism by rat hepatic microsomes of fluorinated ether anesthetics following ethanol consumption.
Author(s): Rice SA Dooley JR, Mazze RI.
Source: Anesthesiology. 1983 Mar,58(3) 237-41
Abstract: The possibility that the metabolism of volatile inhalational anesthetics is altered following chronic ethanol consumption was investigated in male Fischer 344 rats. The hepatic microsomal defluorination rates of methoxyflurane, enflurane, and sevoflurane were determined for pair-fed rats receiving ethanol with normal caloric or with 50% of normal caloric intake. For comparison, the effects of phenobarbital treatment on anesthetic defluorination rates also were examined.

Attachment L – Ethanol

Fourteen days of ad libitum consumption of 16% ethanol resulted in maximal defluorination rates of the above anesthetics. No overt signs of ethanol toxicity were observed. Ethanol-treated rats with a normal caloric intake had significantly increased microsomal defluorination rates per mg protein compared with pair-fed control rats as follows: methoxyflurane, 190% of control; enflurane, 298% of control; and sevoflurane, 301% of control. Ethanol-treated animals with 50% of normal caloric intake showed similar elevations in microsomal defluorination rates when compared with pair-fed controls. Phenobarbital treatment significantly increased the rate of methoxyflurane defluorination (673% of control), whereas the rates of sevoflurane defluorination (127% of control) and enflurane defluorination (86% of control) were not altered significantly. Phenobarbital treatment increased the microsomal content of cytochrome P-450, while ethanol treatment did not. This study demonstrated that regardless of total caloric intake, chronic ethanol consumption increases defluorination of inhalation anesthetics in Fischer 344 rats. It also illustrated that the two enzyme-inducing agents are unique with respect to the degree to which they enhance anesthetic defluorination.

Title: Time course of functional tolerance produced in mice by inhalation of ethanol.
Author(s): Goldstein DB, Zaechelein R.
Source: J Pharmacol Exp Thor. 1983 Oct;227(1):150-3.
Abstract: Continuing our characterization of the ethanol inhalation model for chronic ethanol administration, we have now measured the magnitude and time course of functional tolerance, for comparison with physical dependence and with biochemical and biophysical studies of tissues taken from alcohol-treated mice. Ethanol was administered to mice by inhalation for 1 to 9 days, using pyrazole to maintain continuously elevated blood ethanol levels. At different times after termination of the ethanol administration, a test dose of ethanol was administered by injection and the sensitivity of the mice was assessed by measuring the brain concentration of ethanol at which the animals lost their balance on a horizontal rod. Tolerance, tested at 6 hr after withdrawal and expressed as a ratio of brain ethanol concentrations of mice that had been chronically treated with ethanol and their controls, was measurable after only 1 day of ethanol treatment and increased further in experiments of 3 and 6 days duration, but did not continue to increment during 9 days of alcohol inhalation. The tolerance disappeared rapidly after withdrawal; it was maximal at the earliest test; 2 hr after withdrawal, but decayed progressively and was no longer appreciable 30 hr after withdrawal, The method is suitable for accurate measurement of ethanol sensitivity, even when residual alcohol remains from the chronic treatment.

Title: Hepatic lipid peroxidation and mitochondria! susceptibility to peroxidative attacks

Attachment L – Ethanol

during ethanol inhalation and withdrawal.

Author(s): Rouach H, Clement M, Orfanelli MT, Janvier B, Nordmann J, Nordmann R.
Source: Biochim Biophys Ada. 1983 Oct 11;753(3):439-44.
Abstract: Male Sprague-Dawley rats were exposed to increasing concentrations (15-22 mg/l) of ethanol vapor over a 4-day period. The hepatic lipid peroxide level as well as the sensitivity of mitochondria and microsomes to peroxidative attacks were studied during the early stage of alcohol intoxication, at the end of the inhalation period and, finally, during withdrawal. The level of hepatic lipid peroxide started to increase significantly after the first day of ethanol inhalation, whereas the in vitro mitochondrial sensitivity to peroxidation induced by ADP X Fe3+ in the presence of an 0(2)-generating system was still unaltered after a 2-day inhalation period. Both the hepatic peroxide level and the mitochondria! sensitivity to peroxidation were significantly enhanced at the end of the 4-day inhalation period. Such an enhancement was still apparent 24 h after withdrawal, a time at which no more ethanol was present in the blood. Lipid peroxidation returned to normal values only 48 h after withdrawal. Microsomes were less affected than mitochondria by the ethanol treatment. It is suggested that the alterations of lipid peroxidation are related to the presence and/or the metabolism of ethanol at an early stage of inhalation, whereas changes in the membrane structure would be responsible for the maintenance of enhanced lipid peroxidation 24 h after ethanol withdrawal.

Title: Reproductive toxicity of the industrial solvent 2-ethoxyethanol in rats and interactive effects of ethanol.
Author(s): Nelson BK, Brightwell WS, Setzer JV, O'Donohue TL.
Source: Environ Health Perspect. 1984 Aug;57:255-9.
Abstract: The solvent, 2-ethoxyethanol, induced complete embryomortality in pregnant rats exposed to three times the current Federal permissible exposure limit (PEL). Following exposure to ethoxyethanol at a concentration only one-half the current PEL, the offspring evidenced behavioral and neurochemical deviations from controls. Subsequent studies found that ingestion of ethanol with concomitant inhalation of ethoxyethanol vapors early in pregnancy appeared to reduce the number of both behavioral and neurochemical deviations found for ethoxyethanol. In contrast, the concomitant exposure to ethanol and ethoxyethanol later in gestation potentiated the behavioral and neurochemical effects of ethoxyethanol. This research indicates that the industrial solvent 2-ethoxyethanol presents an occupational reproductive hazard and raises the issue of the importance of an interaction of social habits with occupational exposure to such hazards. The results would suggest that occupational physicians should advise pregnant workers in the chemical industry of the adverse effects of ethanol during pregnancy and of the possible interactions with other chemicals

Attachment L – Ethanol

and should encourage them to be especially cautious with ethanol consumption since they may be at greater risk.

Title: Fatty acid composition of rat liver mitochondrial phospholipids during ethanol inhalation.
Author(s): Rouach H, Clement M, Orfanelli MT, Janvier B, Nordmann. R.
Source: Biochim Biophys Acta. 1984 Aug 15;795(1):125-9.
Abstract: Male Sprague-Dawley rats were exposed to increasing concentrations (15-22 mg/l) of ethanol vapor over a 4-day period. Phospholipids were analyzed in liver mitochondria isolated from ethanol-treated and pair-weighted control animals. After a 2 -day inhalation period, the proportion of monoenoic acids in total phospholipids increased, whereas that of arachidonic acid decreased. These changes were more striking in phosphatidylcholine (PC) than in phosphatidylethanolamine (PE). The decrease in 20:4 may be related to increased lipid peroxidation. After a 4-day inhalation period, quite different changes in phospholipid fatty acids were found. They consisted in a trend towards a more unsaturated system, the proportion of 20:4 being increased in PC and that of 22:6 in PE. This increase in polyunsaturated acids might be related to a direct ethanol effect on lipid structure and/or metabolism that would be linked to the high blood alcohol level present at this stage of ethanol intoxication.

Title: Ethanol-induced genotoxicity.
Author(s): Hayes S.
Source: Mutat Res. 1985 May-Jun;143(1-2):23-7.
Abstract: Unrelated, nondenatured, ethanol preparations, derived synthetically or by fermentation, were found to induce qualitatively similar concentration-dependent toxic and genotoxic effects as measured by RK mutatest. In this system ethanol was found genotoxic above a threshold concentration of 18-19% (v/v) when RK+ selector cells were transiently exposed for 10 min before selection for RK- survivors at 42 degrees C.

Title: Tissue Distribution of Acetaldehyde in Rats following Acetaldehyde Inhalation and Intragastric Ethanol Administration
Author(s): N. Hobara, A. Watanabe; M. Kobayashi, H. Nakatsukasa, H. Nagashima, T. Fukuda and Y. Araki
Source: Bulletin of Environmental Contamination and Toxicology, 1985, Volume 35, Number 1, 393-306
Abstract: None provided.

Title: Inhalation of Ethanol Vapour: A Case Report and Experimental Test Involving the

Attachment L – Ethanol

Spraying of Shellac Lacquer
Author(s): M.J. Lewis
Source: Journal of the Forensic Science Society, Volume 25, Issue 1, January 1985, Pages 5-9
Abstract: The need arose to investigate the effect of inhaled ethanol on blood level, in a subject occupationally exposed to the vapour. Under relatively arduous conditions, a blood alcohol level of 1.3 mg/100 ml was achieved. It was apparent that continued exposure would not have led to a significant increase in that level.

Title: A Theoretical Treatment for the Estimation of Blood Alcohol Concentration Arising from Inhalation of Ethanol Vapour
Author(s): M.J. Lewis
Source: Journal of the Forensic Science Society Volume 25, Issue 1, January 1985, Pages 11-22
Abstract: Little attention has been given to the subject of inhaled ethanol in man, yet it can assume importance in the administration of the law relating to road traffic use. Such experiments as have been reported [1-3] have given results which appear difficult to reconcile with one another, and no theoretical approach to the problem has yet been described. The present work presents a simple theoretical model for the estimation of blood alcohol concentration in a subject exposed to the vapour. Available experimental data are tested against the model and found to be in good agreement.

Title: Acute toxicity of gasoline and ethanol automobile engine exhaust gases.
Author(s): Massad E, Saldiva CD, Cardoso LM, Da Silva R, Saldiva PH, Bohm GM.
Source: Toxicol Lett. 1985 Aug; 26(2-3):187-92.
Abstract: A comparative inhalation exposure study was performed to investigate the potential health effect of gasoline and ethanol engine exhaust fumes. Wistar rats housed in inhalation chambers were exposed to test atmospheres of various concentrations of carbon monoxide (CO) and gasoline and ethanol exhaust fumes diluted with air. CO level, temperature, relative humidity and flow rate were monitored continually to control the gas concentration and the environment. The dilution method gave a concentration within 1.0% of the target. The LC5Os for 3-h exposures were determined for the 3 test atmospheres. The results demonstrated that the acute toxicity, in terms of LC50, of the gasoline-fuelled engine was significantly higher than that of the ethanol-fuelled engine.

Title: Teratological assessment of methanol and ethanol at high inhalation levels in rats.
Author(s): Nelson BK, Brightwell WS, MacKenzie DR, Khan A, Burg JR, Weigel WW, Goad PT.

Attachment L – Ethanol

Source: Fundam Apol Toxicol. 1985 Aug;5(4):727-36.

Abstract: Alcohols are widely used as industrial solvents. In spite of the fact that ethanol is a human teratogen, there has not been systematic investigation of the potential teratogenic effects of other alcohols, particularly using the inhalation route of exposure, as would be appropriate in assessing occupational and environmental types of experience. As part of a large teratological examination of industrial alcohols, methanol and ethanol were administered by inhalation to groups of approximately 15 pregnant Sprague-Dawley rats. Methanol was administered at 20,000 ppm (20ME), 10,000 ppm (10ME), 5000 ppm (5ME), and 0 ppm (MECO) for 7 hr/day on Days 1-19 of gestation (Days 7-15 for 20ME). Ethanol was administered at 20,000 ppm (20ET), 16,000 ppm (16ET), 10,000 ppm (10ET), and 0 ppm (ETCO) for 7 hr/day on Days 1-19 of gestation. Dams were sacrificed on Day 20 (sperm = Day 0). One-half of the fetuses were examined using the Wilson technique for visceral defects, and the other half were examined for skeletal defects. The highest concentration of methanol (20ME) produced slight maternal toxicity and a high incidence of congenital malformations (p less than 0.001), predominantly extra or rudimentary cervical ribs and urinary or cardiovascular defects. Similar malformations were seen in the 10ME group, but the incidence was not significantly different from controls. No adverse effects were noted in the 5ME group. Dams in the 20ET group were narcotized by the end of exposure, and maternal weight gain and feed intake were decreased during the first week of exposure. The 16ET dams had slightly depressed weight gain (p less than 0.01) during the first week of exposure, but there were no significant effects on feed consumption. There was no definite increase in malformations at any level of ethanol, although the incidence in the 20ET group was of borderline significance.

Title: Comparison of behavioral teratogenic effects of ethanol and n-propanol administered by inhalation to rats.

Author(s): Nelson BK, Briohtwell WS, Burg JR.

Source: Neurobehav Toxicol Teratol. 1985 Nov-Dec;7(6):779-83.

Abstract: Despite extensive testing of ethanol, there has been little research on the reproductive effects of other alcohols. We investigated the behavioral teratogenicity of inhalation exposures to ethanol and n-propanol. Groups of 18 male (approximately 450 g) and 15 pregnant female Sprague-Dawley rats were exposed 7 hours/day for six weeks or throughout gestation, respectively, to 16 000, 10 000, or 0 ppm ethanol or to 7 000, 35 000, or 0 ppm n-propanol. Pregnant females exposed to 7 000 ppm n-propanol, but not to ethanol, showed reduced weight gain, and female offspring also had reduced weight gain through three weeks of age; there was also slight teratogenicity observed at this concentration. Exposed males were mated with unexposed females; fertility was

Attachment L – Ethanol

reduced in males exposed to 7 000 ppm n-propanol (two viable litters from 17 matings), but there were no differences from controls in maternal weight gain, feed intake, or water consumption in any other groups. In both maternally- and paternally-exposed groups, litters were culled to four pups of each sex and fostered to untreated females. One female and one male pup per litter were administered tests of neuromotor coordination (ascent on wire mesh screen, rotorod), activity levels (open field, running wheel), or learning ability (avoidance, operant conditioning), but no significant differences from controls were found with either alcohol, despite the reduction in maternal and female offspring body weight and minimal teratogenicity with 7 000 ppm n-propanol. Calculations for predicting blood ethanol levels with inhalation exposure are also presented.

Title: Influence of ethanol induction on the metabolic activation of genotoxic agents by isolated rat hepatocytes.

Author(s): Nets JM, te Brömmelstroet BW, van Gemert PJ, Roelofs HM, Henderson PT.

Source: Arch Toxicol. 1985 Sep;57(4):217-21

Abstract: The effects of ethanol-feeding to rats, over a 6-week period, on the activation of genotoxic compounds of different chemical classes, requiring metabolic conversion to exert their mutagenic activity, were studied in isolated rat hepatocytes. The influence of such treatment on cytochrome P-450 content and N-acetylation in isolated hepatocytes was also investigated. Benzidine (BZ), dimethylnitrosamine (DMN), diethylnitrosamine (DEN), isoniazid (INH) and cyclophosphamide (CP) were more effectively activated to products mutagenic towards Salmonella typhimurium by hepatocytes from ethanol-pretreated rats than by hepatocytes from controls. The mutagenic potency of 2-aminofluorene (2-AF) and 2-acetylaminofluorene (2-AAF) was not influenced by ethanol pretreatment. Ethanol consumption was found to be associated with increased cytochrome P-450 content and enhanced N-acetylation in the isolated hepatocytes. Our results support the hypothesis that an alteration of the hepatic drug-metabolizing system may be responsible for the ethanol-induced increase in susceptibility to certain genotoxic compounds.

Title: Cytotoxic and genotoxic effects of ethanol and acetaldehyde in root-meristem cells of *Allium cepa*

Author(s): Felipe Cortes, Santiago Mateos and Pablo Escalza

Source: Mutation Research/Genetic Toxicology, Volume 171, Issues 2-3, August-September 1986, Pages 139-143

Abstract: The current view is that ethanol itself is not a clastogenic agent or an inducer of sister- chromatid exchanges (SCE) but is mutagenic only when transformed metabolically into acetaldehyde. We have analyzed the cytotoxic effects of ethanol and acetaldehyde, as well as their effectiveness in the induction of

Attachment L – Ethanol

chromosomal aberrations and SCEs in root-tip cells of Allium cepa. The results obtained show that the chemicals differ in their action. For all the parameters analyzed, acetaldehyde treatments resulted in a stronger effect on meristematic cells. These results seem to support the above-mentioned hypothesis that acetaldehyde is the actual mutagenic agent and, on this basis, it can be stated that the mode of metabolism of ethanol in a given system is very important as regards its effectiveness in inducing chromosome damage.

Title: Toxicity of prolonged exposure to ethanol and gasoline autoengine exhaust gases.

Author(s): Massad E, Saldiva PH, Saldiva CD, Caldeira MP, Cardoso LM, de Morals AM, Calheiros DF, da Silva R, Böhm GM.

Source: Environ Res. 1986 Aug; 40(2):479-86.

Abstract: A comparative chronic inhalation exposure study was performed to investigate the potential health effects of gasoline and ethanol engine exhaust fumes. Test atmospheres of gasoline and ethanol exhaust were given to Wistar rats and Balb C mice housed in inhalation chambers for a period of 5 weeks. Gas concentration and physical parameters were continually monitored during the exposure period. Several biological parameters were assessed after the exposure including pulmonary function, mutagenicity, and hematological, biochemical, and morphological examinations. The results demonstrated that the chronic toxicity of the gasoline-fueled engine is significantly higher than that of the ethanol engine.

Title: An inhalation procedure to produce tolerance to the behavioral effects of ethanol

Author(s): Linda Mattucci-Schiavone and Andrew P. Ferko

Source: Physiology & Behavior, Volume 36, Issue 4, 1986, Pages 643-646

Abstract: In male Sprague-Dawley rats the acute effect of ethanol administration (1.0, 2.0, or 3.0 glkg, IP) on motor coordination was measured by the aerial righting reflex. Ethanol in doses of 2.0 and 3.0 g/kg produced significant impairment of motor coordination with corresponding elevated blood ethanol levels. The rate of ethanol dissappearance from the blood was 0.32±0.03 mg/ml/hr. Functional tolerance to the effect of ethanol on motor coordination and hypnosis (sleep time) was produced in rats by a 24 hr period of exposure to ethanol vapor (28 mg/liter of air) in a chamber. Animals tested 48 hr after the ethanol inhalation period showed less motor impairment from acute ethanol (3.0 g/kg, IP) and other animals exhibited a reduced sleep time from ethanol (4.0 g/kg, IP) when they were compared with controls. The rate of ethanol elimination from the blood was unchanged in ethanol vapor treated animals (0.30±0.01 mg/ml/hr) and air-treated animals (0.33±0.02 mg/ml/hr).

Attachment L – Ethanol

Title: Blood Alcohol Concentrations Following the Inhalation of Ethanol Vapour Under Controlled Conditions
Author(s): L. Campbell and H.K. Wilson
Source: Journal of the Forensic Science Society Volume 26, Issue 2, March 1986, Pages 129-135
Abstract: Exposure to ethanol vapour at the UK occupational exposure limit (1900 mg/m^3) does not produce a significant blood alcohol concentration. The results have been derived from a human volunteer study under controlled experimental conditions, and theoretical considerations. The effect of exposure to alcohol vapour in excess of the exposure limit on blood alcohol levels is discussed.

Title: Metabolism of ethanol in vitro produces a compound which induces sister-chromatid exchanges in human peripheral lymphocytes in vitro: acetaldehyde not ethanol is mutagenic.
Author(s): Obe G, Jonas R, Schmidt S.
Source: Mutat Res. 1986 May;174(1):47-51
Abstract: Ethanol (EtOH) in the presence of the EtOH-metabolizing enzyme, alcohol dehydrogenase (ADH) leads to the induction of sister-chromatid exchanges (SCEs) in human peripheral lymphocytes in vitro. Acetaldehyde (AA) induces SCEs, whose frequencies are lowered in the presence of the AA-metabolizing enzyme, aldehyde dehydrogenase (ALDH). EtOH in the presence of ADH produces more SCEs than EtOH in the presence of ADH and ALDH. These data are interpreted to show that not ethanol itself, but its first metabolite acetaldehyde is mutagenic.

Title: Effects of chronic ethanol inhalation on the enhancement of benzodiazepine binding to mouse brain membranes by GABA
Author(s): D.J. de Vries, G.A.R. Johnston, L.C. Ward, P.A. Wilce and B.C. Shanley
Source: Neurochemistry International, Volume 10, Issue 2, 1987, Pages 231-235
Abstract: Chronic ethanol inhalation produced no change in the number or affinity of [^{3}H] flunitrazepam binding sites on well-washed synaptic membranes prepared from male Quackenbush mice, but produced a significant decrease in the capacity of GABA to enhance [^{3}H]flunitrazepam binding. This decrease was characterised by a higher EC_{50} (1.4 μ M compared to 0.6 μ M) and a lower maximal level of enhancement (162% compared to 172%) for tissue from the chronically treated animals compared to tissue from control animals. Acute ethanol treatment or ethanol incubated in vitro with the brain membranes did not produce changes in any of the [^{3}H]flunitrazepam binding parameters. These results support other findings that chronic ethanol may affect the coupling of various sites on GABA-A receptor-ionophore complexes in brain.

Attachment L – Ethanol

Title: Species differences in upper respiratory tract deposition of acetone and ethanol vapors.
Author(s): Morris JB, Clay RJ Cavanagh DG.
Source: Fundam Appl Toxicol. 1986 Nov;7(4):671-80.
Abstract: Regional deposition patterns determine the dose of inhaled gaseous toxicant received by various areas of the respiratory tract. To study potential species differences in upper respiratory tract (URT) deposition of acetone and ethanol vapors, and to determine if deposition of these vapors could be described by a ventilation-perfusion (V-P) model, URT deposition efficiencies of these vapors were measured over a 12- to 18-min period at selected flow rates in the surgically isolated URT of urethane-anesthetized Fischer rats and Hartley guinea pigs. For both gases in both species, deposition efficiencies were significantly dependent upon inspiratory flow rate (p less than 0.0005). The V-P model predicts a linear relationship will exist between the ratio of the deposited to the nondeposited fraction and the inverse of the inspiratory flow rate. Such relationships were observed for deposition of acetone (r = 0.93, p less than 0.0005) and ethanol vapors (r = 0.95, p less than 0.0005) in the guinea pig and for deposition of acetone vapor (r = 0.95, p less than 0.0005) in the rat. In contrast, a linear relationship was not observed for ethanol in the rat suggesting that deposition mechanism(s) differ for this vapor in this species. Despite the fact that the surface area of the URT of the guinea pig is greater than that of the rat, URT deposition of these vapors was as much as twice as efficient in the rat as in the guinea pig (p less than 0.0005). This effect was not due to different blood:air partition coefficients as they were identical in both species. In tote, these results suggest there may be important anatomic and/or physiologic differences in the URT of the Hartley guinea pig and Fischer rat. Such differences may have to be considered when comparing the response(s) of these species to toxic gases or when extrapolating data obtained from these species to the human.

Title: Is ethanol the unknown toxin in smoke inhalation injury?
Author(s): Barillo DJ Rush BF Jr, Goode R, Lin RL, Freda A, Anderson EJ Jr.
Source: Am Surq. 1986 Dec;52(12):641-5.
Abstract: Of the 12,000 fire-related deaths occurring annually in the United States, it is estimated that 60 to 80 per cent are due to smoke inhalation. Plastic and synthetic materials which have been introduced in home construction and furnishings produce a more toxic smoke when burned. Efforts to identify a "supertoxin" in this smoke have been unsuccessful to date. An alternative approach is to examine why victims are unable to escape, and become exposed to smoke for lethal periods of time. The authors examined the circumstances of death in 39 fire victims (27 adults, 12 children) over a 25-month period. Detailed examination of the fire scene, autopsy studies, and toxicologic analysis were

Attachment L – Ethanol

carried out. Position of the victim, and escape efforts were noted. Carbon monoxide was elevated in all victims, with "lethal" levels (= greater than 50%) in 21/39 victims. Cyanide was detected in 24/29 victims, but none had lethal (3 mg/L) levels present. Ethanol was detected in 21/26 adults (80%) and 0/12 children (0%). 18/26 adult victims had ethanol levels above the statutory level of intoxication (10 mg%). Victims found in bed (no escape attempt) had a mean blood ethanol level of 268 mg%, compared with a mean level of 88 mg% in those victims found near an exit (P = .006). Ethanol intoxication significantly impairs the ability to escape from fire and smoke and is a contributory factor in smoke-related mortality.

Title: [Influence of hypoxia on the effects of ethanol and acetaldehyde]. [Article in Russian]
Author(s): Belykh AG, Strelkoy RB.
Source: Farmakol Toksikol. 1987 Nov-Dec;50(6):89-91.
Abstract: During experiments on male SHK mice it was established that a preliminary normobaric normocapnic hypoxic stimulation (30-minute inhalation of a gas mixture containing 10% of oxygen) increased the animal organism resistance to toxic effects of ethanol and its metabolite acetaldehyde.

Title: The organic solvents acetone, ethanol and dimethylformamide potentiate the mutagenic activity of N-methyl-N'-nitro-N-nitrosoguanidine, but have no effect on the mutagenic potential of N-methyl-N-nitrosourea.
Author(s): Gichner T, Veleminskỳ J.
Source: Mutat Res. 1987 Sep;192(1):31-5.
Abstract: The frequency of recessive chlorophyll and embryonic lethals included by N-methyl-N'-nitro-N-nitrosoguanidine (MNNG) in Arabidopsis thaliana was markedly increased when exposure of the seeds to MNNG (3 h) was carried out in the presence of 4-12% acetone, 4-16% ethanol or 8-32% dimethylformamide. The enhancement of MNNG mutagenicity was proportional to the concentrations of these organic solvents. In contrast, neither of them, applied at the same conditions and doses, influenced the mutagenic activity of N-methyl-N-nitrosourea. The solvents without mutagens did not influence the spontaneous rate of mutations and revealed no or very weak toxic effect as measured by the seed germination.

Title: Neurochemical, but not behavioral, deviations in the offspring of rats following prenatal or paternal inhalation exposure to ethanol.
Author(s): Nelson BK, Brightwell WS, MacKenzie-Taylor DR, Burg JR, Massari VJ.
Source: Neurotoxicol Teratol. 1988 Jan-Feb;10(1):1 5-22.
Abstract: In addition to its widespread social use, ethanol is used extensively as an

Attachment L – Ethanol

industrial solvent. Inhalation exposures to ethanol which produce narcosis in maternal rats are not teratogenic. The present study sought to extend the previous research by including offspring from paternal exposures, and testing for behavioral disorders in the offspring following maternal or paternal exposures. Groups of 18 male (approximately 450 g) and 15 female (200-300 g) Sprague-Dawley rats were exposed 7 hours/day for six weeks or throughout gestation to 16000, 10000, or 0 ppm ethanol by inhalation and then mated with untreated rats. Litters were culled to 4 males and 4 females, and were fostered within 16 hours after birth to untreated dams which had delivered their litters within 48 hours previously. Offspring from paternally or maternally exposed animals performed as well as controls on days 10-90 in tests of neuromotor coordination (ascent on a wire mesh screen, rotorod), activity levels (open field, modified-automated open field, and running wheel), and learning ability (avoidance conditioning and operant conditioning). In addition, brains of 10 21-day-old pups were analyzed for neurochemical differences from controls in concentrations of protein and the neurotransmitters acetylcholine, dopamine, norepinephrine, 5-hydroxytryptamine, substance P, Met-enkephalin, and beta-endorphin. Levels of acetylcholine, dopamine, substance P, and beta-endorphin were essentially unchanged in the offspring of rats exposed to ethanol. Complex, but significant changes in levels of norepinephrine occurred only in paternally exposed offspring. 5-Hydroxytryptamine levels were reduced in the cerebrum, and Met-enkephalin levels were increased in all brain regions of offspring from both maternally and paternally exposed rats.

Title: Ethanol-induced enhancement of trichloroethylene metabolism and hepatotoxicity: difference from the effect of phenobarbital.

Author(s): Nakajima T, Okino T, Okuvama 5, Kaneko T, Yonekura I, Sato A.

Source: Toxicol Appl Pharmacol. 1988 Jun 30;94(2):227-37.

Abstract: Male Wistar rats pretreated with ethanol (2.0 g in 80 ml liquid diet/day for 3 weeks) or phenobarbital (PB, 80 mg/kg/day ip for 4 days) were exposed by inhalation to 500, 1000, 2000, 4000, or 8000 ppm trichloroethylene (TRI) for 2 or 8 hr, and the blood concentration of TRI and the urinary concentration of TRI metabolites (trichloroethanol (TCE) and trichloroacetic acid (TCA] were determined at various times. Plasma glutamic-pyruvic transaminase (GPT) activity was measured 22 hr after the end of exposure as an indicator of hepatic damage. Both ethanol and PB enhanced TRI metabolism as evidenced by accelerated disappearance of TRI from the blood and increased excretion of total trichloro compounds (TCE + TCA) in the urine. However, the effects of ethanol and PB were different from each other: ethanol markedly enhanced the metabolism particularly at TRI concentration of 2000 ppm or lower, whereas PB enhanced it only at 4000 ppm or higher. This difference was also reflected in the

Attachment L – Ethanol

effect of TRI on liver: ethanol potentiated TRI hepatotoxicity more markedly than did PB when TRI concentration remained 2000 ppm or lower, whereas PB potentiated the toxicity more markedly than ethanol when the concentration was 4000 ppm or higher. It is noteworthy that ethanol potentiated TRI hepatotoxicity at a TRI concentration as low as 500 ppm. The severity of hepatic damage expressed by plasma GPT activity essentially paralleled the urinary excretion rate of total trichloro compounds during and 4 hr after exposure (r = 0.87 to 0.93). Compared between the contribution of concentration and duration of exposure to the toxicity, a higher concentration of TRI tended to cause more severe liver damage to PB-treated rats than did a prolonged period of exposure, whereas the toxicity in ethanol-treated rats was generally more marked in rats exposed to TRI for a longer period than in rats exposed to a higher concentration.

Title: Antioxidant Protection Systems of Rat Lung after Chronic Ethanol Inhalation
Author(s): Lora E. Rikans, Larry P. Gonzalez
Source: Alcoholism:. Clinical and Experimental Research, 1990, Volume 14, Issue 6, pages 872-877
Abstract: The effect of chronic ethanol administration on pulmonary antioxidant protection systems was investigated in male Sprague-Dawley rats exposed to room air or room air containing ethanol ethanol vapors for 5 weeks. Blood ethanol concentrations in ethanol-exposed rats were usually between 200 and 300 mg/dl. Glutathione, vitamin E, and malondialdehyde concentrations were measured in lung homogenates, and antioxidant enzyme activities (catalase, glutathione peroxidase, Cu/Zn-superoxide dismutase, glutathione reductase) were determined in the supernatant fractions. For comparison, the measurements were also made using liver fractions. Ethanol treatment increased the activities of catalase (117%) and Cu/Zn-superoxide dismutase (25%) in lung but not in liver. Although chronic ethanol inhalation lowered hepatic glutathione (19%) and hepatic vitamin E (30%), there was no increase in malondialdehyde content in either liver or lung of ethanol-exposed rats. The elevation of pulmonary antioxidant enzyme activities could be interpreted to mean that lung is a target for ethanol-induced oxidative stress. However, as there was no loss of pulmonary GSH or vitamin E and no increase in malondialdehyde formation, it appears that long-term ethanol exposure did not produce a significant degree of oxidative stress in rat lung.

Title: Ethanol vapour modulation of Lewis lung carcinoma, a murine pulmonary tumour.
Author(s): Batkin S Tabrah FL.
Source: J Cancer Res Clin Oncol. 1990:116(2):187-9.
Abstract: Pure ethanol in experimental animal studies, may not primarily be carcinogenic, but secondarily it can act chemically and synergistically as a co-carcinogen via its

Attachment L – Ethanol

endogenous metabolites and associated dietary and exogenous factors. However, ethanol, being a small molecule and miscible with water and most lipids, can readily enter cell membranes and directly affect cell function. In an in vitro study where ethanol was added to the culture medium for Lewis lung carcinoma, a dose- dependent suppression of tumour growth occurred. In the in vivo study, sequestration of the Lewis lung carcinoma in the lungs followed tail-vein injection in C57BL/6 mice. Starting on the second day after tumour implantation, primary pulmonary exposure was carried out with inhalations of 0.4% ethanol vapour from an aerosol for 70 min daily, this was continued for 17 days. This resulted in a marked reduction of the pulmonary tumour growth. Exposure to 0.1% ethanol vapour did not significantly affect the growth of Lewis lung carcinoma tumour. The optimum ethanol aerosol applications may thus be tumoricidal. Whether any ethanol secondary metabolites are involved is as yet uncertain.

Title: Ethylene dichloride: The influence of disulfiram or ethanol on oncogenicity, metabolism, and DNA covalent binding in rats

Author(s): Kenneth L. Cheever, James M. Cholakist A. Monaem El-Hawari, Robert M. Kovatch and Elizabeth K. Weisburger

Source: Fundamental and Applied Toxicology, Volume 14, Issue 2, February 1990, Pages 243-261

Abstract: Male and female Sprague-Dawley rats were exposed to 50 ppm ethylene dichloride (EDC) for 7 hr/day, 5 days/week, for 2 years by inhalation. Additional rats were exposed to 50 ppm EDC either with 0.05% disulfiram in the diet or with 5% ethanol in the drinking water. Histopathologic lesions related to the combination of inhaled EDC and dietary disulfiram were observed in the liver, mammary and testicular tissues of rats. This combined exposure resulted in a significant increase in the incidence of intrahepatic bile duct cholangiomas in both male and female rats. Male rats exposed to both EDC and disulfiram also had an increased incidence of subcutaneous fibromas, neoplastic nodules, and interstitial cell tumors in the testes. The female rats exposed to EDC and disulfiram also had a higher incidence of mammary adenocarcinomas. No significant increase in the number of any tumor type was observed in rats exposed to only EDC, disulfiram, or ethanol. Similarly, no significant increase in the number of tumors was observed in rats exposed to inhaled EDC and ethanol in water. At the end of the 2-year period animals from each group were evaluated for EDC metabolism and DNA binding. Blood levels of EDC at the end of a 7-hr exposure period were significantly higher for rats exposed to both EDC and disulfiram than for rats exposed to EDC alone. In addition, the elimination of a single oral dose of radiolabeled EDC was affected. The urinary excretion of ^{14}C from control rats was 47 to 55% of the administered dose with 28 to 30%

Attachment L – Ethanol

detected as unchanged EDC in the breath. In disulfiramtreated rats, only 35 to 36% of the administered ^{14}C was eliminated in the urine with 41 to 55% as unchanged EDC in the breath. The urinary metabolite HPLC profile was qualitatively unchanged by long-term EDC, disulfiram, or ethanol treatment, either alone or in combination, and consisted primarily of thiodiglycolic acid, thiodiglycolic acid sulforxide, and chloroacetic acid.

Title: Antioxidant Protection Systems of Rat Lung after Chronic Ethanol Inhalation
Author(s): Lora E. Rikans, Larry P. Gonzalez
Source: Alcoholism:. Clinical and Experimental Research, 1990, Volume 14, Issue 6, pages 872-877
Abstract: The effect of chronic ethanol administration on pulmonary antioxidant protection systems was investigated in male Sprague-Dawley rats exposed to room air or room air containing ethanol ethanol vapors for 5 weeks. Blood ethanol concentrations in ethanol-exposed rats were usually between 200 and 300 mg/dl. Glutathione, vitamin E, and malondialdehyde concentrations were measured in lung homogenates, and antioxidant enzyme activities (catalase, glutathione peroxidase, Cu/Zn-superoxide dismutase, glutathione reductase) were determined in the supernatant fractions. For comparison, the measurements were also made using liver fractions. Ethanol treatment increased the activities of catalase (117%) and Cu/Zn-superoxide dismutase (25%) in lung but not in liver. Although chronic ethanol inhalation lowered hepatic glutathione (19%) and hepatic vitamin E (30%), there was no increase in malondialdehyde content in either liver or lung of ethanol-exposed rats. The elevation of pulmonary antioxidant enzyme activities could be interpreted to mean that lung is a target for ethanol-induced oxidative stress. However, as there was no loss of pulmonary GSH or vitamin E and no increase in malondialdehyde formation, it appears that long-term ethanol exposure did not produce a significant degree of oxidative stress in rat lung.

Title: Assessment of the mutagenic potential of ethanol auto engine exhaust gases by the Salmonella typhimurium microsomal mutagenesis assay, using a direct exposure method.
Author(s): Lotfi CF, Brentani MM, Böhm GM.
Source: Environ Res. 1990 Aug;52(2):225-30.
Abstract: The mutagenic activity of the new Brazilian fuel, ethanol, was determined by employing the Salmonella typhimurium microsomal mutagenesis assay (TA97, TA98, TA100, TA102, and TA104) and a direct exposure method. This methodology was first used to determine the mutagenic activity of gasoline, revealing mutagenic activity of base-pair substitution without any need for metabolic activation, indicating the presence of direct-action mutagens.

Attachment L – Ethanol

Experiments with ethanol suggest an indirect mutagenic activity of the oxidant type. The exposure system was considered suitable for future studies of gaseous mixtures.

Title: The influence of ethanol on the stem cell toxicity of benzene in mice.
Author(s): Seidel HJ, Bader R, Weber L, Barthel E.
Source: Toxicol Appl Pharmacol. 1990 Aug; 105(1):13-8.
Abstract: BDF1 mice were exposed to 100, 300, and 900 ppm benzene vapor, and the numbers of hematopoietic progenitor cells, early and late erythroid progenitors (BFU-E and CFU-E) and granuloid progenitors (CFU-C), were determined with and without additional exposure to ethanol (5, 10, 15 vol%) in the drinking water. The duration of benzene inhalation was up to 4 weeks, 6 hr per day, 5 days per week. It was shown that the number of CFU-E per femur was depressed in a dose- dependent manner by benzene alone and also by ethanol combined with a given benzene concentration. CFU-E showed rapid regeneration after the end of the exposure, but not BFU-E and CFU-C. Prolongation of the ethanol exposure after withdrawal of benzene had only a marginal effect on progenitor cell regeneration.

Title: Effects of ethanol inhalation on EEG in rats.
Author(s): Ghosh TK, Copeland RL Jr, Pradhan SN.
Source: Pharmacol Biochem Behay. 1991 Feb;38(2):293-7.
Abstract: Effects of ethanol on duration of stages of sleep-wake cycle and EEG power spectra were measured during a 2-h exposure in a dynamic inhalational chamber in rats. Rats were exposed to one of four graded concentrations (approx. 100, 400, 800 and 1600 ppm) of ethanol on different days. Ethanol was found to increase the duration of waking (W) with a decrease in duration of rapid eye movement (REM) sleep at 100 and 400 ppm. No effect was observed at 800 and 1600 ppm on the stages of sleep-wake cycle or at 100-1600 ppm on EEG power spectra from the somatosensory or visual cortices. Results indicate that ethanol administered by inhalation could produce arousal action at low doses, but did not have any effect on EEG power spectrum at the concentrations used.

Title: Prolonged ethanol inhalation decreases gamma-aminobutyric acidA receptor alpha subunit mRNAs in the rat cerebral cortex.
Author(s): Montpied P, Morrow AL, Karanian JW, Ginns EI, Martin BM, Paul SM.
Source: Mol Pharmacol. 1991 Feb;39(2):157-63.
Abstract: Ethanol administration to rats by ethanol vapor inhalation (14 days) results in a 40-50% reduction in the level of gamma-aminobutyric acidA (GABAA) receptor alpha 1 subunit mRNAs [4.4 and 4.8 kilobases (kb)] in the cerebral cortex. The level of alpha 2 subunit mRNA (8.0 kb) was also reduced by 29%, whereas there

Attachment L – Ethanol

was no effect of prolonged ethanol exposure on the level of alpha 3 subunit mRNA (3.1 kb). Ethanol exposure did not alter the steady state levels of cerebral cortical glutamic acid decarboxylase or beta-actin mRNAs. Moreover, no alterations in the levels of total RNA, poly(A)+ RNA, or rRNA were observed, suggesting that the ethanol-induced reductions in GABAA receptor alpha 1 and alpha 2 subunit mRNAs were not the result of a generalized effect of ethanol administration on transcription or mRNA turnover. These ethanol-induced reductions in GABAA receptor alpha subunit mRNAs may underlie alterations in GABAA receptor function or number observed following prolonged ethanol exposure in rats.

Title: Behavioral effects of ethanol inhalation in rats.
Author(s): Ghosh TK, Copeland RL Jr, Alex PK, Pradhan SN.
Source: Pharmacol Biochem Behay. 1991 Apr;38(4):699-704.
Abstract: Behavioral effects of ethanol inhalation were studied on two fixed-ratio (FR) liquid-reinforced schedules and a continuous reinforcement (CRF) schedule intracranial self-stimulation (SS) in rats using the inhalational behavioral chamber designed in our laboratory. In the FR-24 schedule ethanol caused a decrease of reinforcement rate at 161 ppm and higher concentrations. In the FR-50 schedule decreases of the rate were observed at 102 ppm and 203 ppm. In the SS behavior ethanol produced a decrease in the rate of reinforcement at 603 ppm and higher concentrations. In rats of this schedule, blood ethanol concentrations were measured to be 393 micrograms/ml and 545 micrograms/ml after exposure to 600 ppm and 1200 ppm of ethanol respectively. Acute tolerance to ethanol was observed in these experiments, particularly in the FR-24 schedule. Thus ethanol inhalation could produce adequate blood concentrations so as to produce behavioral effects.

Title: Subacute ethanol consumption reverses p-xylene-induced decreases in axonal transport.
Author(s): Padilla S, Lyerly DL, Pope CN.
Source: Toxicology. 1992 Nov 1;75(2):159-67.
Abstract: Human exposure to organic solvents is often complicated by ethanol ingestion and the literature is replete with demonstrations of metabolic interactions between ethanol and organic solvents at a pharmacokinetic level. Because of the possible modulation of xylene toxicity by ethanol consumption, the present group of studies characterizes the effect of ethanol on the p-xylene-induced decrease in axonal transport in the rat optic system previously reported by our laboratory. Long-Evans, hooded, male rats were divided randomly into two groups: those receiving 10% ethanol in their drinking water and those receiving water only. These two groups were further subdivided into two groups which were either

Attachment L – Ethanol

exposed by inhalation to 1600 ppm p-xylene for 6 h/day, 5 days/week for 8 exposure-days or were treated identically except that they were exposed to air while in the inhalation chambers. The ethanol-drinking rats were given ethanol 6 days prior to and on the days of the inhalation exposure. Immediately after removal from the inhalation chambers on the last exposure day, the animals were injected intraocularly with [35S]methionine and [3H]fucose to measure the synthesis and rapid axonal transport of proteins and glycoproteins, respectively, in the retinal ganglion cells. The animals were sacrificed 20 h later, and the amount of radioactivity in different areas of the retinal ganglion cells was determined by liquid scintillation counting. As in previous experiments, the xylene exposure group showed a significant reduction in axonal transport of proteins and glycoproteins, whereas the ethanol exposure alone produced no significant reductions in the transport of either proteins or glycoproteins. In the animals receiving both ethanol and xylene, however, the ethanol treatment prevented the decreased transport characteristic of the xylene only animals, i.e. in all areas of the optic projections the level of transport were similar to the level present in the control groups. These data suggest that the xylene-induced reduction in rapid axonal transport was reversed (or prevented) by subacute ethanol consumption.

Title: Modulation of the mutagenicity of three dinitropyrene isomers in vitro by rat-liver S9, cytosolic, and microsomal fractions following chronic ethanol ingestion.

Author(s): Winston GW, Traynor CA, Shane BS, Halos AK.

Source: Mutat Res. 1992 Jun 16;279(4):289-98.

Abstract: The effects of chronic ethanol feeding of rats on the ability of liver fractions to modulate the bacterial mutagenicity of three dinitropyrene isomers (1,3-, 1,6- and 1,8-DNP), which require bacterial enzymes but not an exogenous enzyme source for activation, were studied. The mutagenicity of the DNP isomers toward S. typhimurium TA98 and TA100 was attenuated in the presence of post-mitochondrial supernatants (S9) from both ethanol-fed and pair-fed rats albeit, that from the ethanol- fed group was more efficient in lowering the mutagenicity. The cytosolic fraction from ethanol-fed rats enhanced the mutagenicity of all of the DNP isomers in TA100. The most notable enhancement was with 1,3-DNP in which a more than 4-fold enhancement was obtained. Cytosol from pair-fed rats enhanced only the mutagenicity of 1,3-DNP, this by 2.9-fold. Cytosolic NADPH-nitroreductase activity from ethanol-treated rats toward 1,6-, 1,8- and 1,3-DNP was increased 2.8-, 1.7- and 1.3-fold, respectively over pair-fed controls. Cytosolic NADH-nitroreductase from ethanol-fed rats was increased with 1,3-DNP (1.7-fold) and 1,8-DNP (1.4-fold) as substrates, but not with 1,6-DNP. Microsomes decreased the mutagenicity of DNP similarly to S9, i.e., fractions from ethanol-fed rats were more efficient than those of pair-fed rats in deactivating all the DNP isomers. Per mg of protein, detoxification of DNP by S9

Attachment L – Ethanol

was more efficient than with microsomes, thus both cytosolic and microsomal enzymes are required for maximal detoxification. In summary, ethanol feeding modulates both the augmented cytosolic activation of DNP to mutagens and the deactivation of the direct-acting mutagenicity of DNP by microsomes. In combination, as is the case with S9, the microsomal detoxifying activity outcompetes the cytosolic activation.

Title: Comparative Acute Inhalation Toxicity of a Saline Suspension and an Ethanol Solution of T -2 Mycotoxin in Mice
Author(s): D. A. Creasia and J. D. Thurman
Source: Inhalation Toxicology, 1993, Vol. 5, No. 1, Pages 33-41
Abstract: We compared retention, distribution, toxicity, and histopathological change in mice after exposure to aerosols of 1-2 suspended in saline or dissolved in ethanol. We found that the LC50 for mice exposed for 10 min to an aerosol of a saline suspension of T-2 was 0.035 0.02 mg T-2 per liter air. which was lower than the LC50 (0.380 ± 0.08 mg 7-2 per liter air) for an aerosol of T-2 dissolved in ethanol. However, within about 15 min postexposure, most of the T-2 deposited in the respiratory tract was translocated from the respiratory tract regardless of whether the T-2 aerosol was from a saline suspension or ethanol solution. Also, although T-2 is an inflammatory agent to dermis and gastrointestinal epithelium, 1-2 from either aerosol did not produce any histological evidence of inflammation in the respiratory tract.

Title: Evaluation of pyrazole and ethanol induced S9 fraction in bacterial mutagenicity testing.
Author(s): Burke DA, Wedd DJ, Herriott D, Bayliss MK, Spalding DJ, Wilcox P.
Source: Mutagenesis. 1994 Jan;9(1):23-9.
Abstract: A major constitutive enzyme in the liver of the uninduced rat is cytochrome P450-2E1. This isozyme has been shown to metabolize a number of carcinogens, including low molecular weight nitrosamines and a number of compounds normally regarded as non-mutagenic in the Ames test, e.g. aniline, urethane and benzene. Using the standard induction procedures [Aroclor 1254 or a combination of phenobarbitone (PB) and beta-naphthoflavone (beta-NF)] the level of CYP2E1 in rat liver is actually suppressed and it has been suggested that this may account for the negative findings with these compounds in the Ames test. S9 fractions were prepared from rats pre-treated with pyrazole or ethanol (inducers of CYP2E1) and then used in the Ames test (or pre-incubation modification) with urethane, acetaminophen, aniline, benzene, procarbazine and Nnitrosopyrrolidine. Both pyrazole and ethanol induced S9 were superior to PB/beta-NF-S9 and uninduced-S9 for the activation of N-nitrosopyrrolidine, a known CYP2E1 substrate. However, there was no evidence of mutagenic activity

Attachment L – Ethanol

with urethane, aniline, benzene, procarbazine or acetaminophen. As these compounds have demonstrated genotoxicity in vivo, additional important metabolic pathways must be required which are not present in rat liver S9 fraction.

Title: Pharmacodynamic and metabolic interactions between ethanol and two industrial solvents (methyl n- butyl ketone and methyl isobutyl ketone) and their principal metabolites in mice.
Author(s): M Sharkawi, C Granvil, A Faci, G L Plaa
Source: Toxicology. 1994 (1-3);187-95
Abstract: MnBK and MiBK prolong the duration of ketamine-, pentobarbital-, thiopental- and ethanol-induced loss of righting reflex (LRR) in mice. In equimolar doses,(5 mmol/kg i.p.), both isomers were equipotent with respect to the enhancement of ketamine-, pentobarbital-, and thiopental-induced LRR. However, MnBK was significantly more effective (twice as effective) than its isomer with respect to enhancing ethanol-induced LRR. An attempt to explain the difference in effectiveness between the two isomers was carried out. The effects of both ketones and their principal metabolites,(2-hexanol (2-HOL), 2,5-hexanedione (2,5-HD), 4- methy1-2-pentanol (4-MPOL) and 4-hydroxy 4-methyl-2-pentanone (HMP)) on ethanol-induced LRR and ethanol elimination were studied in mice. The ketones and their metabolites were dissolved in corn oil and injected intraperitoneally 30 min before 4 g/kg ethanol for LRR and 2 g/kg for ethanol elimination. Ethanol-induced LRR was significantly prolonged by the following dosages (mmollkg), MnBK, 5; MiBK, 5; 2-HOL, 2.5; 4-MPOL, 2.5; and HMP, 2.5; 2,5-HD, 2.5, however exerted no effect, Concentrations of ethanol in blood or brain upon return of the righting reflex were similar in solvent-treated and control animals. The mean elimination rate of ethanol was slower in groups pretreated with MnBK or 2-HOL as compared to control animals. Ethanol elimination in animals pretreated with MiBK, HMP, 4-MPOL, or 2,5-HD was similar to that in control animals. These ketones are known to have some central depressant action on their own. This by itself could lead to prolongation of ethanol-induced LRR. However, MnBK, as well as one of its principal metabolites,(2-HOL), markedly reduced ethanol elimination. This could explain the observation that MnBK has a greater potentiating effect on ethanol-induced LRR that its isomer, MiBK, which does not affect ethanol elimination.

Title: Dose and route dependency of metabolism and toxicity of chloroform in ethanol-treated rats.
Author(s): Wang PY, Kaneko T, Tsukada H, Sato A.
Source: Arch Toxicol. 1994; 69(1):18-23.
Abstract: The effects of a single dose of ethanol on the metabolism and toxicity of

Attachment L – Ethanol

chloroform administered to rats per os (p.o.), intraperitoneally (i.p.), or by inhalation (inh) at different doses were investigated. Rats that had been given either ethanol (2 g/kg) or vehicle (water) alone at 4 p.m. on the previous day were challenged with chloroform at 10 a.m. p.o. (0.01, 0.2, or 0.4 g/kg), i.p. (0, 0.1, 0.2, or 0.4 g/kg), or inh (for 6 h each at 0, 50, 100, or 500 ppm). The ethanol treatment, which had no influence on the intake of food and water, increased chloroform metabolism in vitro about 1.5-fold with no significant influence on liver glutathione content. The treatment had a dose-dependent effect on the metabolism and toxicity of chloroform, and the effect differed depending on the route of administration. Compared at the same dose level, the area under the curve (AUC) of blood chloroform concentration was invariably smaller following p.o. than i.p. administration. In accordance with this, chloroform administered p.o. caused more deleterious hepatic damage than the same amount of chloroform administered i.p. Although ethanol treatment had no significant influence on the AUC at any dose by any route of administration, the toxicity of p.o.-administered chloroform was significantly higher in ethanol-treated rats than in control rats at a dose as low as 0.1 g/kg, whereas no significant difference was observed in toxicity between both groups of rats at such a low dose administered i.p.(ABSTRACT TRUNCATED AT 250 WORDS)

Title: Co-administration of ethanol transiently inhibits urethane genotoxicity as detected by a kinetic study of micronuclei induction in mice.

Author(s): Choy WN, Mandakas G, Paradisin W.

Source: Mutat Res. 1996 Apr 6;367(4):237-44.

Abstract: Urethane (ethyl carbamate) is a genotoxic carcinogen that requires metabolic activation. Ethanol is known to inhibit urethane metabolism and genotoxicity. Since ethanol is eliminated rapidly in animals, the persistence of ethanol inhibition was studied in a mouse bone marrow and a peripheral blood micronucleus assays. In the bone marrow assay, male CD-1 mice were injected intraperitoneally (i.p.) with water (vehicle), urethane (1000 mg/kg), ethanol (2500 mg/kg) or urethane and ethanol (1000 and 2500 mg/kg, respectively) in single injections. Polychromatic erythrocytes (PCE) from bone marrow were obtained at 24 and 48 h after injection and scored for micronuclei. Urethane induced an increase of micronucleated PCE (MN PCE) frequency from 0.19% in the control to 8.63% at 24 h, followed by a decrease to 6.98% at 48 h. When urethane was co-administered with ethanol, the MN PCE frequency was suppressed to 0.49% at 24 h, but markedly increased to 7.35% at 48 h. This delay of MN PCE occurrence indicated that ethanol inhibition was transient. To pinpoint the duration of this delay, a peripheral blood micronucleus assay was conducted to monitor the kinetics of MN PCE induction. In this assay, male CD-1 mice were injected i.p. with water, ethanol, urethane, or urethane and ethanol as described

Attachment L – Ethanol

above. Peripheral blood was scored for MN PCE at 8-h intervals for 4 days. Two additional dose groups injected with urethane or urethane and ethanol were also scored for MN PCE at 8 h intervals, but each blood sampling time was staggered 4 h later from the first four dose groups. The combined data provided MN PCE frequencies at 4-h intervals from 24 to 100 h after injection. Urethane alone induced a peak MN PCE frequency of 11.6% at 52 h. Urethane and ethanol induced a peak MN PCE frequency of 11.2% at 64 h, a delay of 12 h. Thus, ethanol delays but does not diminish urethane genotoxicity.

Title: Potential Air Quality Effects of Using Ethanol-Gasoline Fuel Blends: A Field Study in Albuquerque, New Mexico
Author(s): Jeffrey S. Gaffney and Nancy A. Marley
Source: Environ. Sci. Technol., 1997, 31 (11), pp 3053-3061.
Abstract: The use of alternate fuels has been proposed as a method of improving urban air quality by reducing combustion- related pollution. One such program mandates the use of oxygenates in the wintertime to reduce CO emissions in cities such as Albuquerque, NM. A field study was conducted in Albuquerque to determine the atmospheric impacts of the use of ethanol fuels. Atmospheric concentrations of ozone, oxides of nitrogen, CO, peroxyacetyl nitrate (PAN), aldehydes, and organic acids were measured in the summer of 1993, before the use of ethanol fuels, and in the winters of 1994 and 1995, during the use of 10% ethanol fuel (>99%). Data showed increased levels of peroxyacetyl nitrate (PAN) and aldehydes in winter. The formaldehyde/acetaldehyde ratio was 1.4, indicating an anthropogenic source, and PAN and acetaldehyde levels were anti-correlated over short time periods, indicating primary acetaldehyde emissions. A comparison of data taken at rural sites south of the city indicates that although there is a significant anthropogenic component to the aldehyde concentrations during the winter, there are also contributions from the photochemical oxidation of natural hydrocarbons.

Title: Dose- and route-dependent alterations in metabolism and toxicity of chemical compounds in ethanol-treated rats: difference between highly (chloroform) and poorly (carbon tetrachloride) metabolized hepatotoxic compounds.
Author(s): Wang PY, Kaneko T, Tsukada H, Nakano M, Sato A.
Source: Toxicol Appl Pharmacol. 1997 Jan; 142(1):13-21.
Abstract: The dose and route dependency of the metabolism and the toxicity of chloroform (CHCl3) and carbon tetrachloride (CCl4) was investigated in ethanol-treated rats. Rats that had been kept on either an ethanol (containing ethanol at 2 g/rat/day) or a control (containing no ethanol) liquid diet for 3 weeks were challenged with CHCl3 or CCl4 by inhalation (0, 50, or 500 ppm x 6 hr), or by p.o. or i.p. administration (0, 0.105, or 1.675 mmol/kg). Ethanol consumption, which

Attachment L – Ethanol

increased the in vitro metabolism of both compounds six- to sevenfold, affected the metabolism and toxicity of CCl4 differently from those of CHCl3. Ethanol increased the metabolism and toxicity of CHCl3 at 500 ppm only, whereas it increased the metabolism and toxicity of CCl4 at either 50 or 500 ppm. In addition, the effect of ethanol consumption differed between CHCl3 and CCl4 depending on the route of administration (p.o. or i.p.) and the dose (0.105 or 1.675 mmol/kg). For the p.o. route, ethanol increased the metabolism and toxicity of both compounds at either dose. For the i.p. route, however, ethanol increased the metabolism and toxicity of the high dose of CHCl3 only, but of both CCl4 doses. The dose- and route-dependent differences in the effect of enzyme induction on the metabolism and toxicity between CHCl3 and CCl4 can be explained by a supposition that the hepatic blood flow rate-limits the metabolism of a low dose of CHCl3 (perfusion-limited metabolism), whereas the metabolic capacity of the liver limits the metabolism of CCl4 (capacity-limited metabolism) irrespective of dose.

Title: Cytochrome P-450 2E1 in rat liver, kidney and lung microsomes after chronic administration of ethanol either orally or by inhalation.

Author(s): Zerilli A, Lucas D, Amet Y, Beaude F, Volant A, Floch HH, Berthou F, Menez JF.

Source: Alcohol Alcohol. 1995 May;30(3):357-65.

Abstract: In this study, microsomal cytochrome P-450 2E1 (CYP2E1) contents and activities were tested in liver, kidney and lung from Wistar rats after the following treatments (1) oral administration of a 10% ethanol solution for 4 weeks; (2) pair fed controls; (3) oral administration of a 5% acetone solution for 1 week; (4) inhalation of ethanol vapour for 4 weeks. CYP2E1 activity was measured using chlorzoxazone as substrate and CYP2E1 content was measured using Western blot analysis. In addition, the cellular distribution of CYP2E1 was studied in liver, lung and kidney by immunohistochemistry. Basal liver CYP2E1 was 10-20 times lower in lung and kidney than in liver. Inhalation was clearly the most efficient way of inducing CYP2E1, probably due to the continuous and high alcohol exposure. Among the organs tested, lung appeared to be the tissue least sensitive to induction even after ethanol inhalation, suggesting the absence of local induction. After ethanol intoxication, immunostaining was increased in the centrilobular region of the liver, in the alveolar cells of the lung and in the proximal convoluted tube of the kidney. The CYP2E1 activities decreased to control values in the three tissues tested, within 24 h after cessation of intoxication.

Title: Effects of low-dose ethanol on rat progeny upon inhalation exposure during gestation

Author(s): A. V. Liopo, M. S. Omelyanchik and O. V. Chumakova

Attachment L – Ethanol

Source: Bulletin of Experimental Biology and Medicine, 1996, Volume 121, Number 3, 244-246

Abstract: Effects of ethanol in low concentrations on the course of pregnancy in rats and the development and metabolic characteristics of their progeny are studied: the morphology and function of pathological shifts are assessed and the Level of endogenous ethanol in the blood and acetylcholinesterase activity in erythrocyte membranes measured. Inhalation exposure to ethanol is found to retard the development of the progeny. The consumption of ethanol by the progeny in the experimental group was 1.5 times higher than in the control. The results indicate that even trace amounts of ethanol in the atmosphere are toxic for the organism.

Title: Influence of Aroclor 1254, phenobarbital, p-naphthoflavone, and ethanol pretreatment on the biotransformation of cyclophosphamide in male and female rats

Author(s): P.J.M. Sessink, W.H.J. Vaes, P.H.H. van den Broek, J.H.C. de Roos, J.Noordhoek and R.P. Bos

Source: Toxicology, Volume 112, Issue 2, 16 August 1996, Pages 141-150

Abstract: The aim of the present study is to investigate the influence of the environmental factors, smoking and alcohol, on the biotransformation of cyclophosphamide (CP) in the rat in NMI and in vitro with S9 liver fractions. The biotransformation of CP was studied by the determination of the CP metabolites, nor-nitrogen mustard (NNM), 4-ketocyclophosphamide (KCP) and carboxyphosphamide (CAR). The effect of the environmental factors, smoking and alcohol consumption, on the biotransformation enzymes was mimicked by pretreatment of rats with 13-naphthoflavone and ethanol, respectively. Rats treated with olive oil and water served as controls and rats pretreated with Aroclor 1254 and phenobarbital were used as positive controls. The influence of sex and supplementation with NAD and GSH, mimicking a biological variation in NAD and GSH levels in rat and human liver, was also studied. Pretreatment of rats with Aroclor 1254 decreased the excretion of unmetabolized CP in urine, most likely due to an enhanced biotransformation. The in vitro hepatic biotransformation of CP in rats was strondy influenced by sex, by supplementation with NAD and GSH and by pretreatment with the enzyme-inducers, phenobarbital and Aroclor 1254. No influence of pretreatment with the enzyme-inducers, 3-naphthoflavone and ethanol, was found. The results suggest that the influence of the environmental factors, alcohol consumption and smoking, on the biotransformation of CP in man will be negligible.

Title: Indoor Air Guideline Levels for 2-ethoxyethanol, 2-(2-ethoxyethoxy)ethanol, 2-(2-butoxyethoxy)ethanol and 1-methoxy-2-propanol

Author(s): Gunnar Damgard Nielsen, Lea Frimann Hansen, Bjorn Andersen Nem, Otto

Attachment L – Ethanol

Melchior Poulsen
Source: Indoor Air, Volume 8, Issue S5, pages 37-54, December 1996
Abstract: Three guideline values have been proposed for each of the substances, i.e. one for evaluation of odour, one for sensory irritation and one value covering both the lung and the systemic effects. Genotoxic and carcinogenic effects have also been considered.

Title: Using a four-compartment closed model to describe inhalation of vaporised ethanol on 1-14C-pyruvate kinetics in mice.
Author(s): Wu G.
Source: Arch Toxicol. 1997;71(8):501-7.
Abstract: A four-compartment closed model was set up by means of a system of differential equations. A completely analytical solution of the four-compartment closed model was found by means of Laplace transform and Cramer's rule. 1-14Cpyruvate kinetics were studied in mice without and with inhalation of vaporised ethanol. The 1-14C-pyruvate kinetics were modelled by the four-compartment closed model, i.e. injected site, blood, 14CO2 expired in air, and 14C eliminated in urine. The kinetic parameters were estimated using the analytical solution of the four-compartment closed model to fit expired 14CO2 and 14C eliminated in urine simultaneously. Although the results showed that the inhalation of vaporised ethanol increased the expired 14CO2, the compartmental analysis revealed that the increase of expired 14CO2 is mainly attributed to increased 1-14C-pyruvate transmembrane process. The developed model is useful for toxicokinetic analysis when blood is not easy to obtain. Moreover, the developed model can also be used to model two compartments as urine and faeces when the toxin is not eliminated through air.

Title: A comparison of physiologically based pharmacokinetic model predictions and experimental data for inhaled ethanol in male and female B6C3F1 mice, F344 rats, and humans.
Author(s): Pastino GM, Asgharian B, Roberts K, Medinsky MA, Bond JA.
Source: Toxicol Appl Pharmacol. 1997 Jul;145(1):147-57.
Abstract: Ethanol is added to unleaded gasoline as an oxygenate to decrease carbon monoxide automobile emissions. This introduces inhalation as a new possible route of environmental exposure to humans. Knowledge of the pharmacokinetics of inhaled ethanol is critical for adequately assessing the dosimetry of this chemical in humans. The purpose of this study was to characterize the pharmacokinetics of inhaled ethanol in male and female B6C3F1 mice and F344 rats and to develop a physiologically based pharmacokinetic (PBPK) model for inhaled ethanol in mice, rats, and humans. During exposure to 600 ppm for 6 hr, steady-state blood ethanol concentrations (BEC) were reached within 30 min in

Attachment L – Ethanol

rats and within 5 min in mice. Maximum BEC ranged from 71 microM in rats to 105 microM in mice. Exposure to 200 ppm ethanol for 30 min resulted in peak BEC of approximately 25 microM in mice and approximately 15 microM in rats. Peak BEC of about 10 microM were measured following exposure to 50 ppm in female rats and male and female mice, while blood ethanol was undetectable in male rats. No sex-dependent differences in peak BEC at any exposure level were observed. Species-dependent differences were found following exposure to 200 and 600 ppm. A blood flow limited PBPK model for ethanol inhalation was developed in mice, rats, and humans which accounted for a fractional absorption of ethanol. Compartments for the model included the pulmonary blood and air, brain, liver, fat, and rapidly perfused and slowly perfused tissues. The PBPK model accurately simulated BEC in rats and mice at all exposure levels, as well as BEC reported in human males in previously published studies. Simulated peak BEC in human males following exposure to 50 and 600 ppm ranged from 7 to 23 microM and 86 and 293 microM, respectively. These results illustrate that inhalation of ethanol at or above the concentrations expected to occur upon refueling results in minimal BEC and are unlikely to result in toxicity.

Title: Use of a five-compartment closed model to describe the effects of ethanol inhalation on the transport and elimination of injected pyruvate in the rat.
Author(s): Wu G.
Source: Alcohol Alcohol. 1997 Sep-Oct;32(5):555-61.
Abstract: A five-compartment closed model was established using the system of differential equations. The completely analytical solution of a five-compartment closed model was found using the Laplace transform. 2-[14C]Pyruvate kinetics were studied in rats without and with inhalation of vaporized ethanol, and were modelled by the five-compartment closed model, i.e. injected site, blood, eliminated 14CO2 in air, eliminated 14C in urine and faeces. The kinetic parameters were estimated using the analytical solution of the five-compartment closed model to fit eliminated 14CO2, and 14C in urine and faeces simultaneously. The compartmental analysis showed that the inhalation of vaporized ethanol can increase 2-[14C]pyruvate trans-membrane, trans-tissue processes and oxidation rate. The model developed is useful for general pharmacokinetic and toxicokinetic analysis as well as for studies on ethanol.

Title: Effect of subchronic ethanol ingestion on styrene-induced damage to the tracheal and pulmonary epithelium of the rat.
Author(s): Coccini T, Fenoglio C, Maestri L, Costa LG, Manzo L.
Source: J Apol Toxicol. 1998 Sep-Oct;18(5):349-56.
Abstract: Previous studies have indicated that ethanol may affect styrene metabolism and toxicity in target tissues (e.g. brain). Morphological and biochemical changes

Attachment L – Ethanol

have been reported in the respiratory tract of laboratory animals exposed to styrene either by inhalation or i.p. injection. The aim of the present study was, therefore, to investigate the influence of subchronic ethanol administration (5% in a Lieber-DeCarli liquid diet) on the morphological alterations of the respiratory tract induced by styrene inhalation (300 ppm, 6 h day(-1), 5 days a week for 2 weeks) in rats. Levels of reduced glutathione (GSH) in lung and liver tissues as well as in erythrocytes and whole blood were studied as indicators of overall GSH status, and urinary levels of the styrene metabolites-mandelic acid and phenylglyoxylic acid-were also measured as indicators of styrene-absorbed dose. Rats exposed to 300 ppm styrene presented morphological alterations throughout the respiratory tract. Electron microscopy analysis showed diffuse cell damage involving the tracheal, bronchiolar and alveolar epithelium. These abnormalities were accompanied by 40% depletion of GSH in the lung tissue and also 35% depletion in hepatic GSH in the absence of alteration of the GSH content in blood. Styrene metabolism was apparently induced by subchronic ethanol treatment, as indicated by an increased excretion of urinary mandelic (+140%, $P < 0.05$) and phenylglyoxylic (+50%) acids. However, repeated ethanol administration did not exacerbate the lung GSH depletion nor the damaging effect to the respiratory tract induced by the 2-week exposure to styrene alone. The lack of effects of ethanol on styrene pulmonary toxicity after combined exposure may be due to the different tissue distribution of the cytochrome P-450 isoforms involved in the styrene biotransformation to styrene-7,8-oxide, and their different induction by ethanol.

Title: The effects of the nitric oxide synthase inhibitor 7-nitroindazole on ethanol pharmacokinetics in rats after acute and chronic ethanol administration.

Author(s): Vassiliev V, Kalda A, Pokk P, Vali M, Zharkovsky A.

Source: Alcohol Alcohol. 1998 Nov-Dec; 33(6):609-15.

Abstract: The aim of this work was to study the effects of the nitric oxide synthase (NOS) inhibitors 7-nitroindazole (7-NI) and NG-nitro-L-arginine (L-NOARG) on the effects and pharmacokinetics of ethanol in rats. Ethanol at a dose of 4 g/kg, i.p. induced sleep in rats (sleep time: 117.2+/-30.7 min). Administration of the NOS inhibitors 7-NI (20 mg/kg, i.p.) and L-NOARG (20 mg/kg, i.p.) 30 min before ethanol significantly increased the duration of ethanol-induced sleep. L-NOARG also significantly increased the toxicity of ethanol as evidenced by increased post-experimental lethality. Ethanol at a dose of 2 g/kg (i.p.) did not induce sleep in vehicle-treated rats; however, the combined administration of ethanol (2 g/kg) and 7-NI at doses of 40, 80, and 120 mg/kg caused sleep, for 49.4+1-3.7, 204.0+/-13.3, and 447.5+/-62.8 min, respectively. L-NOARG (20 mg/kg) had no effect on ethanol concentrations in blood after acute ethanol administration (4 g/kg). 7-NI in lower doses (20 and 40 mg/kg) had no effect and in higher doses

Attachment L – Ethanol

(80 and 120 mg/kg) significantly slowed ethanol clearance during the 12 h after ethanol administration. The effect of 7-NI (20 mg/kg) on ethanol pharmacokinetics after chronic ethanol administration (inhalation for 18 days) was also studied. The administration of 7-NI immediately after the end of ethanol exposure had a pronounced effect on ethanol pharmacokinetics; in 7-NI-treated rats the fall in ethanol concentrations was significantly slower as compared with vehicle-treated rats. In 7-NI-treated rats, blood-ethanol levels were higher at 3, 6, 9, and 12 h after the end of ethanol exposure.

Title: In vitro dermal and transdermal delivery of doxycycline from ethanollmigliol 840 vehicles.

Author(s): Perkins NC, Heard CM.

Source: Int .3 Pharm. 1999 Nov 15;190(2):155-64.

Abstract: This work investigated the feasibility of dermal and transdermal delivery of doxycycline from vehicles containing Migliol 840 (M840) and ethanol. Delivery of the drug via the skin would provide a useful alternative to oral delivery, which has many undesirable side-effects, such as oesophageal ulceration and disturbance of the normal gut flora. Potential applications include malaria prophylaxis, and the treatment of acne vulgaris, Lyme disease and Reiter syndrome. Experiments were performed to determine the permeation of doxycycline across excised full-thickness human skin and heat-separated epidermal membranes from saturated solutions in ethanol, 1:1 and 2:1 ethanol/M840. Unusual burst behaviour was observed using an ethanol vehicle, possibly as a result of the formation of dimers at saturation. Doxycycline permeated to a higher degree from ethanolic vehicles when M840 is present, suggesting that M840 is capable of enhancing the permeation of doxycycline. The flux across full-thickness skin was highest from a 2:1 ethanol:M840 vehicle (2.41 microg cm(-2) h(-1)), sufficient to deliver 282 microg l(-1) using an area of application of 30 cm(2). The data also produced unexpected results in that permeability across heat separated skin was an order of magnitude greater than across full-thickness skin (28.75 microg cm(-2) h(-1) for the 2:1 ethanol:M840 vehicle). Depth profiling indicated that the drug distributed quite evenly throughout the epidermis. The mean amount of doxycycline recovered from the epidermis at the end of a permeation experiment was 458.4 microg ml(-1). This was far higher than the volume of extractable lipid present in the same unit area, approximately 52.3 microg ml(-1) and indicated that a large proportion of the drug must have been located within the proteinaceous domain. The data therefore suggest (1) significant amounts of doxycycline can be administered into and across the skin; (2) M840 is a potentially useful enhancing vehicle; and (3) the transcellular route was of significance.

Attachment L – Ethanol

Title: Damaging effects of the calcium paradox are reduced in isolated hearts from ethanol- dependent rats: paradoxic effects of dihydropyridine drugs.

Author(s): Guppy LJ, Littleton JM.

Source: J Cardiovasc Pharmacol. 1999 Dec;34(6):765-71.

Abstract: Previous experiments showed that isolated hearts from ethanol-exposed rats show a marked increase in sensitivity to anoxic myocardial damage, and we suggested that this may be due to excess calcium entry through L-type voltage-operated calcium channels (L-VOCCs). To challenge this hypothesis, we investigated the effect of ethanol treatment ex vivo on a damaging stimulus, the "calcium paradox," which is associated with removal of calcium from the perfusate. Adult male Sprague-Dawley rats were exposed to intoxicating concentrations of ethanol for 6-10 days by inhalation. Isolated hearts from these animals were perfused with Krebs-Henseleit buffer by using a modified Langendorff technique, and the calcium paradox induced by a 10-min period of perfusion with calcium-free buffer, followed by reperfusion with calcium-containing buffer. Compared with controls, hearts from ethanol-exposed rats were significantly protected against myocardial damage, as shown by a marked reduction in release of intracellular proteins (lactate dehydrogenase, creatine phosphokinase, and myoglobin) during the reperfusion phase. These indices of myocardial damage were modified by the presence of the dihydropyridine (DHP) calcium channel antagonist nitrendipine (10(-6) M) and the DHP L-VOCC activator Bay K 8644 (10(7) M) in the perfusate during the calcium paradox. Paradoxically, both drugs appeared to increase the damaging effects of calcium-free perfusion, with this effect being generally greater in the preparations from ethanol-exposed rats. As a result, the difference between these hearts and those from control rats was reduced, although a significant degree of protection against the calcium paradox remained. The results support the hypothesis that long-term exposure to ethanol in vivo produces marked alterations in the toxic effects of changes in myocardial calcium concentration. The increased sensitivity to DHP drugs of isolated hearts from ethanol-treated rats is consistent with previous experiments showing increased DHP radioligand-binding sites in these tissues.

Title: In vitro genotoxicity of ethanol and acetaldehyde in human lymphocytes and the gastrointestinal tract mucosa cells.

Author(s): Blasiak J, Trzeciak A, Malecka-Panas E, Drzewoski J, Wolewódzka M.

Source: Toxicol In Vitro. 2000 Aug;14(4):287-95.

Abstract: The influence of ethanol and acetaldehyde on DNA in human lymphocytes, gastric mucosa (GM) and colonic mucosa (CM) was investigated by using the comet assay. All kinds of cells were exposed to ethanol and acetaldehyde in two regimens: the cells were incubated with either chemical and analysed or they were exposed first to ethanol, washed and then exposed to acetaldehyde and

Attachment L – Ethanol

analysed. Lymphocytes were exposed to ethanol at final concentrations of 30 mM and acetaldehyde at 3 mM. GM cells were incubated with ethanol at 1 M and acetaldehyde at 100 mM. CM cells were exposed to ethanol at 10 mM and acetaldehyde at 100 mM. In combined exposure, the cells were subsequently exposed to ethanol and acetaldehyde at all combination of the concentrations of the agents. Ethanol caused DNA strand breaks, which were repaired during 4 hr, except when this agent was applied in GM cells at a concentration of 1 M. A dose- dependent decrease in the tail moment of all types of acetaldehyde-treated cells was observed. Similar results were obtained when a recognized DNA crosslinking agent, formaldehyde, was used. These results suggest that acetaldehyde may form crosslinks with DNA. These crosslinks were poorly repaired. CM cells showed the highest sensitivity of all cell types to ethanol than lymphocytes and GM cells. There were no differences in the sensitivity to acetaldehyde of all the cell types. Our results clearly indicate that ethanol and acetaldehyde can contribute to cancers of the digestive tract.

Title: Effect of CYP2E1 induction by ethanol on the immunotoxicity and genotoxicity of extended low-level benzene exposure.

Author(s): Daiker DH, Shipp BK, Schoenfeld HA, Klimpel GR, Witz G, Moslen MT, Ward JB Jr.

Source: J Toxicol Environ Health A. 2000 Feb 11;59(3):181-96.

Abstract: Potential additive effects of ethanol consumption, a common life-style factor, and low-level benzene exposure, a ubiquitous environmental pollutant, were investigated. Ethanol is a potent inducer of the cytochrome P-450 2E1 (CYP2E1) enzyme, which bioactivates benzene to metabolites with known genotoxicity and immunotoxicity. A liquid diet containing 4.1% ethanol was used to induce hepatic CYP2E1 activity by 4-fold in female CD-1 mice. Groups of ethanol-treated or pair-fed control mice were exposed to benzene or filtered air in inhalation chambers for 7 h/d, 5 d/wk for 6 or 11 wk. The initial experiment focused on immunotoxicity endpoints based on literature reports that ethanol enhances high-dose benzene effects on spleen, thymus, and bone marrow cellularity and on peripheral red blood cell (RBC) and white blood cell (WBC) counts. No statistically significant alterations were found in spleen lymphocyte cellularity, subtype profile, or function (mitogen-induced proliferation, cytokine production, or natural killer cell lytic activity) after 6 wk of ethanol diet, 0.44 ppm benzene exposure, or both. This observed absence of immunomodulation by ethanol alone, a potential confounding factor, further validates our previously established murine model of sustained CYP2E1 induction by dietary ethanol. Subsequent experiments involved a 10-fold higher benzene level for a longer time of 11 wk and focused on genotoxic endpoints in known target tissues. Bone marrow and spleen cells were evaluated for DNA-protein cross-links, a sensitive transient

Attachment L – Ethanol

index of genetic damage, and spleen lymphocytes were monitored for hprt-mutant frequency, a biomarker of cumulative genetic insult. No treatment-associated changes in either genotoxic endpoint were detected in animals exposed to 4.4 ppm benzene for 6 or 11 wk with or without coexposure to ethanol. Thus, our observations suggest an absence of genetic toxicity in CD-1 mice exposed to environmentally relevant levels of benzene with or without CYP2E1 induction.

Title: Combined effects of exposure to styrene and ethanol on the auditory function in the rat.
Author(s): Loquet G, Campo P Lataye R Cossec B, Bonnet P.
Source: Hear Res. 2000 Oct;148(1-2):173-80.
Abstract: In order to study the auditory effects of a metabolic interaction between ethanol and styrene, a first group of rats was gavaged once a day with ethanol (4 g/kg), a second group was exposed to 750 ppm styrene by inhalation, and a third group was exposed to both ethanol and styrene (5 days/week, 4 weeks). Auditory function was tested by recording brainstem (inferior colliculus) auditory evoked potentials, and cochlear hair cell loss was estimated by light microscopy. Cytochrome P450 2E1 and the main urinary styrene metabolites, namely mandelic, phenylglyoxylic and hippuric acids, were measured by high-performance liquid chromatography to check the effects of ethanol on styrene metabolism. In our experimental conditions, ethanol alone did not have any effect on auditory sensitivity, whereas styrene alone caused permanent threshold shifts and outer hair cell damage. Hearing and outer hair cell losses were larger after the exposure to both ethanol and styrene than those induced by styrene alone, indicating a clear potentiation of styrene ototoxicity by ethanol. As expected, metabolic data showed that ethanol alters styrene metabolism and can therefore be considered a modifying factor of styrene toxicokinetics.

Title: Environmental Implications on the Oxygenation of Gasoline with Ethanol in the Metropolitan Area of Mexico City
Author(s): I. Schifter, M. Vera, L. Diaz, E. Guzman, F. Ramos, and E. Lopez-Salinas
Source: Environ. Sci. Technol., 2001, 35 (10), pp 1893-1901
Abstract: Motor vehicle emission tests were performed on 12 in-use light duty vehicles, made up of the most representative emission control technologies in Mexico City: no catalyst, oxidative catalyst, and three way catalyst. Exhaust regulated (CO, $NO_{„}$, and hydrocarbons) and toxic (benzene, formaldehyde, acetaldehyde, and 1,3-butadiene) emissions were evaluated for MTBE (5 vol %)- and ethanol (3, 6, and 10 vol %)-gasoline blends. The most significant overall emissions variations derived from the use of 6 vol % ethanol (relative to a 5% MTBE base gasoline) were 16% decrease in CO, 28% reduction in formaldehyde, and 80% increase in

Attachment L – Ethanol

acetaldehyde emissions. A 26% reduction in CO emissions from the oldest fleet (<MY 1991, without catalytic converter), which represents about 44% of the in-use light duty vehicles in Mexico city, can be attained when using 6 vol % ethanol-gasoline, without significant variation in hydrocarbons and NO,, emissions, when compared with a 5% vol MTBE-gasoline. On the basis of the emissions results, an estimation of the change in the motor vehicle emissions of the metropolitan area of Mexico city was calculated for the year 2010 if ethanol were to be used instead of MTBE, and the outcome was a considerable decrease in all regulated and toxic emissions, despite the growing motor vehicle population.

Title: Is ethanol genotoxic? A review of the published data.
Author(s): Phillips BJ, Jenkinson P.
Source: Mutagenesis. 2001 Mar;16(2):91-101.
Abstract: A great many studies have been carried out on the toxicology of ethanol, the majority in the context of the effects of the consumption of alcohol in beverages. Published information relevant to the assessment of the possible genotoxic potential of ethanol has been reviewed and evaluated in terms of the safety of ethanol as an industrial chemical, rather than as a component of beverages. The available data on ethanol from standard genotoxicity test methods are incomplete. There is clear evidence that ethanol is not a bacterial or mammalian cell mutagen but in vitro assays for chromosome aberration, although mostly negative, have generally not included exogenous metabolic activation. Evidence from the use of ethanol as a vehicle control suggests that it is not mutagenic or clastogenic in vitro. Reported tests for chromosome aberration induction in vivo are all negative and only a minority of micronucleus tests are positive. Conflicting results have been reported for the dominant lethal assay, although an inter-laboratory study performed to OECD guidelines was negative. There is some evidence that ethanol induces SCE in vivo and can also act as an aneugen at high doses. Many in vivo studies were designed to model alcoholism and used very high doses, sometimes for long periods. Outcomes may have been affected by disturbances of metabolism giving rise to secondary effects. It is concluded that there is no significant evidence that ethanol is a genotoxic hazard according to the criteria normally applied for the purpose of classification and labelling of industrial chemicals. Some degree of genotoxicity may result from excessive alcohol drinking, but this is not considered relevant to any conceivable exposure obtainable by either inhalation or dermal exposure in the workplace.

Title: Inhalation delivery of proteins from ethanol suspensions.
Author(s): Choi WS Murthv GG, Edwards DA Langer R, Klibanov AM.
Source: Proc Natl Aced Sci U S A. 2001 Sep 25;98(20):11103-7.

Attachment L – Ethanol

Abstract: To circumvent inherent problems associated with pulmonary administration of aqueous-solution and dry-powder protein drugs, inhalation delivery of proteins from their suspensions in absolute ethanol was explored both in vitro and in vivo. Protein suspensions in ethanol of up to 9% (wt/vol) were readily aerosolized with a commercial compressor nebulizer. Experiments with enzymic proteins revealed that nebulization caused no detectable loss of catalytic activity; furthermore, enzyme suspensions in anhydrous ethanol retained their full catalytic activity for at least 3 weeks at room temperature. With the use of Zn(2+)-insulin, conditions were elaborated that produced submicron protein particles in ethanol suspensions. The latter (insulin/Et0H) afforded respirable-size aerosol particles after nebulization. A 40-min exposure of laboratory rats to 10 mg/ml insulin/Et0H aerosols resulted in a 2-fold drop in the blood glucose level and a marked rise in the serum insulin level. The bioavailability based on estimated deposited lung dose of insulin delivered by inhalation of ethanol suspension aerosols was 33% (relative to an equivalent s.c. injection), i.e., comparable to those observed in rats after inhalation administration of dry powder and aqueous solutions of insulin. Inhalation of ethanol in a relevant amount/time frame resulted in no detectable acute toxic effects on rat lungs or airways, as reflected by the absence of statistically significant inflammatory or allergic responses, damage to the alveolar/capillary barrier, and lysed and/or damaged cells.

Title: Dermal tolerance and effect on skin hydration of a new ethanol-based hand gel.
Author(s): Kampf G, Muscatiello M, Hantschel D, Rudolf M.
Source: J Hosp Infect. 2002 Dec;52(4):297-301.

Abstract: We studied the dermal tolerance (repetitive occlusive patch test; ROPT) and the skin hydrating properties of a new ethanol- based gel [85% (w/w)1, Sterillium Gel. For the ROPT, 53 participants were studied. Gel was applied to one site on the back under an occlusive patch during an induction phase (nine applications over three weeks) and two weeks later to a virgin site on the back during a challenge phase (one application). Twenty-four hours after the removal of the patches (induction phase and challenge phase), then 48 and 72 h later (challenge phase) sites were graded for skin reactions using a standardized scale. In the induction phase none of the 53 participants had a skin reaction. In the challenge phase one participant had a barely perceptible skin reaction, and one had mild erythema at one time point. To evaluate skin hydrating properties of the gel, treated skin of 21 participants was compared to untreated skin. The gel was applied twice a day to the forearm for 14 days. Control corneometer values were taken before application of the gel (mean: 32.7 +/- 5.0) and after one (36.3 +/- 4.4) and two weeks (36.1 +/- 5.4). Relative skin hydration on treated skin in comparison with an untreated control field was significantly higher after one week by 6.85% (P = 0.0031; paired t -test for dependent samples) and after two weeks

Attachment L – Ethanol

by 4.47% (P = 0.0153). Sterillium Gel did not demonstrate a clinically relevant potential for dermal irritation or sensitization, and significantly increased skin hydration after repetitive use, and so could enhance compliance with hand hygiene among healthcare workers.

Title: Analysis of solvent central nervous system toxicity and ethanol interactions using a human population physiologically based kinetic and dynamic model.
Author(s): MacDonald AJ, Rostami-Hodiegan A, Tucker GT, Linkens DA.
Source: Requl Toxicol Pharmacol. 2002 Apr; 35(2 Pt 1):165-76.
Abstract: The effect of acute ethanol-mediated inhibition of m-xylene metabolism on central nervous system (CNS) depression in the human worker population was investigated using physiologically based pharmacokinetic (PBPK) models and probabilistic random (Monte Carlo) sampling. PBPK models of inhaled m-xylene and orally ingested ethanol were developed and combined by a competitive enzyme (CYP2E1) inhibition model. Human interindividual variability was modeled by combining estimated statistical distributions of model parameters with the deterministic PBPK models and multiple random or Monte Carlo simulations. A simple threshold pharmacodynamic model was obtained by simulating m-xylene kinetics in human studies where CNS effects were observed and assigning the peak venous blood m-xylene concentration (C(V,max)) as the dose surrogate of toxicity. Probabilistic estimates of an individual experiencing CNS disturbances given exposure to the current UK occupational exposure standard (100 ppm time-weighted average over 8 h), with and without ethanol ingestion, were obtained. The probability of experiencing CNS effects given this scenario increases markedly and nonlinearly with ethanol dose. As CYP2E1-mediated metabolism of other occupationally relevant organic compounds may be inhibited by ethanol, simulation studies of this type should have an increasingly significant role in the chemical toxicity risk assessment.

Title: Relevance of the developmental toxicity of ethanol in the occupational setting: a review.
Author(s): Irvine LF.
Source: J Appl Toxicol. 2003 Sep-Oct; 23(5): 289-99.
Abstract: Numerous studies have been conducted investigating the reproductive toxicology of ethanol, the overwhelming majority concerning the adverse effects of consuming alcohol in beverages during pregnancy. Because many of the in vivo studies were designed to model alcoholism, they used comparatively high doses and assessed relatively few endpoints. Outcomes may have been affected by disturbances of metabolism at such high exposures, giving rise to secondary effects on development. The available data on ethanol from "conventional" developmental toxicity study test methods of the type used for regulatory hazard

Attachment L – Ethanol

assessment of chemicals are limited. It is in this context, however, i.e. the use of ethanol as an industrial chemical rather than as a component of beverages, that this review is based. Using the usual criteria applied for the purpose of hazard assessment of industrial chemicals, it is concluded that there is no evidence that industrial exposure to ethanol is a developmental toxicity hazard. Developmental toxicity may result from drinking alcoholic beverages, the threshold level for all aspects of which has yet to be de fi ned. This is not, however, considered relevant to the low blood alcohol concentrations resulting from any conceivable inhalation or dermal exposure in the workplace or through the directed use of any consumer product containing ethanol.

Title: Neuromotor Effects of Acute Ethanol Inhalation Exposure in Humans: A Preliminary Study

Author(s): Veronique Nadeau, Daniel Lamoureux, Anne Beuter, Michel Charbonneau and Robert Tardif

Source: Journal of Occupational Health Vol. 45 (2003), No. 4 215-222

Abstract: Ethanol (ETOH) is added to unleaded gasoline to decrease environmental levels of carbon monoxide from automobiles emissions. Therefore, addition of ETOH in reformulated fuel will most likely increase and the involuntarily human exposure to this chemical will also increase. This preliminary study was undertaken to evaluate the possible neuromotor effects resulting from acute ETOH exposure by inhalation in humans. Five healthy non-smoking adult males, with no history of alcohol abuse, were exposed by inhalation, in a dynamic, controlled-environment exposure chamber, to various concentrations of ETOH (0, 250, 500 and 1,000 ppm in air) for six hours. Reaction time, body sway, hand tremor and rapid alternating movements were measured before and after each exposure session by using the CATSYS^{Tm} 7.0 system and a diadochokinesimeter. The concentrations of ETOH in blood and in alveolar air were also measured. ETOH was not detected in blood nor in alveolar air when volunteers were exposed to 250 and 500 ppm, but at the end of exposure to 1,000 ppm, blood and alveolar air concentrations were 0.443 mg/100m/ and 253.1 ppm, respectively. The neuromotor tests did not show conclusively significant differences between the exposed and non-exposed conditions. In conclusion, this study suggests that acute exposure to ethanol at 1,000 ppm or lower or to concentrations that could be encountered upon refueling is not likely to cause any significant neuromotor alterations in healthy males.

Title: Dermal Exposure During Filling, Loading and Brushing with Products Containing 2-(2- Butoxyethoxy)ethanol

Author(s): Jose H.J. Gusbers, Erik Tielemans, Derk H. Brouwer and Joop J. Van Hemmen

Source: Oxford Journals Medicine, The Annals of Occupational Hygiene. 2004.

Attachment L – Ethanol

Volume 48, Issue 3 pp, 219-227

Abstract: Introduction: Limited quantitative information is available on dermal exposure to chemicals during various industrial activities. Therefore, within the scope of the EU-funded RISKOFDERM project, potential dermal exposure was measured during three different tasks: filling, loading and brushing. DEGBE (2-(2-butoxyethoxy) ethanol) was used as a 'marker' substance to determine dermal exposure to the products that workers were handling. Methods: Potential whole body exposure was measured using self-constructed cotton sampling pads on 11 body locations. Cotton gloves were used to determine the contamination of both hands. Bulk samples were collected to determine the concentration of DEGBE so as to be able to calculate exposure to the handled product. Results: A total of 94 task-based measurements were performed, 30 on filling, 28 on loading and 36 on brushing, which resulted in potential dermal hand exposure to the handled product of 4.1-18 269 mg [geometric mean (GM) 555.4, n = 30], 0.3-27745 mg (GM 217.0, is = 28) and 11.3-733.3 mg (GM 98.4, 17 = 24) for each of the scenarios, respectively. Potential whole body exposure to the product during filling and loading ranged from 1.67 to 155.0 (GM 15.2, is = 9) and <LOD to 176.2 (GM 0.30, is = 10). Because of sampling and analytical problems, whole body exposure during brushing could not be determined. Conclusion: Dermal exposure during filling and loading were of the same order of magnitude, while brushing resulted in much lower exposure levels, probably due to differences in work activities and work precision. For each of the scenarios, contamination was mainly found on the hands, representing up to 96% of the total exposure for filling. For filling and loading the most important source of variability in exposure was due to between-company variability rather than to either between- worker or within-worker variability. The pooled between-worker variability was the most important source of variability in dermal exposure levels for the brushing scenario.

Title: Exhaled ethanol and acetaldehyde in human subjects exposed to low levels of ethanol.

Author(s): Tardif R, Liu L, Raizenne M.

Source: Inhal Toxicol. 2004 Apr;16(4):203-7.

Abstract: With the increased use of ethanol-blend gasoline as an alternative to gasoline, there is a demand for new data to assess the potential health risk of ethanol exposure. Currently, there is very limited information from the scientific database on the adverse effects of inhaled low doses of ethanol (ETOH). The aim of this study was to determine the concentration of ETOH and acetaldehyde (ACTDH), a toxic metabolite of ETOH, in the alveolar air (AA) of subjects exposed to low ETOH vapor concentrations by inhalation. Five healthy adults (2 women, 3 men), nonsmoking, 25-55 yr of age, were exposed for 6 consecutive hours to 25, 100,

Attachment L – Ethanol

or 1000 ppm ETOH and were asked to provide AA samples at various intervals during and after each exposure session, for ETOH and ACTDH measurements. Results showed that the concentrations of ACTDH and ETOH in AA measured after 2 h of exposure at 25 ppm were 0.06 ppm and 7.5 ppm, respectively. Overall, there was a significant correlation between ETOH exposure and ETOH ($R(2) = .92$, $p < .001$) and ACTDH ($R(2) = .99$, $p < .001$) in AA. The ratios between ACTDH and ETOH in AA after 4 h of exposure to 25.9 ppm, 101.7 ppm, and 990.8 ppm ETOH were 0.005, 0.008, and 0.006, respectively. In conclusion, this study showed that inhalation of ETOH at low concentrations resulted in measurable levels of ACTDH in AA and suggests that ACTDH may be used, with good toxicological relevance, as an indicator of exposure to low levels of ETOH in air.

Title: Comparative studies on the effects of water, ethanol and water/ethanol mixtures on chemical partitioning into porcine stratum corneum and silastic membrane

Author(s): D. Van der Merwe and J. E. Riviere

Source: Toxicology in Vitro, Volume 19, Issue 1, February 2005, Pages 69-77

Abstract: The effects of water and ethanol vehicles on stratum corneum and silastic membrane partitioning of 11 industrial and agricultural compounds were studied to aid in characterizing and assessing risk from skin exposure. Zero percent, 50% and 100% aqueous ethanol solutions were used as solvents for ^{14}C labeled phenol, 4-nitrophenol, pentachlorophenol, dimethyl parathion, parathion, chloropyrifos, fenthion, triazine, atrazine, simazine and propazine. Compound partitioning between the solvents and porcine stratum corneum/silastic membrane were estimated. Stratum corneum was exposed to aqueous ethanol ranging from 0% to 100% v/v ethanol in 20% increments and Fourier transform infrared spectroscopy (FT-IR) was used to obtain an index of lipid disorder. Gravimetry and FT-IR were used to demonstrate lipid extraction in aqueous ethanol solutions. Partitioning patterns in silastic membranes resembled those in stratum corneum and were correlated with octanol/water partitioning. Partitioning was highest in water and was higher from 50% ethanol than from 100% ethanol, except for parathion, 4-nitrophenol, atrazine and propazine. Correlation existed between molecular weight and partitioning in water, but not in ethanol and ethanol/water mixtures. Lipid order, as reflected in FT-IR spectra, was not altered. These studies suggest that stratum corneum partitioning of the compounds tested is primarily determined by relative compound solubility between the stratum corneum lipids and the donor solvent. Linear relationships existed between octanol/water partitioning and stratum corneum partitioning. Partitioning was also correlated with molecular weight in water solvent systems, but not in ethanol and ethanol/water mixtures. Ethanol and ethanollwater mixtures altered the stratum corneum through lipid extraction, rather than through

Attachment L – Ethanol

disruption of lipid order.

Title: Effects of an ethanol-gasoline mixture: results of a 4-week inhalation study in rats.

Author(s): Chu I, Poon R, Valli V, Yaqminas A, Bowers WJ Seeqal R Vincent R.

Source: J Appl Toxicol. 2005 May-Jun;25(3):193-9.

Abstract: The inhalation toxicity of an ethanol-gasoline mixture was investigated in rats. Groups of 15 male and 15 female rats were exposed by inhalation to 6130 ppm ethanol, 500 ppm gasoline or a mixture of 85% ethanol and 15% gasoline (by volume, 6130 ppm ethanol and 500 ppm gasoline), 6 h a day, 5 days per week for 4 weeks. Control rats of both genders received HEPA/charcoal-filtered room air. Ten males and ten females from each group were killed after 4 weeks of treatment and the remaining rats were exposed to filtered room air for an additional 4 weeks to determine the reversibility of toxic injuries. Female rats treated with the mixture showed growth suppression, which was reversed after 4 weeks of recovery. Increased kidney weight and elevated liver microsomal ethoxyresorufin-O-deethylase (EROD) activity, urinary ascorbic acid, hippuric acid and blood lymphocytes were observed and most of the effects were associated with gasoline exposure. Combined exposure to ethanol and gasoline appeared to exert an additive effect on growth suppression. Inflammation of the upper respiratory tract was observed only in the ethanol-gasoline mixture groups, and exposure to either ethanol and gasoline had no effect on the organ, suggesting that an irritating effect was produced when the two liquids were mixed. Morphology in the adrenal gland was characterized by vacuolation of the cortical area. Although histological changes were generally mild in male and female rats and were reversed after 4 weeks, the changes tended to be more severe in male rats. Brain biogenic amine levels were altered in ethanol- and gasoline-treated groups; their levels varied with respect to gender and brain region. Although no general interactions were observed in the brain neurotransmitters, gasoline appeared to suppress dopamine concentrations in the nucleus accumbens region co-exposed to ethanol. It was concluded that treatment with ethanol and gasoline, at the levels studied, produced mild, reversible biochemical hematological and histological effects, with some indications of interactions when they were co-administered.

Title: Feasibility studies of dermal delivery of paclitaxel with binary combinations of ethanol and isopropyl myristate: role of solubility, partitioning and lipid bilayer perturbation

Author(s): Ramesh Panchagnula, Hariraghuram Desu, Amit Jain and Sateesh Khandavilli

Source: Il Farmaco, Volume 60, Issues 11-12, November-December 2005, Pages 894-899

Attachment L – Ethanol

Abstract: In the current investigation, paclitaxel (PCL) delivery into the different layers of skin, vehicle optimization and relationship between vehicle composition and the relative contribution of solubility, partition and diffusion towards drug transport has been outlined. Saturation solubility of PCL was determined in ethanol (EtOH), isopropyl myristate (IPM) and their binary combinations, and partition studies performed to study the probability of skin depot formation. Epidermal and dermal partitioning was carried from PCL saturated vehicles. Skin permeation of PCL was studied using the rat skin. FT-IR has been utilized to study the skin barrier perturbation, and the localization of PCL and isopropyl myristate (IPM) in epidermis. High K_{app} value in mineral oil/buffer indicated the tendency of PCL to form a reservoir in skin, and an inverse relationship between PCL solubility in different solvent systems and partitioning into epidermis was found. Maximum $K_{epidermis}$ for PCL was observed with IPM, while PCL in Et0H/IPM (1:1) showed high partitioning into dermis. Maximum flux of PCL was observed with EtOH/IPM (1:1). For lipophilic drug like PCL modulation of vehicle seems to be effective approach to increase the permeability across the skin. With a binary combination of EtOH/IPM (1:1) higher concentration of PCL can be delivered to deeper layer of skin whereas with IPM higher concentration of PCL could be localized in the epidermis. While engineering the delivery vehicle selection of solvents should be such that one of them is miscible in both hydrophilic and lipophilic phase like ethanol and another should be lipophilic in nature (IPM in this case) so that an optimum balance between 'push—pull' and 'blending' effect can be achieved.

Title: Effect of bax deletion on ethanol sensitivity in the neonatal rat cerebellum.
Author(s): Heaton MB Paiva M, Madorsky I, Siler-Marsiolio K, Shaw G.
Source: J Neurobiol. 2006 Jan;66(1):95-101.
Abstract: The developing cerebellum is highly sensitive to ethanol during discrete neonatal periods. This sensitivity has been linked to ethanol-induced alterations in molecules of the Bcl-2 survival-regulatory gene family. Ethanol exposure during peak periods of cerebellar sensitivity, for example, results in increased expression of proapoptotic proteins of this family, while overexpression of the antiapoptotic Bcl-2 protein in the nervous system protects against ethanol neurotoxicity. For the present study, neonatal mice with a targeted deletion of the proapoptotic bax gene were used to determine whether elimination of this protein would mitigate ethanol toxicity. bax knock-out and wild-type mice pups were exposed to ethanol via vapor inhalation during the maximal period of neonatal cerebellar ethanol sensitivity and cerebellar tissue was subsequently assessed for Purkinje and granule cell number and ethanol-mediated generation of reactive oxygen species (ROS). The results revealed that: (1) ethanol exposure during the peak period of cerebellar vulnerability resulted in substantial loss of Purkinje cells in wild-type animals, but not in bax knock-outs; (2) granule cells in the bax

Attachment L – Ethanol

gene-deleted animals were not similarly protected from ethanol effects; and (3) levels of ROS following acute ethanol exposure were appreciably enhanced in the wild-type animals but not in the bax knock-outs. These results imply that Bax is important to ethanol-induced Purkinje cell death during critical neonatal periods, but that ethanol effects on granule cells may function at least partially independent of this apoptosis agonist. Amelioration of ethanol-mediated increases in ROS production in the knock-outs may contribute to the observed effects.

Title: Ethanol-induced increase in the metabolic clearance of 1,1,1-trichloroethane in human volunteers.

Author(s): Johns DO, Daniell WE, Shen DO, Kalman DA, Dills RL, Morgan MS.

Source: Toxicol Sci. 2006 Jul;92(1):61-70.

Abstract: This study evaluated the effect of moderate doses of ethanol over a short period of time on the toxicokinetics of an organic solvent, 1,1,1-trichloroethane. A group of 10 moderate drinkers were recruited and exposed via inhalation for 2 h to a low concentration of 1,1,1-trichloroethane (175 ppm) on two separate occasions. Subjects were administered ethanol (0.35 g/kg body weight) on each of the 7 days preceding one of the exposures. Blood and urine samples were collected during and following each exposure, with blood analyzed for 1,1,1-trichloroethane and urine analyzed for the metabolites of 1,1,1- trichloroethane: trichloroethanol and trichloroacetic acid. Prior ethanol consumption resulted in a significant increase in apparent metabolic clearance of 1,1,1-trichloroethane (mean increase = 25.4%). The results of this study demonstrate that ethanol consumption over time can affect the rate at which an organic solvent is cleared through metabolism in humans. For chemicals with toxic metabolic products, this inductive effect of ethanol consumption on the rate of biotransformation could be potentially harmful to exposed individuals. Metabolic clearance of compounds with high hepatic extraction may not be affected by enzyme induction as it is likely that these compounds are essentially completely metabolized while passing through the liver.

Title: Does the clinical use of ethanol-based hand sanitizer elevate blood alcohol levels? A prospective study

Author(s): Michael A. Miller, Alex Rosin, Marc E. Levsky, Manish M. Patel, Timothy J.D. Gregory, Chad S. Crystal.

Source: American Journal of Emergency Medicine, Volume 24. Issue 7, Pages 815-817, November 2006

Abstract: **Background-**Ethanol-based hand sanitizers (EBHSs) are used in most health care facilities in the United States. Infection control personnel advocate the use of generous quantities of EBHS before and after contact with patients. Although it

Attachment L – Ethanol

is assumed that little systemic absorption of ethanol occurs during EBIIS use, many alcohols are absorbed to varying degrees via the transdermal route. Ethanol intoxication by employees in the medical workplace is a potentially serious finding, and it is of forensic and medical-legal importance to elucidate the effects of frequent use of EBHS upon serum blood ethanol levels (BEL.$). To investigate the effect of frequent use of EBHS upon serum blood ethanol concentrations, we prospectively studied 5 volunteers undergoing frequent application of EBHS.
Methods-Enrolled subjects applied 5 rnL of the product (62% denatured ethyl alcohol manufactured by Kimberley-Clark, Roswell, GA) to both hands and rubbed until dry. This activity was repeated 50 times over 4 hours. Participants had their blood drawn before as well as after completing the study. Each participant was without alcohol exposure during the 12 hours preceding the study.
Results-Five volunteers were enrolled. All had an initial blood ethanol level of less than 5 mg/clL. All 5 participants completed the 4-hour study. There were no noted adverse reactions during the study. Blood ethanol level upon completion of the 50 applications of EBHS was less than 5 Ing/dl. in all 5 study participants.
Conclusion-The results of this study demonstrate that use of ethanol-based hand sanitizers, when frequently used in accordance with labeling, do not raise serum blood ethanol levels.

Title: Quantity of ethanol absorption after excessive hand disinfection using three commercially available hand rubs is minimal and below toxic levels for humans.

Author(s): Kramer A, Below H, Bieber N, Kampf G, Toma CD, Huebner NO, Assadian O.

Source: BMC Infect Dis. 2007 Oct 11;7:117.

Abstract: BACKGROUND: Despite the increasing promotion of alcohol-based hand rubs and the worldwide use of ethanol-based hand rubs in hospitals only few studies have specifically addressed the issue of ethanol absorption when repeatedly applied to human skin. The aim of this study was to assess if ethanol absorption occurs during hygienic and surgical hand disinfection using three different alcohol-based hand-rubs, and to quantify absorption levels in humans.
METHODS: Twelve volunteers applied three hand-rubs containing 95% (hand-rub A), 85% (hand-rub B) and 55% ethanol (hand-rub C; all w/w). For hygienic hand disinfection, 4 mL were applied 20 times for 30 s, with 1 minute break between applications. For surgical hand disinfection, 20 mL of each hand rub was applied to hands and arms up to the level of the elbow 10 times for 3 minutes, with a break of 5 minutes between applications. Blood concentrations of ethanol and acetaldehyde were determined immediately prior and up to 90 minutes after application using head space gas chromatography.
RESULTS: The median of absorbed ethanol after hygienic hand disinfection was

Attachment L – Ethanol

1365 mg (A), 630 mg (B), and 358 mg (C). The proportion of absorbed ethanol was 2.3% (A), 1.1% (B), and 0.9% (C). After surgical hand disinfection, the median of absorbed ethanol was 1067 mg (A), 1542 mg (B), and 477 mg (C). The proportion of absorbed ethanol was 0.7% (A), 1.1% (B), and 0.5% (C). The highest median acetaldehyde concentration after 20 hygienic hand disinfections was 0.57 mg/L (hand-rub C, after 30 min), after 10 surgical hand disinfections 3.99 mg/L (hand-rub A, after 20 minutes).
CONCLUSION: The overall dermal and pulmonary absorption of ethanol was below toxic levels in humans and allows the conclusion that the use of the evaluated ethanol-based hand-rubs is safe.

Title: Effects of ethanol (E85) versus gasoline vehicles on cancer and mortality in the United States.
Author(s): Jacobson MZ.
Source: Environ Sci Technol. 2007 Jun 1;41(11):4150-7.
Abstract: Ethanol use in vehicle fuel is increasing worldwide, but the potential cancer risk and ozone-related health consequences of a large-scale conversion from gasoline to ethanol have not been examined. Here, a nested global-through-urban air pollution/weather forecast model is combined with high-resolution future emission inventories, population data, and health effects data to examine the effect of converting from gasoline to E85 on cancer, mortality, and hospitalization in the United States as a whole and Los Angeles in particular. Under the base-case emission scenario derived, which accounted for projected improvements in gasoline and E85 vehicle emission controls, it was found that E85 (85% ethanol fuel, 15% gasoline) may increase ozone-related mortality, hospitalization, and asthma by about 9% in Los Angeles and 4% in the United States as a whole relative to 100% gasoline. Ozone increases in Los Angeles and the northeast were partially offset by decreases in the southeast. E85 also increased peroxyacetyl nitrate (PAN) in the U.S. but was estimated to cause little change in cancer risk. Due to its ozone effects, future E85 may be a greater overall public health risk than gasoline. However, because of the uncertainty in future emission regulations, it can be concluded with confidence only that E85 is unlikely to improve air quality over future gasoline vehicles. Unburned ethanol emissions from E85 may result in a global-scale source of acetaldehyde larger than that of direct emissions.

Title: Effects of Ethanol (E85) versus Gasoline Vehicles on Cancer and Mortality in the United States
Author(s): Mark Z. Jacobson
Source: Environ. Sci. Technol., 2007, 41 (11), pp 4150-4157
Abstract: Ethanol use in vehicle fuel is increasing worldwide, but the potential cancer risk

Attachment L – Ethanol

and ozone- related health consequences of a large-scale conversion from gasoline to ethanol have not been examined. Here, a nested global-through-urban air pollution/weather forecast model is combined with high-resolution future emission inventories, population data, and health effects data to examine the effect of converting from gasoline to E85 on cancer, mortality, and hospitalization in the United States as a whole and Los Angeles in particular. Under the base-case emission scenario derived, which accounted for projected improvements in gasoline and E85 vehicle emission controls, it was found that E85 (85% ethanol fuel, 15% gasoline) may increase ozone-related mortality, hospitalization, and asthma by about 9% in Los Angeles and 4% in the United States as a whole relative to 100% gasoline. Ozone increases in Los Angeles and the northeast were partially offset by decreases in the southeast. E85 also increased peroxyacetyl nitrate (PAN) in the U.S. but was estimated to cause little change in cancer risk. Due to its ozone effects, future E85 may be a greater overall public health risk than gasoline. However, because of the uncertainty in future emission regulations, it can be concluded with confidence only that E85 is unlikely to improve air quality over future gasoline vehicles. Unburned ethanol emissions from E85 may result in a global-scale source of acetaldehyde larger than that of direct emissions.

Title: Influence of ethanol-diesel blended fuels on diesel exhaust emissions and mutagenic and genotoxic activities of particulate extracts.

Author(s): Song CL, Zhou YC, Huang RJ, Wang YQ, Huang OF, LO G, Liu KM.

Source: J Hazard Mater. 2007 Oct 22;149(2):355-63.

Abstract: This study was aimed at evaluating the influence of ethanol addition on diesel exhaust emissions and the toxicity of particulate extracts. The experiments were conducted on a heavy-duty diesel engine and five fuels were used, namely: E0 (base diesel fuel), E5 (5%), E10 (10%), E15 (15%) and E20 (20%), respectively. The regulated emissions (THC, CO, NOx, PM) and polycyclic aromatic hydrocarbon (PAH) emissions were measured, and Ames test and Comet assay, respectively, were used to investigate the mutagenicity and genotoxicity of particulate extracts. From the point of exhaust emissions, the introduction of ethanol to diesel fuel could result in higher brake specific THC (BSTHC) and CO (BSCO) emissions and lower smoke emissions, while the effects on the brake specific NOx (BSNOx) and particulate matters (BSPM) were not obvious. The PAH emissions showed an increasing trend with a growth of ethanol content in the ethanol-diesel blends. As to the biotoxicity, E20 always had the highest brake specific revertants (BSR) in both TA98 and TA100 with or without metabolizing enzymes (S9), while the lowest BSR were found in E5 except that of TA98-S9. DNA damage data showed a lower genotoxic potency of E10 and E15 as a whole.

Attachment L – Ethanol

Title: Organ-specific inflammation following acute ethanol and burn injury.
Author(s): Bird MD, Kovacs EJ.
Source: J Leukoc Biol. 2008 Sep;84(3):607-13. Epub 2008 Mar 24.
Abstract: Clinical and experimental evidence demonstrates that ethanol exposure prior to injury alters local and systemic inflammatory responses, increasing morbidity and mortality. Moreover, the aberrant inflammatory responses can directly and indirectly lead to the poor prognosis after injury by altering leukocyte infiltration into the wound site and remote organs and by suppressing immunity leading to increased susceptibility to opportunistic infections. Recent studies from our laboratory have focused on inflammatory responses at the wound site and in other distal organs after exposure to acute ethanol and burn injury. This combined insult leads to increased mortality after dermal or intratracheal pseudomonas infection, relative to infected mice given ethanol or burn injury alone. The increased mortality in mice given ethanol and burn injury parallels elevated serum levels of proinflammatory cytokines, IL-6 and TNF-alpha, marked infiltration of leukocytes into the lung and gut, as well as immunosuppression at the sites of infection. Bacterial translocation from the gut is likely to be responsible, in part, for the aberrant accumulation of leukocytes in the lungs of ethanol-exposed, burn-injured mice. Additionally, other factors, such as expression of adhesion molecules, increased chemokine production, and leakiness of the vascular endothelium, may also be involved.

Title: Ethyl glucuronide excretion in humans following oral administration of and dermal exposure to ethanol.
Author(s): Rosano TG, Lin J.
Source: J Anal Toxicol. 2008 Oct;32(8):594-600.
Abstract: Ethyl glucuronide (EtG) is a direct ethanol biomarker and U.S. Department of Health and Human Services has advised that specificity studies at low EtG levels are needed for distinction of ethanol consumption and incidental exposure. The authors report urinary EtG excretion with ethanol abstinence, dermal exposure and oral consumption. EtG concentration by sensitive liquid chromatography-tandem mass spectrometry measurement in 39 urine specimens from adult alcohol abstainers (< 10-62 microg/L) and in urine from 13 children (< 10-80 microg/L) indicates either unrecognized ethanol exposure or endogenous ethanol metabolism. With repetitive daily dermal exposure to hand sanitizer (60% ethanol) by 9 adults, EtG concentration ranged from < 10 to 114 microg/L in 88 first-morning void specimens. EtG excretion following a 24 g ethanol drink by 4 adults revealed maximum urine EtG concentration (12,200-83,200 microg/L) at 3 to 8 h postdose and an EtG detection window up to 25-39 h, compared to an ethanol window of only 2 to 4 h. Oral ethanol use also showed an increase in the

Attachment L – Ethanol

percent (molar equivalent) ethanol excreted as EtG with increasing oral ethanol doses. Human excretion studies show 1. EtG detectable at low concentration (< 100 microg/L) when ethanol use or exposures is not evident, 2. EtG concentration less than 120 microg/L in first morning specimens from adults with repeated dermal exposure to ethanol, 3. EtG levels maximally elevated within 3-8 h and above baseline for up to 39 h after a 24 g ethanol drink, and 4. a dose-dependent increase in the percentage of ethanol excreted as EtG with increasing oral ethanol use.

Title: Ethanol poisoning together with organophosphate exposure: a difficult clinical diagnosis because of physician anchoring.

Author(s): Li Y Yu X, Wane Z, Wane H.

Source: Alcohol Alcohol. 2008 Nov-Dec;43(6)

Abstract: AIMS: Organophosphorus pesticide poisoning occurs frequently in China and can be diagnosed easily based on the history of ingestion and the cholinergic toxic syndrome. Yet, when combined with other toxins, organophosphorus poisoning may appear different.
METHODS: Here, we present a case of acute ethanol poisoning together with a dermal organophosphorus exposure.
RESULTS: Based on the history and a misinterpretation of the physical examination, the patient was treated as an organophosphorus poisoning. Ultimately, serum analysis helped clarify the diagnosis.
CONCLUSIONS: Toxicologist should be aware of the error known as anchoring and take appropriate precautions to limit its occurrence.

Title: Transdermal resorption of an ethanol- and 2-propanol-containing skin disinfectant.

Author(s): Kirschner MH, Lang RA, Breuer B, Breuer M, Gronover CS, Zwinciers T, Böttrich JG, Arndt A, Brauer U Hintzpeter M Burmeister MA Fauteck JD.

Source: Langenbecks Arch Surq. 2009 Jan;394(1):151-7.

Abstract: BACKGROUND AND AIMS: Ethanol- or 2-propanol-containing disinfectant agents are widely used in medical practice, particularly in the surgical environment. It was the primary objective of this phase I study to comparatively investigate the transdermal resorption of ethanol and 2-propanol within 1 h after dermal application of the two agents as single preparations and a commercial product containing both alcohols in combination, respectively. The secondary objective was to examine whether a mutual influence of the two alcohols in combination exists.
MATERIALS AND METHODS: Following the double-blind, randomized, three-times cross-over design for this clinical trial, 20 ml of three different alcohol-containing disinfectants were applied on a 200-cm(2) gauze swab on skin areas,

Attachment L – Ethanol

identical in size and location, of 14 healthy volunteers for 10 min to investigate the absorption rate of ethanol and 2-propanol with special focus on the question whether the two alcohols might influence each other's absorption rate when being applied in combination.
RESULTS: No clinically relevant enhancement of dermal absorption, with respect to ethanol and 2-propanol, could be observed within 1 h after application, neither when used as single preparations, nor in combination.
CONCLUSION: Therefore, the use of ethanol- and 2-propanol-containing disinfectants in the medical environment can be considered as safe.

Title: An assessment of potential cancer risk following occupational exposure to ethanol.
Author(s): Bevan RJ Slack RJ, Holmes P Levy LS.
Source: J Toxicol Environ Health B Crit Rev. 2009 Mar;12(3):188-205.
Abstract: Recognition of the carcinogenic properties of ethanol has resulted from comprehensive evidence regarding the effect of consumption of alcohol; indeed, ethanol in alcoholic beverages is now considered a Group 1 carcinogen by the International Agency for Research on Cancer. However, there is little information on the effects of ethanol following exposure via the occupationally relevant routes of inhalation and dermal exposure. This review therefore focuses on these exposure routes, to assess potential carcinogenic risk associated with occupational exposure to ethanol. Inhalatory exposure at the current occupational exposure limit (OEL) for the United Kingdom (1000 ppm ethanol over an 8-h shift) was estimated to be equivalent to ingestion of 10 g ethanol (approximately 1 glass of alcohol) per day. However, in the occupational setting the dose-rate delivery of this amount of ethanol is low, allowing for its rapid and effective elimination, for the majority of individuals. Similarly, while dermal absorption in an occupational setting could potentially add to overall body ethanol burden, additional carcinogenic risk of such exposure is considered negligible. Thus, on balance, there appears little cause to suppose occupational exposure at or below the current OEL associates with any appreciable increase in risk of cancer. However, available occupational exposure data to confirm this view are currently limited. It is also suggested that adoption of a more flexible classification regime, considering risk in the context of hazard and exposure (such as that adopted by the German MAK commission), would represent an improvement over traditional occupational risk assessment practices.

Title: Ethanol fuel use in Brazil: air quality impacts
Author(s): Garry G. Anderson
Source: Energy Environ. Sci., 2009, 2, 1015-1037
Abstract: Brazil has a long history in the development of ethanol for use as a liquid fuel for

Attachment L – Ethanol

vehicles. They have developed one of most efficient and economical systems for producing ethanol in the world. Brazil provides an example that many other countries would like to emulate. Using ethanol as a vehicle fuel has significant potential air quality impacts. This paper will review the available air quality and vehicle emissions data in Brazil, specifically focusing on vehicle related pollutants that may be impacted by the use of large quantities of ethanol in the fuel. The atmospheric concentrations of acetaldehyde (CH_3CHO) and ethanol in Brazil are much higher than those in other areas of the world, while the concentrations of the single ring aromatic compounds and small carboxylic acids are more typical of observations elsewhere. Acetaldehyde and ethanol increase in vehicle emissions and nitrogen oxides (NO,) may increase when ethanolfuels are used. Both CH3CHO and NO, are very important contributors to photochemical air pollution and ozone (0_3) formation. There are very significant 0_3 air quality problems in Brazil, most studied in the larger cities of Sao Paulo and Rio de Janeiro. These are issues that must be evaluated for other areas of the world that are considering the use of high ethanol content vehicle fuels.

Title: Percutaneous absorption of volatile solvents following transient liquid exposures II. Ethanol

Author(s): Siladitya Ray Chaudhuri, Rachna M. Gajjar, William B. Krantz and Gerald B. Kasting

Source: Chemical Engineering Science, Volume 64, Issue 8, 15 April 2009, Pages 1665-1672

Abstract: The permeation of neat ethanol through split-thickness cadaver skin was measured in non-occluded Franz cells placed in a fume hood. The test compound spiked with ^{14}C radiolabel was applied to skin using four doses ranging from 6.33 to 50.6 pL/cm^2 (5-40 pL over an area of 0.79 cm^2). Additional gravimetric experiments were conducted with ethanol and benzene to determine the evaporation mass transfer coefficient. The experimental data were analyzed by non-linear regression analysis using a previously developed diffusion model in order to ascertain the optimal values of two adjustable parameters, the fractional deposition depth (f_{dep}) and the permeant diffusivity inside the stratum corneum (D_{sc}). Constant diffusivity and variable diffusivity models were considered, Both models were able to describe the combined observations from absorption data from three of the four applied doses. The best correlation between the experimental data and model predictions was observed with the variable diffusivity model.

Attachment L – Ethanol

Title: Chronic candidosis and oral cancer in APECED-patients: production of carcinogenic acetaldehyde from glucose and ethanol by Candida albicans.

Author(s): Uittamo J, Siikala E, Kaihovaara P, Salaspuro M, Rautemaa R.

Source: Int J Cancer. 2009 Feb 1;124(3):754-6.

Abstract: The aim of this study was to compare the production of acetaldehyde (ACH) from ethanol (EtOH) and glucose by oral *Candida* isolates from autoimmune polyendocrinopathy-candidiasis-ectodermal dystrophy (APECED) patients with control isolates. A total of 67 clinical oral isolates and one reference strain (ATCC 90029) of *Candida albicans* were selected for this study [Finland]. Of these, 44 were isolated from 21 APECED patients during the years 1994-2007. From 14 APECED patients multiple isolates (2-4 per patient) from years apart were tested. There was no significant difference in the ACH production from EtOH or glucose between the earlier and more recent isolates from 6 patients. There were some differences between the earlier and more recent isolates from 8 patients, mostly when incubated in glucose, but there was no obvious temporal trend nor were the differences significant. This study was able to show that *C. albicans* isolated from APECED patients produced potentially mutagenic amounts of ACH when incubated in 100 mM glucose. The amount of ACH produced was significantly higher when compared to *C. albicans* strains isolated from groups of patients with oral cancer or from healthy controls.

Title: Examining the temperature dependence of ethanol (E85) versus gasoline emissions on air pollution with a largely-explicit chemical mechanism

Author(s): Diana L. Ginnebaugh, Jinyou Liang and Mark Z. Jacobson

Source: Atmospheric Environment, Volume 44, Issue 9, March 2010, Pages 1192-1199

Abstract: The increased use of ethanol in transportation fuels warrants an investigation of its consequences. An important component of such an investigation is the temperature dependence of ethanol and gasoline exhaust chemistry. We use the Master Chemical Mechanism (MCM, version 3.1, LEEDS University) with the SMVGEAR II chemical ordinary differential solver to provide the speed necessary to simulate complex chemistry to examine such effects. The MCM has over 13,500 organic reactions and 4600 species. SMVGEAR II is a sparse-matrix Gear solver that reduces the computation time significantly while maintaining any specified accuracy. Although we use a box model for this study, we determine and demonstrate in a separate study that the speed of the MCM with SMVGEAR II allows the MCM to be modeled in 3-dimensions. We also verified the accuracy of the model in comparison with smog chamber data. We then use the model with species-resolved tailpipe emissions data for E85 (15% gasoline, 85% ethanol fuel blend) and gasoline vehicles to compare the impact of each on nitrogen oxides, organic gases, and ozone as a function of ambient temperature and background concentrations, using Los Angeles in 2020 as a base case. We

Attachment L – Ethanol

use two different emissions sets — one is a compilation of exhaust and evaporative data taken near 24 °C and the other from exhaust data taken at —7 °C — to determine how atmospheric chemistry and emissions are affected by temperature. We include diurnal effects by examining two day scenarios. We find that, accounting for chemistry and dilution alone, the average ozone concentrations through the range of temperatures tested are higher with E85 than with gasoline by ~7 part per billion volume (ppbv) at higher temperatures (summer conditions) to ~39 ppbv at low temperatures and low sunlight (winter conditions) for an area with a high nitrogen oxide (NOx) to non-methane organic gas (NMOG) ratio. The results suggest that E85's effect on health through ozone formation becomes increasingly more significant relative to gasoline at colder temperatures due to the change in exhaust emission composition at lower temperatures. Acetaldehyde and formaldehyde concentrations are also much higher with h E85 at cold temperatures, which is a concern because both are considered to be carcinogens. These could have implications for wintertime use of E85. Peroxyacetyl nitrate (PAN), another air pollutant of concern, increases with E85 by 0.3-8 ppbv. The sensitivity of the results to box size, initial background concentrations, background emissions, and water vapor were also examined.

Title: Oil-frozen W(1)/ONV(2) double emulsions for dermal biomacromolecular delivery containing ethanol as chemical penetration enhancer.

Author(s): Jaimes-Lizcano YA, Lawson LB, Papadopoulos KD.

Source: J Pharm Sci. 2010 Oct 19. [Epub ahead of print]

Abstract: Oil-frozen water-in-oil-in-water (W(1)/O/W(2)) double emulsions (DE) containing ethanol up to 40% (w/v) in the external aqueous W(2) phase exhibited external coalescence upon thawing of the oil phase, releasing up to 85% of the encapsulated protein of the internal aqueous phase. These emulsions were studied in vitro as potential dermal macromolecular delivery formulations, achieving fluorescein isothiocyanate-labeled bovine serum albumin (FITC-BSA) penetration of up to 86 pm into porcine skin, reaching the viable epidermis where the immunocompetent Langerhans cells are located. Enzyme-linked immunosorbent assay was performed to observe the effect of the emulsification process and ethanol content on the ability of BSA to form antigen-antibody complexes; results indicated that ethanol content and the emulsification process did not diminish the BSA-antibody complex formation when compared with a BSA standard aqueous solution. Therefore, it is shown that oil-frozen VV(1)/ONV(2) DE, with penetration-enhancing ethanol in the W(2) phase, can potentially be used for cutaneous vaccine delivery formulations

Title: Transdermal absorption of ethanol- and 1-propanol-containing hand

Attachment L – Ethanol

disinfectants.

Author(s): Lane RA, Egli-Gany D, Brill FH, Böttrich JG, Breuer M, Breuer B, Kirschner MH.

Source: Langenbecks Arch Surg. 2010 Dec 1. [Epub ahead of print]

Abstract: BACKGROUND AND AIMS: Ethanol- or 1-propanol-containing hand disinfectants are widely used as surgical hand antisepsis. The primary objective of this study was to investigate transdermal absorption of ethanol and 1-propanol from combination of 45% ethanol and 18% 1-propanol with skin protecting ingredients (Softa-Mantl) within 1 h after application in comparison to the absorption of these alcohols from the product in the absence of the cosmetic additives. The secondary objective was to evaluate the dermal tolerability.
MATERIALS AND METHODS: Following the double-blind, randomized cross-over design for this clinical trial, 20 ml of two different alcohol-containing disinfectants was applied with a 200-cm(2) gauze swab on a skin area, identical in size and location, of 14 healthy volunteers for 10 min to investigate the absorption rate of ethanol and 1-propanol. Local dermal tolerability was evaluated using a four-point erythema scale.
RESULTS: No clinically relevant dermal absorption, with respect to ethanol and 1-propanol, could be observed within 1 h after application. Disinfectant-related mild local skin erythema was observed in three cases.
CONCLUSION: The use of the tested formulations containing ethanol and 1-propanol can be considered as safe. The tested formulation containing skin protecting additives (Softa-Mane) does not result in more alcoholic absorption than the formulation without protective additives.

Title: The in vitro genotoxicity of ethanol and acetaldehyde

Author(s): M.A. Kayani and J.M. Parry

Source: Toxicology in Vitro, Volume 24, Issue 1, February 2010, Pages 56-60

Abstract: Ability of ethanol to produce chromosomal changes has been controversial in past many years; nevertheless many recent studies have shown that ethanol itself produces genotoxic effects like acetaldehyde. This study was carried out to evaluate the ability of ethanol and its metabolite acetaldehyde to induce chromosomal changes using in vitro CBMN assay (Cytokinesis Blocked Micronucleus assay) in conjunction with immunofluorescent labeling of kinetochores. Kinetochore staining was used with a view to differentiate, between the genotoxic effects of both chemicals, and ascertain the mechanisms of genotoxicity induction by ethanol and acetaldehyde. Both ethanol and acetaldehyde produced statistically significant ($P < 0.05$) dose dependent increase in MN induction as compared with the controls over the dose range tested. Kinetochore analysis proved that the MN induced in ethanol were originated by an aneugenic mechanism, whereas in the case of acetaldehyde most of the MN had originated by a clastogenic mechanism. This not only

Attachment L – Ethanol

confirms the ability of ethanol to produce DNA damage in vitro but it also establishes the efficacy of CBMN assay to detect and differentiate between the genotoxic effects of different genotoxins. Here we report that ethanol is itself genotoxic, at least in vitro, and produces genotoxic effects mainly through an aneugenic mechanism whereas its metabolite acetaldehyde is a clastogen.

Title: Fibroblast function and wound breaking strength is impaired by acute ethanol intoxication.

Author(s): Ranzer MJ, Chen L, DiPietro LA.

Source: Alcohol Clin Exp Res. 2011 Jan;35(1):83-90.

Abstract: BACKGROUND: Alcohol intoxication occurs in nearly half of all trauma patients and increases the morbidity, mortality, and healing complications of these patients. Prior studies in our laboratory and elsewhere have demonstrated impairments in reepithelialization, angiogenesis, and inflammation in wounds following acute ethanol exposure. Clinically, acute ethanol exposure has been shown to cause an increased breakdown of wounds. To date, the mechanisms by which acute ethanol exposure modifies wound strength have received little experimental attention.
METHODS: To examine how ethanol influences functions critical to the development of wound strength, the effect of ethanol exposure on fibroblast proliferation and extracellular matrix production was examined. Normal human dermal fibroblasts (NHDF) were exposed to ethanol (100 mg/dl) and then examined for proliferative capacity and mRNA production of collagen I, collagen III, and lysyl oxidase (LOX). In in vivo studies, the wound breaking strength, LOX activity, collagen, and hyaluronic acid (HA) contents of wounds of ethanol-exposed (100 mg/dl) mice were examined.
RESULTS: At 24, 48, and 72 hours after acute ethanol exposure (8 hours duration), NHDF displayed a significant impairment in proliferative capacity (up to 50% at 24 hours $p < 0.001$). After ethanol exposure, NHDF produced less collagen I and LOX mRNA, but more collagen III mRNA than control fibroblasts ($p < 0.05$). Ethanol exposure in vivo caused a reduction in wound breaking strength of up to 40% when compared to control mice ($p < 0.01$). LOX activity, collagen, and HA contents in the wounds of ethanol-exposed mice were significantly reduced ($p < 0.01$).
CONCLUSIONS: These studies reveal that a single exposure to ethanol prior to injury can cause a significant decrease in wound breaking strength. Our studies suggest that ethanol directly impairs fibroblast function, leading to decreased collagen production. The results provide a possible explanation for how acute ethanol exposure might increase in wound complications and wound failure.

Title: Ethyl glucuronide, ethyl sulfate, and ethanol in urine after sustained exposure to

Attachment L – Ethanol

an ethanol-based hand sanitizer.

Author(s): Reisfield GM Goldberger BA, Crews BO, Pesce AJ, Wilson GR, Teitelbaum SA, Bertholf RL.

Source: J Anal Toxicol. 2011 Mar;35(2):85-91.

Abstract: To assess the degree of ethanol absorption and subsequent formation of urinary ethyl glucuronide (EtG) and ethyl sulfate (EtS) following sustained application of hand sanitizer, 11 volunteers cleansed their hands with Purell(TM) hand sanitizer (62% ethanol) every 5 min for 10 h on three consecutive days. Urine specimens were obtained at the beginning and end of each day of the study, and on the morning of the fourth day. Urinary creatinine, ethanol, EtG, and EtS concentrations were measured. EtG was undetectable in all pre-study urine specimens, but two pre-study specimens had detectable EtS (73 and 37 ng/mL). None of the pre-study specimens had detectable ethanol. The maximum EtG and EtS concentrations over the course of the study were 2001 and 84 ng/mL, respectively, and nearly all EtG- and EtS-positive urine specimens were collected at the conclusion of the individual study days. Only two specimens had detectable EtG at the beginning of any study day (96 and 139 ng/mL), and only one specimen had detectable EtS at the beginning of a study day (64 ng/mL), in addition to the two with detectable EtS prior to the study. Creatinine-adjusted maximum EtG and EtS concentrations were 1998 and 94 pg/g creatinine, respectively. In patients being monitored for ethanol use by urinary EtG concentrations, currently accepted EtG cutoffs do not distinguish between ethanol consumption and incidental exposures, particularly when urine specimens are obtained shortly after sustained use of ethanolcontaining hand sanitizer. Our data suggest that EtS may be an important complementary biomarker in distinguishing ethanol consumption from dermal exposure.

Attachment L – Ethanol

Welfare Effects Studies (toxicity in aquatic organisms, toxicity in terrestrial species, toxicity to plants/bacteria, effects on visibility/weather/climate, effects on materials, economic effects, effects on personal comfort and well-being)

Title: Effects of endosulfan and ethanol on the reproduction of the snail Biomphalaria tenagophila: a multigeneration study.
Author(s): Oliveira-Filho EC, Grisolia CK, Paumoartten FJ.
Source: Chemosphere. 2009 Apr;75(3):398-404. Epub 2009 Jan 12.
Abstract: Endosulfan (END) is an insecticide used in agriculture and as a wood preservative. Since END is practically insoluble in water, ethanol (ETOH) is often employed as a carrier solvent to spike it in the test medium in aquatic toxicity assays. In this study were investigated the effects of END and ETOH on the reproduction of the freshwater snail Biomphalaria tenagophila exposed over three successive generations. END (0, 0.001, 0.01, 0.1 mg L(-1)) was dissolved in the medium water using ETOH (up to 19.8 mg L(-1)) as carrier solvent. ETOH (19.8, 198, 1980 mg L(-1)) alone was tested as well. Adult snails (F(0) - generation) were exposed to END and ETOH for 8 weeks. The F(1)-generation continued to be exposed from embryo to reproductive maturity, while their descendants (F(2)) were exposed until day 10 after spawning. Effects on the fecundity (8- week production of eggs and egg-masses) of mature F(0) and F(1) snails were evaluated. Developmental toxicity was investigated in F(1) and F(2) embryos. END at the highest level tested (0.1 mg L(-1)) inhibited egg production by F(0) and F (1) snails. ETOH at levels 198 mg L(-1) also reduced fecundity of F(0) and F(1) an effect that was apparently aggravated by exposure over successive generations. END 0.1 mg L(-1) increased mortality and malformations and decreased hatching among F(1) embryos. ETOH drastically reduced the proportion of hatchings among F(2) embryos. The study-derived NOECs (no-observed-effect-concentrations) for END was 0.01 mg L(-1) (reduction in fecundity), and for ETOH were 19.8 mg L(-1) for reduction in fecundity and <19.8 mg L(-1) for developmental toxicity (hatching retardation).

Title: Effect of Ethanol on Branchial Adenosine Triphosphatases in Oreochromis mossambicus (Peters).
Author(s): Bhanu SV Philip B.
Source: Toxicol Int. 2011 Jan;18(1):27-30.
Abstract: The aim of this work was to determine the toxicity of ethanol in an aquatic system by means of bioassays with Oreochromis mossambicus (Peters) as a test organism. The study revealed changes in the gill ATPase activities. The results obtained indicated that ethanol brought about a decrease in the body weight, followed by significant inhibition on total ATPase, Na (+)/K(+) ATPase, Ca(2+) ATPase and Mg(2+) ATPase activities. The studies also indicated that these can

Attachment L – Ethanol

be employed as suitable biomarkers in ethanol related toxicity studies.

Title: Developmental exposures to ethanol or dimethylsulfoxide at low concentrations alter locomotor activity in larval zebrafish: implications for behavioral toxicity bioassays.

Author(s): Chen TH, Wanq YH, Wu YH.

Source: Aquat Toxicol. 2011 Apr;102(3-4):162-6. Epub 2011 Feb 2.

Abstract: Ethanol and dimethylsulfoxide (DMSO) are commonly used as carrier solvents for lipophilic chemicals in aquatic toxicity bioassays. However, very little information has been reported on the behavioral effects of these solvents. In this study, we examined the effects of ethanol and DMSO on development and locomotor activity by a zebrafish embryo-larval bioassay. The zebrafish were exposed to different concentrations (control, 0.01, 0.1, and 1%) of ethanol or DMSO from blastula stage to 144 hour-post-fertilization (hpf). Hatchability, survival, and abnormalities were monitored every 12h, and locomotor activity of the larvae was analyzed at 144 hpf. Hatchability was not affected by the ethanol or DMSO treatments. No effect on survival was observed except the 1% ethanol group suffered 89% mortality during 108-120 hpf. No developmental defects were observed in any of the solvents at the 0.01 and 0.1% concentrations, but significantly higher deformity rates occurred with 1% ethanol and DMSO groups. Hyperactivity and less tortuous swimming paths were observed in all ethanol and DMSO concentrations. Based on this study, we suggest that data of behavioral toxicity bioassays using ethanol or DMSO as carrier solvents should be interpreted cautiously, because the solvents at low concentrations could alter locomotor activity of larval zebrafish without causing any observable developmental defects.

Attachment M – Isopropylcyclopentane

Welfare Effects Studies (toxicity in aquatic organisms, toxicity in terrestrial species, toxicity to plants/bacteria, effects on visibility/weather/climate, effects on materials, economic effects, effects on personal comfort and well-being)

Title: Biodegradation of effluent contaminated with diesel fuel and gasoline

Author(s): P.A. Vieira, R.B. Vieira, F.P. de Franca and V.L. Cardoso

Source: Journal of Hazardous Materials, Volume 140, Issues 1-2, 9 February 2007, Pages 52-59

Abstract: We studied the effects of fuel concentration (diesel and gasoline), nitrogen concentration and culture type on the biodegradation of synthetic effluent similar to what was found at inland fuel distribution terminals. An experimental design with two levels and three variables (2^3) was used. The mixed cultures used in this study were obtained from lake with a history of petroleum contamination and were named culture C_1 (collected from surface sediment) and C_2 (collected from a depth of approximately 30 cm). Of the parameters studied, the ones that had the greatest influence on the removal of total petroleum hydrocarbons (TPH) were a nitrogen concentration of 550 mg/L and a fuel concentration of 4% (v/v) in the presence of culture C_1. The biodegradability study showed a TPH removal of 90 ± 2% over a process period of 49 days. Analysis using gas chromatography identified 16 hydrocarbons. The aromatic compounds did not degrade as readily as the other hydrocarbons that were identified.

Attachment N – Methacrolein

Health Effects Studies (acute toxicity, subchronic toxicity, chronic toxicity – e.g., including reproductive/developmental toxicity, neurotoxicity, immunotoxicity, organ system effects, mutagenicity/genotoxicity, carcinogenicity)

Title: Mutagenicity and chemical reactivity of epoxidic intermediates of the isoprene metabolism and other structurally related compounds

Author(s): P.G. Gervasi, L. Citti, M. Del Monte, V. Longo and D. Benetti

Source: Mutation Research/Genetic Toxicology, Vol 156, Issues 1-2, April-May 1985, Pages 77-82

Abstract: The mutagenic activities of the epoxidic intermediates of the isoprene biotransformation were investigated using Salmonella typhimurium and compared with those of other structurally related epoxides. The compound 2-methyl-1,2,3,4-diepoxybutane, chemically analogous to the well known carcinogenic 1,2,3,4-diepoxybutane, was found to be as mutagenic as the latter. Moreover, the mutagenic activities of oxiranes were correlated to their alkylating powers towards nicotinamide and to their half-lives for spontaneous hydrolysis. The relationship between alkylating power and mutagenicity was found to hold for the stable epoxides that react mainly by an SN_2 substitution mechanism.

Title: The lipid peroxidation product 4-hydroxynonenal is a potent inducer of the SOS response.

Author(s): Benamira M, Marnett LJ.

Source: Mutat Res. 1992 Nov; 293(1):1-10.

Abstract: An important aspect of bacterial mutagenesis by several difunctional carbonyl compounds appears to be the induction of the SOS system. We tested the ability of a series of carbonyl compounds to induce expression of the SOS-regulated umu operon in Salmonella typhimurium TA1535/pSK1002. SOS-inducing potencies varied widely among the carbonyl compounds tested. 4-Hydroxynonenal, a product of lipid peroxidation, was the most potent SOS-inducer, with maximal induction observed at concentrations of 0.1-1 microM. Acrolein, crotonaldehyde and methacrolein induced little increase over background umu expression. Malondialdehyde, another product of lipid peroxidation, was a very weak SOS-inducer with a maximal response induced at a concentration of 28 mM. Substitution at the alpha-position of malondialdehyde, which abolishes frameshift mutagenicity, did not abolish SOS-inducing activity. Substitution of the hydroxyl group of malondialdehyde and alpha-methyl-malondialdehyde by a better leaving group (benzoyloxy) resulted in an approximately 250-fold higher SOS-inducing potency. Comparison of the present results to literature reports on bacterial mutagenicity indicates a poor correlation of the two properties between different classes of difunctional carbonyl compounds and even within the same class of difunctional carbonyl compounds.

Attachment N – Methacrolein

Title: Effects of methacrolein on the respiratory tract in mice.
Author(s): Larsen ST, Nielsen GD.
Source: Toxicol Lett. 2000 Apr 3; 114(1-3):197-202.
Abstract: The acute respiratory effects of airborne exposure to methacrolein were studied in a recent refinement of the standard test method with mice (ASTM, 1984. American Society for Testing and Materials, Philadelphia). Irritation of the upper respiratory tract caused a concentration-dependent decrease in the respiratory rate of 2-26 ppm methacrolein. In this range, only a minor airflow limitation occurred in the lower respiratory tract, suggesting that the main effect of methacrolein is sensory irritation. During exposure, the sensory irritation response maintained the same level, i.e. no desensitisation occurred. The concentration 10.4 ppm methacrolein reduced the respiratory rate by 50% (RD50). The extrapolated threshold for the respiratory depressing effect, RDO, was 1.3 ppm. The sensory irritation effect of methacrolein was compared with results from closely related compounds in order to elucidate the mechanism of the interaction between methacrolein and the sensory irritant receptor.

Title: Effects of 1,3-butadiene, isoprene, and their photochemical degradation products on human lung cells.
Author(s): Doyle M, Sexton KG, Jeffries H, Bridge K, Jaspers I.
Source: Environ Health Perspect. 2004 Nov; 112(15): 1488-95.
Abstract: Because of potential exposure both in the workplace and from ambient air, the known carcinogen 1,3-butadiene (BD) is considered a priority hazardous air pollutant. BD and its 2-methyl analog, isoprene (ISO), are chemically similar but have very different toxicities, with ISO showing no significant carcinogenesis. Once released into the atmosphere, reactions with species induced by sunlight and nitrogen oxides convert BD and ISO into several photochemical reaction products. In this study, we determined the relative toxicity and inflammatory gene expression induced by exposure of A549 cells to BD, ISO, and their photochemical degradation products in the presence of nitric oxide. Gas chromatography and mass spectrometry analyses indicate the initial and major photochemical products produced during these experiments for BD are acrolein, acetaldehyde, and formaldehyde, and products for ISO are methacrolein, methyl vinyl ketone, and formaldehyde; both formed < 200 ppb of ozone. After exposure the cells were examined for cytotoxicity and interleukin-8 (IL-8) gene expression, as a marker for inflammation. These results indicate that although BD and ISO alone caused similar cytotoxicity and IL-8 responses compared with the air control, their photochemical products significantly enhanced cytotoxicity and IL-8 gene expression. This suggests that once ISO and BD are released into the environment, reactions occurring in the atmosphere transform these hydrocarbons into products that induce potentially greater adverse health effects

Attachment N – Methacrolein

than the emitted hydrocarbons by themselves. In addition, the data suggest that based on the carbon concentration or per carbon basis, biogenic ISO transforms into products with proinflammatory potential similar to that of BD products.

Title: Emissions of Toxic Pollutants from Compressed Natural Gas and Low Sulfur Diesel-Fueled Heavy-Duty Transit Buses Tested over Multiple Driving Cycles

Author(s): Norman Y. Kado, Robert A. Okarnoto, Paul A. Kuzmicky, Reiko Kobayashi, Alberto Ayala, Michael E. Gebel, Paul L. Rieger, Christine Maddox, and Leo Zafonte

Source: Environ. Sci. Technol., 2005, 39 (19), pp 7638-7649

Abstract: The number of heavy-duty vehicles using alternative fuels such as compressed natural gas (CNG) and new low-sulfur diesel fuel formulations and equipped with after-treatment devices are projected to increase. However, few peer-reviewed studies have characterized the emissions of particulate matter (PM) and other toxic compounds from these vehicles. In this study, chemical and biological analyses were used to characterize the identifiable toxic air pollutants emitted from both CNG and low-sulfur-diesel-fueled heavy-duty transit buses tested on a chassis dynamometer over three transient driving cycles and a steady-state cruise condition. The CNG bus had no after-treatment, and the diesel bus was tested first equipped with an oxidation catalyst (DC) and then with a catalyzed diesel particulate filter (DPF). Emissions were analyzed for PM, volatile organic compounds (VOCs; determined on-site), polycyclic aromatic hydrocarbons (PAHs), and mutagenic activity. The 2000 model year CNG- fueled vehicle had the highest emissions of 1,3-butadiene, benzene, and carbonyls (e.g., formaldehyde) of the three vehicle configurations tested in this study. The 1998 model year diesel bus equipped with an OC and fueled with low-sulfur diesel had the highest emission rates of PM and PAHs. The highest specific mutagenic activities (revertants/pg PM, or potency) and the highest mutagen emission rates (revertants/mi) were from the CNG bus in strain TA98 tested over the New York Bus (NYB) driving cycle. The 1998 model year diesel bus with DPF had the lowest VOCs, PAH, and mutagenic activity emission. In general, the NYB driving cycle had the highest emission rates (g/mi), and the Urban Dynamometer Driving Schedule (ODDS) had the lowest emission rates for all toxics tested over the three transient test cycles investigated. Also, transient emissions were, in general, higher than steady-state emissions. The emissions of toxic compounds from an in-use CNG transit bus (without an oxidation catalyst) and from a vehicle fueled with low-sulfur diesel fuel (equipped with DPF) were lower than from the low-sulfur diesel fueled vehicle equipped with OC. All vehicle configurations had generally lower emissions of toxics than an uncontrolled diesel engine. Tunnel backgrounds (measurements without the vehicle running) were measured throughout this study and were helpful in determining the incremental increase in

Attachment N – Methacrolein

pollutant emissions. Also, the on-site determination of VOCs, especially 1,3-butadiene, helped minimize measurement losses due to sample degradation after collection.

Title: The effect on human eye blink frequency of exposure to limonene oxidation products and methacrolein.
Author(s): Nøjgaard JK, Christensen KB, Wolkoff P.
Source: Toxicol Lett. 2005 Apr 10; 156(2):241-51.
Abstract: Oxidation products of terpenes (e.g. limonene) contain unidentified irritants, which may be responsible for a fraction of the reported eye and airway complaints in indoor environments. Here we report exposure to parts per billion (ppb) levels of limonene oxidation products (LOPs) and the terpene oxidation product methacrolein using blink frequency (BF) as a measure of trigeminal stimulation of the human eye. Ten male subjects averaging 43 (standard deviation 10.5) years were exposed for 20 min to LOPs, methacrolein, and clean air, respectively. A baseline BF was measured prior to and following each exposure (8 min and 4 min, respectively). The subjects were exposed locally in the non-dominant eye and single blind at 20% relative humidity (RH), while viewing an educational film. Blinking was video recorded and evaluated for full sessions of 36 min. Mean BF increased significantly during exposure to LOPs and methacrolein compared to the baseline of clean air, and the findings coincided with weak eye irritation symptoms. Lowest observed effect levels were 286 ppb methacrolein and a 10-min-old LOPs mixture of initially 92 ppb limonene and 101 ppb ozone (03), which increased the BF comparably by 18% (p=0.001) and 17% (p=0.003), respectively. The increase in BF was smaller, although not significantly different, during exposure to LOPs at 50% RH to 20% RH in mixtures prepared from ca. 350 ppb limonene and 300 ppb 03. LOPs may cause trigeminal stimulation and possibly eye irritation at 03 and limonene concentrations, which are close to high-end values measured in indoor settings. The effects may be exacerbated by low RH.

Title: A sensitive method for the quantification of acrolein and other volatile carbonyls in ambient air.
Author(s): Seaman VY, Charles MJ, Cahill TM.
Source: Anal Chem. 2006 Apr 1; 78(7):2405-12.
Abstract: Acrolein, an unsaturated aldehyde found in both indoor and outdoor air, is considered one of the greatest noncancer health risks of all organic air pollutants. Current methods for determining acrolein often employ sorbent-filled cartridges containing a carbonyl derivatizing agent (e.g., dinitrophenylhydrazine). These methods are of limited use for unsaturated compounds due to the formation of unstable derivatives, coelution of similar compounds, long sample collection

Attachment N – Methacrolein

times, and ozone interferences that result in poor sensitivity, selectivity, and reproducibility. The goal of this research was to develop an analytical method for determining ppt concentrations of acrolein and other carbonyls in air with short sampling times (10 min). The method uses a mist chamber to collect carbonyls by forming water-soluble carbonyl-bisulfite adducts. The carbonyls are then liberated from the bisulfite, derivatized, and quantified by gas chromatography/electron capture negative ionization mass spectrometry. The method was applied to determine atmospheric acrolein concentrations at three sites in northern California reflecting hemispheric background concentrations, biogenic-dominated regions, and urban environments. The resulting acrolein concentrations were 0.056, 0.089, and 0.29 microg/m3, respectively, which are all above the EPA Reference Concentration of 0.02 microg/m3. The minimum detection limit of 0.012 microg/m3 is below that of other published methods. Methacrolein, methyl vinyl ketone, crotonaldehyde, glyoxal, methyl glyoxal, and benzaldehyde were also quantified.

Title: Volatile aldehydes in the mainstream smoke of the narghile waterpipe.
Author(s): Al Rashidi M, Shihadeh A, Saliba NA.
Source: Food Chem Toxicol. 2008 Nov;46(11):3546-9.
Abstract: Very little is known about the quality and quantity of toxicants yielded by the narghile, a subject of increasing importance as this method of tobacco smoking has become popular all over the world. This study is concerned with the identification and quantification of volatile aldehydes in the gas and particle phases of mainstream narghile smoke generated using a popular type of flavored ma'ssel tobacco mixture. These compounds were analyzed based on a modified version of the Environmental Protection Agency compendium method TO-11A. Using a standardized smoking machine protocol consisting of 171 puffs, 2.6s puff duration and 17s inter puff interval, the average yields of formaldehyde, acetaldehyde, acrolein, propionaldehyde and methacrolein were 630, 2520, 892, 403, and 106 microg/smoking session, respectively. The results showed that none of the aldehydes identified in this study are found in the particulate phase of the smoke, except for formaldehyde for which the partitioning coefficient was estimated as Kp = 3.3 x 10(-8) microg/m3. Given previously reported lung absorption fractions of circa 90% for volatile aldehydes, the yields measured in this study are sufficient to induce various diseases depending on the extent of exposure, and on the breathing patterns of the smokers.

Attachment N – Methacrolein

Title: Comparison of carcinogen, carbon monoxide, and ultrafine particle emissions from narghile waterpipe and cigarette smoking: Sidestream smoke measurements and assessment of second-hand smoke emission factors.

Author(s): Daher N, Saleh R, Jaroudi E, Sheheitli H, Badr T, Sepetdjian E, Al Rashidi M, Saliba N, Shihadeh A.

Source: Atmos Environ. 2010 Jan 1; 44 (1):8-14.

Abstract: The lack of scientific evidence on the constituents, properties, and health effects of second-hand waterpipe smoke has fueled controversy over whether public smoking bans should include the waterpipe. The purpose of this study was to investigate and compare emissions of ultrafine particles (UFP, <100 nm), carcinogenic polyaromatic hydrocarbons (PAH), volatile aldehydes, and carbon monoxide (CO) for cigarettes and narghile (shisha, hookah) waterpipes. These smoke constituents are associated with a variety of cancers, and heart and pulmonary diseases, and span the volatility range found in tobacco smoke. Sidestream cigarette and waterpipe smoke was captured and aged in a 1 m(3) Teflon-coated chamber operating at 1.5 air changes per hour (ACH). The chamber was characterized for particle mass and number surface deposition rates. UFP and CO concentrations were measured online using a fast particle spectrometer (TSI 3090 Engine Exhaust Particle Sizer), and an indoor air quality monitor. Particulate PAH and gaseous volatile aldehydes were captured on glass fiber filters and DNPH-coated SPE cartridges, respectively, and analyzed off-line using GC-MS and HPLC-MS. PAH compounds quantified were the 5- and 6-ring compounds of the EPA priority list. Measured aldehydes consisted of formaldehyde, acetaldehyde, acrolein, methacrolein, and propionaldehyde.We found that a single waterpipe use session emits in the sidestream smoke approximately four times the carcinogenic PAH, four times the volatile aldehydes, and 30 times the CO of a single cigarette. Accounting for exhaled mainstream smoke, and given a habitual smoker smoking rate of 2 cigarettes per hour, during a typical one-hour waterpipe use session a waterpipe smoker likely generates ambient carcinogens and toxicants equivalent to 2-10 cigarette smokers, depending on the compound in question. There is therefore good reason to include waterpipe tobacco smoking in public smoking bans.

Title: Toxic emissions from open burning

Author(s): Carl Renan Estrellan and Fukuya Iino

Source: Chemosphere, Volume 80, Issue 3, June 2010, Pages 193-207

Abstract: This review compiled the data from recent actual and simulation studies on toxic emissions from open burning and categorized into sources, broadly as biomass and anthropogenic fuels. Emission factors, in mass of pollutant per mass of material being burned, and actual concentrations, in mass of pollutant per unit volume have been compared based on source classifications. In addition to

Attachment N – Methacrolein

gaseous emissions, this review presents the updated data on emissions to air in the form of particulate matter, and emissions to soil and water environment. Data from forest fires, accidental fires such as vehicle fires, house fires, and unintentional landfill fires are included in this review as well as combustion involving traditional and recreational activities.

Attachment N – Methacrolein

Welfare Effects Studies (toxicity in aquatic organisms, toxicity in terrestrial species, toxicity to plants/bacteria, effects on visibility/weather/climate, effects on materials, economic effects, effects on personal comfort and well-being)

Title: Influence of sagebrush terpenoids on mule deer preference
Author(s): Robert O. Bray, Carl L. Wambolt and Rick G. Kelsey
Source: Journal of Chemical Ecology, 1991, Volume 17, Number 11, 2053-2062
Abstract: The effect on mute deer (Odocoileus hemionus hemionus Rafinesque) preference of compounds in mountain big sagebrush [Artemisia tridentata Nutt. ssp. Vaseyana (Rydb.) Beetle], Wyoming big sagebrush (A. t. ssp. Wyomingensis Beetle and Young), basin big sagebrush (A. t. ssp.Tridentata), and black sagebrush (A. nova Nets.) was compared using a two-choice preference test. Compounds tested included:p-cymene, 1,8-cineole, methacrolein (two concentrations), and the nonvolatile crude terpenoid fraction (NVCTF) from each taxon. The compounds were tested by applying them to chopped alfalfa hay at concentrations similar to those found in nature. The intake of the treated hay was compared with that of an untreated control. Eight deer were used as test animals in an 8 - 8 Latin- square design. AR compounds tested significantly deterred ingestion (P 0.05). Compound influence on preference, in order of increasing deterrence, was as follows: 50% methacrolein, mountain big sagebrush NVCTF, methacrolein, basin big sagebrush NVCTF,p-cymene, Wyoming big sagebrush NVCTF, black sagebrush NVCTF, and 1,8-cineole. Methacrolein appears to be an important preference determinant among big sagebrush subspecies, andp-cymene between black sagebrush and big sagebrush. The NVCTFs containing sesquiterpene lactones as one of their constituents were closely related to the preference of all four taxa. Future studies of animal preference for sagebrush should consider all of the potential defensive chemicals in the foliage.

Title: Atmospheric fate of methyl vinyl ketone and methacrolein
Author(s): Tomasz Gierczak, James B. Burkholder, Ranajit K. Talukdar, A. Melloukl, S. B. Barone and A. R. Ravishankarab
Source: Journal of Photochemistry and Photobiology A: Chemistry, Volume 110, Issue 1, 15 October 1997, Pages 1-10
Abstract: The rate coefficients for the reaction of OH with methyl vinyl ketone (MVK,$CH_3C(0)CHCH_2$) and methacrolein (MACR, $CH_2C(CH_3)CHO$) between 232 and 378 K were measured using the pulsed laser photolysis-pulsed laser induced fluorescence (PP-PLIF) technique. The rate coefficient data can be expressed in the Arrhenius form as k_l(OH + MVK) = 2.67±0.45) x 10^{-12}exp((452±130)/7) and k_2(OH + MACR) = (7.73±0.65) x 10^{-12}exp((379±46)/7) cm^3 molecule $^{-1}$ s^{-1}, where the error limits are 2a and include estimated systematic error. The UV absorption

Attachment N – Methacrolein

cross-sections of MVK and MACR were measured over the wavelength range 250-395 nm using a diode array spectrometer. Absolute quantum yields for loss of MVK and MACR were measured at 308, 337, and 351 nm. The MACR quantum yield, $01)_{mAcR}$, was <0.01. The MVK quantum yield was both pressure and wavelength dependent and is represented by the expression:: $0_0(A,P) < \exp [-0.055 (A-308)]/(5.5 + 9.2 \times 10^{-19}N)$ where A is measured in nm and N is the number density in molecule cm^{-3}. Atmospheric loss rate calculations using these results show that the primary loss process for both MVK and MACR is the reaction with OH radicals throughout the troposphere.

Title: Uptake of Methacrolein and Methyl Vinyl Ketone by Tree Saplings and Implications for Forest Atmosphere

Author(s): Akira Tani, Seita Tobe, and Sachie Shimizu

Source: Environ. Sci. Technol., 2010, 44 (18), pp 7096-7101

Abstract: Methacrolein (MACR) and methyl vinyl ketone (MVK) are oxygenates produced from isoprene which is abundantly emitted by trees. The uptake rate of these compounds by leaves of three different Quercus species, Q. acutissima, Q. myrsinaefolia, and Q. phillyraeoides, at typical concentrations within a forest (several part per billion by volume) were determined. The rates of uptake of croton aldehyde (CA) and methyl ethyl ketone (MEK) were also investigated for comparison. The rates of uptake of the two aldehydes MACR and CA were found to be higher than those of the two ketones. In particular, the rate of MEK uptake for Q. myrsinaefolia was exceptionally low. The ratio of intercellular to fumigated concentrations, Ci/Ca, for MACR and CA was found to be low (0-0.24), while the ratio for the two ketones was 0.22-0.90. To evaluate the contribution of tree uptake as a sink for the two isoprene-oxygenates within the forest canopy, loss rates of the compounds due to uptake by trees and by reactions with hydroxyl radicals (OH radicals) and 0_3 were calculated. The loss rate by tree uptake was the highest, followed by the reaction with OH radicals, even at a high OH concentration (0.15 pptv) both for MACR and MVK, suggesting that tree uptake provides a significant sink.

Attachment O – Methanol

Health Effects Studies (acute toxicity, subchronic toxicity, chronic toxicity – e.g., including reproductive/developmental toxicity, neurotoxicity, immunotoxicity, organ system effects, mutagenicity/genotoxicity, carcinogenicity)

Title: Influence of ethanol and methanol gasoline blends on the mutagenicity of particulate exhaust extracts
Author(s): Charles R. Clark, John S. Dutcher, Roger O. McClellan, Ted M. Naman and Donald E. Seizinger
Source: Archives of Environmental Contamination and Toxicology, 1983, Volume 12, Number 3, 311-317
Abstract: The Salmonella mutagenicity test was used to evaluate the influence of alcohol fuel extenders on the genetic toxicity of particulate exhaust extracts. Four spark-ignition engine equipped vehicles were operated on gasoline alone, 10% blends of ethanol or methanol in gasoline, and a commercially available gasohol. The tests were conducted on a chassis dynomometer and the particulate exhaust was collected on high volume filters after dilution in a tunnel. The vehicles used were a 1980 Chevrolet Citation, a 1980 Mercury Monarch, a 1981 Ford Escort and a 1981 Oldsmobile Cutlass. Dichloromethane extracts of the exhaust particles from all tests were mutagenic inSalmonella typhimurium strains TA 100 and TA 98. The extracts were less mutagenic in the nitroreductase deficient strains TA 98NR and TA 98DNPR suggesting that nitro substituted polycyclic aromatic hydrocarbons may be responsible for part of the mutagenicity. In all the alcohol blended fuel tests, the mass of particle associated organics emitted from the exhaust was lower than that observed during the control tests using gasoline alone. Thus, in most cases, estimates of the emission of mutagenic combustion products from the exhaust were lower in the alcohol blend tests.

Title: Solvent toxicity: isopropanol, methanol, and ethylene glycol.
Author(s): Smith MS.
Source: Ear Nose Throat J 1983 Mar;62(3):126-35.
Abstract: None provided.

Title: Methanol metabolism and toxicity
Author(s): Tephly, TR, Martin, KE
Source: Food Science and Technology Bulletin: Functional Foods, 1984, Vol 12, pp. 111-140
Abstract: Methanol is commonly used in industry for organic synthetic procedures or as a solvent. As a result, it is accessible to the general public in a variety of products such as antifreeze, fuels (Sterna), duplicating machine fluids, and in gasoline as a fuel extender. Methanol and other alcohols have been employed as sources of energy or fuel for many years, particularly in times of war. Methanol's use as an

Attachment O – Methanol

automobile fuel, as well as other proposed uses for energy production, will increase human methanol contact from a limited laboratory or industrial exposure to a general environments: exposure. Although methanol theoretically represents a "clean" substance capable of oxidation to water and carbon dioxide, in humans biochemical reactions produce metabolites that are clearly toxic. A consideration of the toxicity of methanol, especially in species which demonstrate signs and symptoms, seems appropriate for several reasons. First, humans are sensitive to methanol poisoning, and limits of tolerance must be considered. Second, nutritional factors may play an important role (e.g.. foate deficiency) in determining susceptibility. Our current understanding of the mechanisms involved in methanol toxicity is described.

Title: Postsynaptic actions of ethanol and methanol in crayfish neuromuscular junctions
Author(s): W. Finger and H. Stettmeier
Source: Pflügers Archiv European Journal of Physiology, 1984, Volume 400, Number 2, 113-120
Abstract: Actions of ethanol and methanol on excitatory postsynaptic channels activated by quisqualate were investigated in opener muscles from the first walking leg and the claw of crayfish. Both ethanol and methanol reduced the elementary currents I i I that flow through channels operated by quisqualate in a concentration-dependent manner but did not affect the apparent mean open time, Tnoise, of the channels estimated from power spectra. 0.26 moll ethanol, or 1 mold methanol, respectively, reduced 1i I e-fold. Ethanol also markedly decreased the size and the decay time constant r(sEPSCs) of spontaneous excitatory postsynaptic currents (sEPSCs). At ten fibres, on the average, 0.26 motif ethanol decreased -T(sEPSC5) by a factor 1.56±0.24 (SD). T(sIPSCs) and Tnoise of inhibitory postsynaptic currents apparently were not affected by ethanol. Moreover the size of elementary inhibitory postsynaptic currents did not decrease in the presence of this alcohol. Thus, in crayfish opener muscles ethanol seems to selectively depress excitatory postsynaptic currents.

Title: Role of hepatic tetrahydrofolate in the species difference in methanol toxicity.
Author(s): KA Black, JT Eells, PE Noker, CA Hawtrey, and TR Tephly
Source: Proc Natl Acad Sci U S A. 1985 June; 82(11): 3854-3858.
Abstract: The susceptibility of various species to methanol toxicity is inversely related to the rate of tetrahydrofolate (H4folate)-dependent formate oxidation to carbon dioxide. Thus, the levels of various folate derivatives and folate-dependent enzyme activities present in the livers of monkeys, which are sensitive to methanol, and rats, which are not, were compared in order to investigate the biochemical basis of this species difference. Hepatic H4folate levels in monkeys

Attachment O – Methanol

were 60% of those in rats, and formylated-H4folate derivatives were 2-fold higher in monkeys than in rats. No significant difference between monkeys and rats in the levels of total hepatic folate or 5-methyl-H4folate was observed. The activities of formyl-H4folate synthetase (EC 6.3.4.3) and formyl-H4folate dehydrogenase (EC 1.5.1.6) were 4- and 2-fold higher, respectively, in monkeys than in rats. There was no significant difference between monkeys and rats in methionine synthetase activity (EC 2.1.1.13). Dihydrofolate reductase activity (EC 1.5.1.3) in monkeys was 20% of that in rats. 5,10-Methylene-H4folate reductase (NADPH) activity (EC 1.1.1.171) in monkeys was 40% and 25% of that in rats when the rates of the forward and reverse reactions, respectively, were compared. Serine hydroxymethyltransferase activity (EC 2.1.2.1) was 2-fold higher in monkeys than in rats. The differences in the activities of methylene-H4folate reductase and serine hydroxymethyl-transferase between monkeys and rats may have contributed to the difference in hepatic H4folate levels. The 40% lower level of hepatic H4folate in monkeys, as compared to rats, relates well to the 50% lower maximal rate of formate oxidation in monkeys. Thus, the species difference in susceptibility to methanol may be explained by the difference in the level of hepatic H4folate.

Title: Neonatal behavioral toxicity in rats following prenatal exposure to methanol.
Author(s): Infurna R, Weiss B.
Source: Teratology. 1986 Jun;33(3):259-65.
Abstract: Although methanol (MEOH) may assume a significant role as a fuel, which implies wide availability, little is known of its toxicity apart from acute poisoning episodes in human adults. Even less is known about its toxicity in developing organisms. This experiment studied the early behavioral development of rats whose mothers had consumed MEOH during gestation by measuring the responses of suckling (postnatal day 1) and nest-seeking (postnatal day 10). Primigravida Long-Evans rats were divided into three groups (N = 10). Two of the groups consumed drinking solutions of 2% MEOH instead of distilled water either on gestational days 15-17 (MEOH 1) or 17-19 (MEOH 2). No maternal toxicity was apparent as measured by weight gain, gestational duration, and daily fluid intake. Daily MEOH consumption averaged 2.5 gm/kg over the 3-day period in both MEOH groups. Litter size, birth weight, and infant mortality did not differ among the three groups. Postnatal growth and date of eye opening were unaffected. MEOH pups required longer than controls to begin suckling on postnatal day 1. On postnatal day 10, they required more time to locate nesting material from their home cages. These data suggest that prenatal MEOH exposure induces behavioral abnormalities early in life that are unaccompanied by overt toxicity.

Attachment O – Methanol

Title: Subchronic inhalation toxicity of methanol.
Author(s): Andrews LS Clary JJ Terrill JB, Bolte HF.
Source: J Toxicol Environ Health. 1987;20(1-2):117-24.
Abstract: The subchronic inhalation toxicity of methanol was evaluated in rats and monkeys. Animals were exposed to 0, 500, 2000, and 5000 ppm methanol vapor for 6 h/d, 5 d/w, for 4 wk. The only treatment-and dose-related effect noted was that of mucoid nasal discharge in rats, which was considered reflective of upper respiratory tract irritation. No consistent treatment-related effects were found for organ or body weights or for histopathologic or ophthalmoscopic examinations. Overall, these findings support the use of the present American Council of Governmental Industrial Hygienists threshold limit value (TLV) of 200 ppm and short-term exposure limit (STEL) of 250 ppm for exposure to methanol vapor.

Title: The interaction of methanol, rat-liver S9 and the aromatic amine 2,4-diaminotoluene produces a new mutagenic compound.
Author(s): Cunningham ML Burka LT Matthews HB.
Source: Mutat Res. 1990 Aug;244(4):273-7.
Abstract: Methanol is a widely used solvent for organic compounds and a human toxicant. In our studies of the metabolism of aromatic amines in the Ames/Salmonella assay, we observed a rapid and quantitative conversion of the mutagenic and carcinogenic aromatic amine 2,4-diaminotoluene (2,4-DAT) to a single product. This product was only produced in the presence of methanol, and not other organic solvents. Isolation of this product showed that it was highly mutagenic in Salmonella TA98 with S9 activation. Characterization of the product of the interaction of methanol and 2,4-DAT indicated that methanol is activated to a reactive intermediate, probably formaldehyde, by the 9000 X g supernatant used in the Ames/Salmonella assay. The formaldehyde subsequently reacts with 2,4-DAT to form the mutagenic product, identified as bis-5,5'(2,4,2',4'-tetraaminotolyl)methane. Results of this study demonstrate that methanol may be an inappropriate solvent for mutation and metabolism studies of aromatic amines and possibly other chemicals, and that solvent-xenobiotic interactions may in some cases lead to the misinterpretation of results.

Title: The Toxicity of Inhaled Methanol Vapors
Author(s): Robert Kavet, Sc.D. and Kathleen M. Nauss, Ph.D.
Source: Kavet R & Nauss KM (1990) The toxicity of inhaled methanol vapors CRC Crit Rev Toxicol, 21:21-50
Abstract: Methanol could become a major automotive fuel in the U.S., and its use may result in increased exposure of the public to methanol vapor. Nearly all of the available information on methanol toxicity in humans relates to the consequences of acute, rather than chronic, exposures. Acute methanol toxicity evolves in a

Attachment O – Methanol

well-understood pattern and consists of an uncompensated metabolic acidosis with superimposed toxicity to the visual system. The toxic properties of methanol are rooted in the factors that govern both the conversion of methanol to formic acid and the subsequent metabolism of formate to carbon dioxide in the folate pathway. In short, the toxic syndrome sets in if formate generation continues at a rate that exceeds its rate of metabolism. Current evidence indicates that formate accumulation will not challenge the metabolic capacity of the folate pathway at the anticipated levels of exposure to automotive methanol vapor.

Title: The interaction of methanol, rat-liver S9 and the aromatic amine 2,4-diaminotoluene produces a new mutagenic compound
Author(s): Michael L. Cunningham, Leo T. Burka and H.B. Matthews
Source: Mutation Research Letters, Volume 244, Issue 4, August 1990, Pages 273-277
Abstract: Methanol is a widely used solvent for organic compounds and a human toxicant. In our studies of the metabolism of aromatic amines in the Ames/Salmonella assay, we observed a rapid and quantitative conversion of the mutagenic and carcinogenic aromatic amine 2,4-diaminotoluene (2,4-DAT) to a single product. This product was only produced in the presence of methanol, and not other organic solvents. Isolation of this product showed that it was highly mutagenic in Salmonella TA98 with S9 activation. Characterization of the product of the interaction of methanol and 2,4-DAT indicated that methanol is activated to a reactive intermediate, probably formaldehyde, by the 9000 x g supernatant used in the Ames/Salmonella assay. The formaldehyde subsequently reacts with 2,4-DAT to form the mutagenic product, identified as bis-5,5' (2,4,2',4'-tetraaminotolyl)methane. Results of this study demonstrate that methanol may be an inappropriate solvent for mutation and metabolism studies of aromatic amines and possibly other chemicals, and that solvent-xenobiotic interactions may in some cases lead to the misinterpretation of results.

Title: Impact of methanol and CNG fuels on motor-vehicle toxic emissions
Author(s): Black, F, Gabele, P
Source: Environmental Protection Agency, 1991 Jan 01, Technical Report No. PB-92-110378/XAB
Abstract: The 1990 Clean Air Act Amendments require that the Environmental Protection Agency investigate the need for reduction of motor vehicle toxic emissions such as formaldehyde, acetaldehyde, benzene, 1,3-butadiene, and polycyclic organic matter. Toxic organic emissions can be reduced by utilizing the control technologies employed for regulated THC (NMHC) and CO emissions, and by changing fuel composition. The paper examines emissions associated with the use of methanol and compressed natural gas fuels. Both tailpipe and evaporative emissions are examined at varied ambient temperatures ranging from 20 C to

Attachment O – Methanol

105 F. Tailpipe , emissions are also examined over a variety of driving cycles with average speeds ranging from 7 to 48 mph. Results suggest that an equivalent ambient temperatures and average speeds, motor vehicle toxic emissions are generally reduced with methanol and compressed natural gas fuels relative to those with gasoline, except for formaldehyde emissions, which may be elevated. As with gasoline, tailpipe toxic emissions with methanol and compressed natural gas fuels generally increase when ambient temperature or average speed decreases (the sensitivity to these variables is greater with methanol than with compressed natural gas). Evaporative emissions generally increase when fuel volatility or ambient temperature increases (however, the relative contribution of evaporative sources to the aggregate toxic compound emissions is small).

Title: Effect of ethanol and methanol on the motility of Saccostrea commercialis sperm and sperm models

Author(s): Dr. F. C. Molinia, M. A. Swan

Source: Molecular Reproduction and Development, Volume 30, Issue 3, pages 241-249, November 1991

Abstract: The organic solvents methanol and ethanol at concentrations of 2.5% and 5% (v/v), respectively, were found to significantly ($P < 0.001$) decrease the radius of curvature and track velocity of S. commercialis sperm. To observe the effects of the solvent directly on the axoneme, S. commercialis sperm models were prepared by extraction with Triton X-100 and reactivation with ATP in media containing acetate anions, DTT, magnesium, and CAMP. Concentrations of 0.1% Triton X-100 demembranated sperm while 0.01% and 0.05% Triton X-100 permeabilized sperm. Sperm models were successfully produced after reactivation with 1 mM ATP. At pH 8.25, 1% (v/v) ethanol or methanol was observed to increase waveform asymmetry and significantly ($P < 0.001$) decrease track velocity of 0.1% Triton X-100 demembranated sperm models. Similarly 1% (v/v) ethanol increased tailwave asymmetry and decreased track velocity of 0.01% and 0.05% Triton X-100 permeabilized sperm models. Reactivated motility of 0.05% Triton X-100 permeabilized sperm models prepared at pH 7.8 were poor and improved after treatment with 7% (v/v) ethanol, which increased waveform asymmetry and doubled the track velocity of sperm. This stimulatory effect of ethanol was unchanged in the presence of the alcohol dehydrogenase inhibitor pyrazole. Concerning the precise mechanism of action of ethanol on the axoneme, we conclude that a stimulatory or inhibitory effect of ethanol is dependent on the pH of the sperm model system used.

Title: The toxicity of methanol.

Author(s): Teo TR.

Attachment O – Methanol

Source: Life Sci, 1991;48(11):1031-41.

Abstract: Methanol toxicity in humans and monkeys is characterized by a latent period of many hours followed by a metabolic acidosis and ocular toxicity. This is not observed in most lower animals. The metabolic acidosis and blindness is apparently due to formic acid accumulation in humans and monkeys, a feature not seen in lower animals. The accumulation of formate is due to a deficiency in formate metabolism which is, in turn, related, in part, to low hepatic tetrahydrofolate (H4 folate). An excellent correlation between hepatic H4 folate and formate oxidation rates has been shown within and across species. Thus, humans and monkeys possess low hepatic H4 folate levels, low rates of formate oxidation and accumulation of formate after methanol. Formate, itself, produces blindness in monkeys in the absence of metabolic acidosis. In addition to low hepatic H4 folate concentrations, monkeys and humans also have low hepatic 10- formyl H4 folate dehydrogenase levels, the enzyme which is the ultimate catalyst for conversion of formate to carbon dioxide. This review presents the basis for the role of folic acid-dependent reactions in the regulation of methanol toxicity.

Title: Methanol-induced visual toxicity in the rat.

Author(s): Eelis JT.

Source: J Pharmacol Exp Ther. 1991 Apr;257(1):56-63.

Abstract: Human methanol poisoning is characterized by formic acidemia, metabolic acidosis and blindness or serious visual impairment. Nonprimate species are ordinarily resistant to the accumulation of formate and the associated metabolic and visual toxicity. A nonprimate model of methanol-induced visual toxicity was developed using rats treated with subanesthetic concentrations of nitrous oxide to inhibit the oxidation of methanol's toxic metabolite, formic acid. Methanol - intoxicated rats developed formic acidemia, metabolic acidosis and visual toxicity within 36 hr of methanol administration analogous to the human methanol poisoning syndrome. Visual dysfunction was measured as reductions in the flash-evoked cortical potential and electroretinogram, which occurred coincident with blood formate accumulation. Alterations in the electroretinogram occurred at formate concentrations lower than those associated with other visual changes and provide functional evidence of direct retinal toxicity in methanol poisoning.

Title: Methanol and formic acid toxicity: biochemical mechanisms.

Author(s): Liesivuori J, Savolainen H.

Source: Pharmacol Toxioal. 1991 Sep,69(3):157-63.

Abstract: Metabolism of methanol, methyl ethers, esters and amides give rise to formic acid. This acid is an inhibitor of the mitochondria! cytochrome oxidase causing histotoxic hypoxia. Formic acid is a weaker inhibitor than cyanide and

Attachment O – Methanol

hydrosulphide anions. The body burden of formate in methanol poisoning is high enough to cause acidosis, and other clinical symptoms. Part of the protons can be attributed to formic acid whereas the most significant acid load results from the hypoxic metabolism. The acidosis causes e.g. dilatation of cerebral vessels, facilitation of the entry of calcium ions into cells, loss of lysosomal latency and deranged production of ATP, The latter effect seems to impede parathormone-dependent calcium reabsorption in the kidney tubules. Besides, urinary acidification is affected by formic acid. Its excretion causes continuous recycling of the acid by the tubular cell Cl-/formate exchanger. This sequence of events may partially explain an accumulation of formate in urine. Occupational exposure to vapours of methanol and formic acid can be quantitatively monitored by urinary formic acid determinations. Formic acid toxicity may prove a suitable model for agents causing histotoxic hypoxia.

Title: A case of percutaneous industrial methanol toxicity.
Author(s): Downie A Khattab TM, Malik MI, Samara IN.
Source: Occup Med (Londj. 1992 Feb;42(1):47-9.
Abstract: Methanol (CH3OH) is a chemical feedstock of increasing importance as well as a commonly used solvent. In the early 1980s methanol production was introduced at a new petrochemical complex in the Saudi port of Jubail. A case is presented of a consultant supervising tank cleaning prior to methanol loading. He wore positive pressure breathing apparatus but no protective clothing. After 2-3 hours working in the confined space of the tank, he worked on deck and continued to wear his methanol-soaked clothing which eventually dried out. Visual symptoms of acute methanol toxicity presented some 8 hours after exposure. The appropriate treatment (with ethanol provided by the ship bond) was carried out in hospital and the individual recovered completely. Most reported cases of methanol toxicity are social in origin, arising from ingestion. This particular case, though unusual, does present some interesting lessons.

Title: Toxicity to rats of methanol-fueled engine exhaust inhaled continuously for 28 days.
Author(s): Maejima K, Suzuki T Niwa K, Numata H, Maekawa A, Naoase 8, Ishinishi N.
Source: J Toxicol Environ Health. 1992 Oct;37(2):293-312.
Abstract: Fischer 344 rats were exposed to three concentrations of exhaust generated by an M85 methanol-fueled engine (methanol with 15% gasoline) without catalyst for 8 h/d, 7 d/wk for 7, 14, 21, or 28 d. Concentration- and time-dependent yellowing of the fur was prominent in all treated groups. Concentration-dependent increases in the erythrocyte count, hematocrit, hemoglobin concentration, formaldehyde in plasma, and carboxyhemoglobin in the erythrocytes, and decrease in serum alkaline phosphatase activity were seen

Attachment O – Methanol

after all exposure periods. Histopathologically, lesions were found in the nasal cavity and lungs after 7 d of exposure. Squamous metaplasia of the respiratory epithelium of level 1 (level of the posterior edge of the upper incisor teeth) lining of the nasoturbinate and/or maxilloturbinate and infiltration of neutrophils into the submucosa, and decreases of Clara cells in the terminal bronchiolus and of cilia in the bronchiolar epithelium, were observed in the high-concentration group (carbon monoxide, 94 ppm; formaldehyde, 6.9 ppm; methanol, 17.9 ppm; nitrogen oxides, 52.7 ppm; nitrogen dioxide, 10.6 ppm). The histopathological extents of several lesions increased slightly with the exposure time. Slight squamous metaplasia and hyperplasia of the respiratory epithelium at level 1 were also observed in the medium-concentration group (one in three of the high-concentration group). No histopathological changes were found in the olfactory epithelium of the nasal cavity. In the low-concentration group (one in nine of the high-concentration group), no marked histopathological changes in these organs were observed. These results may suggest that the lesions observed in the nasal cavity of rats exposed to methanol-fueled engine exhaust were mainly caused by formaldehyde, although other components in the exhaust also may have affected nasal cavity and/or lungs to less extent.

Title: Methanol toxicity. Agency for Toxic Substances and Disease Registry.
Author(s): None listed.
Source: Am Fam Physician. 1993 Jan;47(1):163-71.
Abstract: Methanol is used in a variety of commercial and consumer products. Increased use of methanol as a motor fuel may lead to higher ambient air levels and a greater potential for ingestion from siphoning accidents. Methanol toxicity initially is not characterized by severe toxic manifestations. Pathophysiologically, methanol toxicity represents a classic example of "lethal synthesis," in which toxic metabolites can cause fatality after a characteristic latent period. Methanol is well absorbed following inhalation, ingestion or cutaneous exposure. It is oxidized in the liver to formaldehyde, then to formic acid, which contributes to the profound metabolic acidosis occurring in acute methanol poisoning. The metabolic products of methanol can produce a syndrome of delayed-onset acidosis, obtundation, visual disturbance and death. Intravenous sodium bicarbonate therapy should be considered if the patients blood pH is below 7.2. Symptoms and history determine whether intravenous ethanol therapy and hemodialysis should be instituted.

Title: The developmental toxicity of inhaled methanol in the CD-1 mouse, with quantitative dose-response modeling for estimation of benchmark doses.
Author(s): Rogers JM, Mole ML, Chernoff N, Barbee BD, Turner CI, Logsdon TR, Kavlock RJ.

Attachment O – Methanol

Source: Teratology. 1993 Mar;47(3):175-88.

Abstract: The developmental toxicity of the alternative motor vehicle fuel methanol was assessed in mice by the inhalation route. Pregnant CD-1 mice were exposed to 1,000, 2,000, 5,000, 7,500, 10,000, or 15,000 ppm methanol for 7 hr/day on days 6 -15 of gestation. Sham-exposed controls were exposed to filtered air under similar conditions. Additional control groups were left in their home cages either unhandled or food-deprived for 7 hr/day to match the food deprivation experienced by the exposed mice. Dams were observed twice daily and weighed on alternate days during the exposure period. Blood methanol concentrations were determined in some mice on gestation days 6, 10, and 15. On day 17, the remaining mice were weighed and killed and the gravid uteri removed. Implantation sites, live and dead fetuses and resorptions were counted, fetuses were examined externally and weighed as a litter. Half of each litter was examined for skeletal morphology and the other half of each litter was examined for internal soft tissue anomalies. One dam died in each of the 7,500, 10,000, and 15,000 ppm methanol exposure groups, but no dose-response relationship was evident for maternal death. The sham-exposed and food-deprived controls as well as all methanol exposed dams gained less weight than did unexposed dams fed ad libitum, but methanol did not exacerbate this effect. Significant increases in the incidence of exencephaly and cleft palate were observed at 5,000 ppm and above, increased embryo/fetal death at 7,500 ppm and above (including an increasing incidence of full-litter resorptions), and reduced fetal weight at 10,000 ppm and above. A dose-related increase in cervical ribs or ossification sites lateral to the seventh cervical vertebra was significant at 2,000 ppm and above. Thus, the NOAEL for the developmental toxicity in this study was 1,000 ppm. A log-logistic dose response model was applied to the incidence data for exencephaly, cleft palate, resorption and cervical rib, and maximum likelihood estimates (MLEs) and benchmark dosages (BDs, the lower 95% confidence interval of the MLEs) corresponding to 1% and 5% added risk above background were calculated. The MLE for 5% added combined risk of having either exencephaly or cleft palate or being resorbed was 3667 ppm, and the corresponding BD was 3,078 ppm. For cervical rib, the 5% added risk values for the MLE and BD were 824 and 305 ppm, respectively. The BDs for 1% added risk were 1915 ppm for exencephaly, cleft palate or resorption, and 58 ppm for cervical rib.(ABSTRACT TRUNCATED AT 400 WORDS)

Title: Methanol intoxication. How to help patients who have been exposed to toxic solvents.

Author(s): Pamies RJ, Sugar D, Rives LA Herold AH.

Source: Postgrad Med. 1993 Jun;93(8):183-4, 189-91, 194.

Abstract: Methanol intoxication can be a challenge, in part because it is relatively

Attachment O – Methanol

uncommon but also because of the pharmacokinetics involved. A patient may not experience symptoms and thus may not present for treatment for several hours, or even a day or two, after exposure to the toxic substance. Yet, the interval between ingestion and treatment is one of the most important factors in determining patient outcome. Typical symptoms of methanol intoxication include lethargy, vertigo, vomiting, blurred vision, and decreased visual acuity. Treatment focuses on prevention of methanol conversion to its toxic metabolites, correction of metabolic acidosis, and elimination of the toxic substances from the system. Ethanol and bicarbonate administration and hemodialysis have been effective.

Title: Developmental toxicity of methanol in whole embryo culture: a comparative study with mouse and rat embryos.

Author(s): Andrews JE, Ebron-McCoy M, Logsdon TR, Mole LM, Kavlock RJ, Rogers JM.

Source: Toxicology. 1993 Aug 27;81(3):205-15.

Abstract: Methanol (MeOH), a widely used industrial solvent, has been proposed as an alternative motor vehicle fuel. Inhaled Me0H is developmentally toxic in both rats and mice but the mouse is more sensitive than is the rat. The contribution of the embryo to this differential sensitivity was studied in whole embryo culture (WEC) using equivalent stage rat (day 9) and mouse (day 8) embryos (plug day = day 0). Rat embryos were explanted and cultured in 0, 2, 4, 8, 12 or 16 mg Me0H/m1 rat serum for 24 h and then transferred to rat serum alone for 24 h. Embryonic development of the 2 and 4 mg Me0H/mlgroups was not significantly different from the controls whereas the higher concentrations resulted in a concentration related decrease in somite number, head length and developmental score. The 12 mg/ml dose resulted in some embryolethality as well as dysmorphogenesis, while the highest dose was embryolethal. Me0H was dysmorphogenic in vitro in rat embryos at a Me0H concentration comparable to that reported in maternal serum following teratogenic in vivo exposures. Day 8 mouse embryos were explanted and cultured in 0, 2, 4, 6 or 8 mg Me0H/mIculture medium (75% rat serum, 25% Tyrode's salt solution) for 24 h. Embryonic development in the 2 mg/ml Me0H group was not significantly different from the controls but all higher concentration groups had a significant decrease in developmental score and crown-rump length. The high concentration group also suffered 80% embryolethality. Thus, mouse embryos were affected at Me0H concentrations which were not dysmorphogenic or embryotoxic in the rat, suggesting that the higher sensitivity of the mouse to the developmental toxicity of inhaled methanol is due, at least in part, to greater intrinsic embryonal sensitivity of this species to methanol.

Attachment O – Methanol

Title: Marked variability in methanol toxicity.
Author(s): Roeggla G, Wagner A, Frossard M Roegdla H.
Source: Am Fam Physician. 1993 Oct;48(5):731.
Abstract: None provided.

Title: Phase-specific developmental toxicity in mice following maternal methanol inhalation.
Author(s): Bolon B, Dorman DC, Janszen 0, Morgan KT, Welsch F.
Source: Fundam Appl Toxicol. 1993 Nov;21(4):508-16.
Abstract: Methanol is toxic to embryos of mice and rats when inhaled by dams at high concentrations. The present studies examined methanol-induced developmental toxicity following inhalation exposure (6 hr/day) of pregnant CD-1 mice to 5000, 10,000 or 15,000 ppm either throughout organogenesis (GD 6-15), during the period of neural tube development and closure (GD 7-9), or during a time of potential neural tube reopening (GD 9-11). Transient neurologic signs and reduced body weights were observed in up to 20% of dams exposed to 15,000 ppm. Examination of near-term fetuses revealed embryotoxicity (increased resorptions, reduced fetal weights, and/or fetal malformations) at 10,000 and 15,000 ppm, while 3-day exposures at 5000 ppm yielded no observable adverse effects. Terata included neural and ocular defects, cleft palate, hydronephrosis, deformed tails, and limb (paw and digit) anomalies. Neural tube defects and ocular lesions occurred after methanol inhalation between GD 7-9, while limb anomalies were induced only during GD 9-11; cleft palate and hydronephrosis were observed after exposure during either period. These findings were consistent with prior reports that maternal methanol inhalation at high levels induces developmental toxicity in a concentration-dependent manner. Furthermore, our data indicate that the spectrum of teratogenic effects depended upon both the timing (i.e., stage of embryonic development) and the number of methanol exposures.

Title: Inhalational and percutaneous methanol toxicity in two firefighters.
Author(s): Aufderheide TP, White SM, Brady WJ, Stueven HA.
Source: Ann Emerg Med. 1993 Dec;22(12):1916-8.
Abstract: We present two cases of adult inhalational and percutaneous methanol toxicity resulting from transient exposure to vaporized methanol. Both patients complained only of a mild headache at the time of the emergency department evaluation and had normal physical examinations, normal anion gaps, and peak methanol levels of 23 and 16 mg/dL, respectively. Emergency physicians should recognize the potential for toxic transcutaneous absorption of methanol. Because of the varying relationship between clinical symptoms, physical examination findings, and anion gap values to potentially toxic methanol exposures,

Attachment O – Methanol

acquisition of empiric serum methanol levels appears warranted in appropriate situations.

Title: Methanol inhalation toxicity.
Author(s): Frenia ML, Schauben JL.
Source: Ann Emerg Med. 1993 Dec;22(12):1919-23.
Abstract: Significant toxicity can result from intentional methanol inhalation. We report seven cases, involving four patients, of intentional inhalation of GARB-MEDIC carburetor cleaner containing toluene (43.8%), methanol (23.2%), methylene chloride (20.5%), and propane (12.5%). Patients arrived at the emergency department with central nervous system depression, nausea, vomiting, shortness of breath, photophobia, and/or decreased visual acuity. Treatment included correction of acidosis, leucovorin and/or folic acid, ethanol infusions, and supportive care. Hemodialysis was necessary in three cases. Measured blood methanol levels ranged from 50.4 to 128.6 mg/dL. Blood formic acid levels were 120, 193, and 480 micrograms/mL, respectively, in three patients. Ophthalmic examinations revealed hyperemic discs and decreased visual acuity in one patient. One individual was found pulseless with several GARB-MEDIC cans nearby. Attempts at revival were unsuccessful. Clinicians should be aware that significant blood methanol and formic acid levels may occur after inhalation of methanol.

Title: Animal model for the study of methanol toxicity: comparison of folate-reduced rat responses with published monkey data.
Author(s): Lee EW, Garner CD, Terzo TS.
Source: J Toxicol Environ Health. 1994 Jan;41(1):71-82.
Abstract: We attempted to develop a rodent model that exhibits characteristics of human methanol toxicities such as acidosis and visual dysfunction, which are correlated with an accumulation of formate, a toxic metabolite of methanol. Initially three groups of Long-Evans rats with different levels of liver folate were prepared and examined for formate accumulation after methanol administration (3.5 g/kg). The folate-reduced (FR) rats prepared by feeding a folate-deficient diet with 1% succinylsulfathiazole yielded blood formate levels equivalent to those found in methanol-intoxicated humans and developed signs of the visual system toxicity (a manuscript on the latter aspect is in preparation). Responses of FR rats to a variety of methanol exposure scenarios were then investigated, and the results were compared with those reported in the literature for monkeys. Formate accumulation and/or lethality were used as toxic parameters for this comparative evaluation. In FR rats dosed orally with 3 g/kg, the blood formate concentration was 9.2 mmol/L at 24 h postadministration and increased to 15.6 mmol/L at 48 h. The same dose given to monkeys yielded a plateau of 7.4 mmol/L at 12 h after

Attachment O – Methanol

methanol administration, and stayed at this level for an additional 12 h. The area under the concentration vs. time curve for blood formate in FR rats was 2.5-fold greater than that in monkeys when 2.0 g/kg methanol was administered. After a 6-h exposure to 1200 ppm and 2000 ppm methanol, the blood formate concentrations in FR rats were increased by 370% and 636% above the endogenous level, respectively. However, blood formate did not accumulate above the endogenous level when monkeys were exposed to methanol up to 2000 ppm for 6 h. Under acute inhalation exposure conditions, FR rats exposed to 3000 ppm methanol, 20 h/d, could not survive more than 4 d. On the other hand, monkeys exposed to 3000 ppm, 21 h/d, out-lived 20 d. Moreover, monkeys survived for more than 4 d even after an exposure to 10,000 ppm. Thus, these results indicate that FR rats are more sensitive to methanol challenges than monkeys, and suggest that the FR rat could be a congruous animal model for evaluating the health effects of methanol in humans.

Title: Subchronic (12-week) inhalation toxicity study of methanol-fueled engine exhaust in rats.

Author(s): Maelima K, Suzuki T, Numata H, Maekawa A, Naciase S, Ishinishi N.

Source: J Toxicol Environ Health. 1994 Mar;41(3):315-27.

Abstract: To evaluate the inhalation toxicity to rats of exhaust at low concentration for longer periods, Fischer 344 rats were exposed to 3 concentrations of exhaust generated by an M85 methanol-fueled engine (methanol with 15% gasoline) without catalyst for 8 h/d, 6 d/wk for 4, 8, or 12 wk. Concentration- and time-dependent increase carboxyhemoglobin in the erythrocytes and decrease in cytochrome P-450 in the lungs were observed in all treated groups. Furthermore, significant increases in plasma formaldehyde were observed in all treated groups. Furthermore, significant increases in plasma formaldehyde were observed in the group exposed to the highest concentration of exhaust (carbon monoxide, 89.8 ppm; formaldehyde, 2.3 ppm; methanol, 8.1 ppm; nitrogen oxides, 22.9 ppm; nitrogen dioxide, 1.1 ppm) for 8 or 12 wk. No change of plasma folic acid was observed in any group, and no methanol or formic acid was detected in the plasma in any animals. Histopathologically, exposure-related changes were found only in the nasal cavity of the high-concentration group. Slight hyperplasia/squamous metaplasias of the respiratory epithelium lining the nasoturbinate and maxilloturbinate were observed after 4 wk of exposure, and the incidences and degrees of these lesions increased slightly with the exposure time. No changes were found in the olfactory epithelium of the nasal cavity. As judged by optical microscopy, the exhaust concentration with no effect on the nasal cavity under the experimental conditions was concluded to be the medium concentration level containing 0.55 ppm formaldehyde. In the present study, however, concentration- and time- dependent increase of carboxyhemoglobin in

Attachment O – Methanol

the erythrocytes and decrease of the lung P-450 level were observed. Therefore, further study on more long-term inhalation of lower concentrations of exhaust might be needed.

Title: Inhalation toxicity study of methanol, toluene, and methanol/toluene mixtures in rats: effects of 28-day exposure.
Author(s): Poon R, Chu I, Biarnason S Potvin M, Vincent R Miller RB Valli VE.
Source: Toxicol Ind Health, 1994 May-Jun;10(3):231-45.
Abstract: The inhalation toxicity of methanol and toluene was investigated in rats. Young Sprague Dawley rats of both sexes were exposed to vapors of methanol (300 ppm, 3000 ppm), toluene (30 ppm, 300 ppm) or methanol/toluene (300/30 ppm, 300/300 ppm, 3000/30 ppm, and 3000/300 ppm) six hrs per day, five days/week for four weeks. Control animals inhaled air only. Increased serum alkaline phosphatase activity was observed in males exposed to high-dose toluene, and decreased creatinine was noted in the group exposed to high-dose methanol/toluene. The thyroid gland in females appeared to be a target organ for inhaled methanol, toluene, and methanol/toluene, although the changes were confined to a mild, and occasionally moderate, reduction in follicle size. Histopathological changes of the nasal passages, consisting of subepithelial nonsuppurative inflammation, occurred in higher incidences in rats exposed to methanol/toluene than in those exposed to the individual vapors. Inhalation of methanol, toluene, or methanol/toluene produced no changes in liver weights, hepatic mixed-function oxidases, or serum aspartate transaminase activities, and onlly minimal changes in liver histopathology. The only liver changes were decreased liver weight and increased cytoplasmic density of the periportal areas in females exposed to high-dose methanol/toluene. These data indicated that exposure to methanol, toluene, or a mixture of both produced mild biochemical effects and histological changes in the thyroid and nasal passage. No apparent interactive effects were observed.

Title: Breath monitoring of inhalation and dermal methanol exposure
Author(s): Franzblau, A, Batterman, S, D'Arcy, JB, Sargent, NE, Gross, KB, Schreck, RM
Source: Applied Occupational & Environmental Hygiene, Vol. 10, no. 10, pp. 833-839. 1995.
Abstract: A fundamental assumption of monitoring breath for a toxicant is that the concentration of the toxicant in breath is proportional to the concentration in blood. The present study was designed, in part, to assess the conditions under which measurement of methanol in breath would be useful for estimating the blood concentration of methanol following inhalation or dermal exposures to methanol. Paid volunteer subjects underwent controlled inhalation exposure to methanol vapor at various concentrations for 8 hours, or dermal exposures

Attachment O – Methanol

(without inhalation exposure) to methanol for varying periods of time. Blood and end-expiratory air were analyzed for methanol from samples obtained prior to exposures, and at various times during and after exposures. The results demonstrate that blood and breath concentrations of methanol are disproportional for varying periods of time during and following cessation of methanol exposure, depending on the route of exposure (dermal versus inhalation).

Title: Short-term inhalation toxicity of methanol, gasoline, and methanol/gasoline in the rat.

Author(s): Poon R, Chu I, Bjarnason S, Vincent R, Potvin M, Miller RB, Valli VE.

Source: Toxicol Ind Health. 1995 May-Jun;11(3):343-61.

Abstract: Four- to five-week-old male and female Sprague Dawley rats were exposed to vapors of methanol (2500 ppm), gasoline (3200 ppm), and methanol/gasoline (2500/3200 ppm, 570/3200 ppm) six hours per day, five days per week for four weeks. Control animals were exposed to filtered room air only. Depression in body weight gain and reduced food consumption were observed in male rats, and increased relative liver weight was detected in rats of both sexes exposed to gasoline or methanol/gasoline mixtures. Rats of both sexes exposed to methanol/gasoline mixtures had increased relative kidney weight and females exposed to gasoline and methanol/gasoline mixtures had increased kidney weight. Decreased serum glucose and cholesterol were detected in male rats exposed to gasoline and methanol/gasoline mixtures. Decreased hemoglobin was observed in females inhaling vapors of gasoline and methanol/gasoline at 570/3200 ppm. Urine from rats inhaling gasoline or methanol/gasoline mixtures had up to a fourfold increase in hippuric acid, a biomarker of exposure to the toluene constituent of gasoline, and up to a sixfold elevation in ascorbic acid, a noninvasive biomarker of hepatic response. Hepatic mixed-function oxidase (aniline hydroxylase, aminopyrine Ndemethylase and ethoxyresorufin 0-deethylase) activities and UDP-glucuronosyltransferase activity were elevated in rats exposed to gasoline and methanol/gasoline mixtures. Histopathological changes were confined to very mild changes in the nasal passages and in the uterus, where decreased incidence or absence of mucosal and myometrial eosinophilia was observed in females inhaling gasoline and methanol/gasoline at 570/3200 ppm. It was concluded that gasoline was largely responsible for the adverse effects, the most significant of which included depression in weight gain in the males, increased liver weight and hepatic microsomal enzyme activities in both sexes, and suppression of uterine eosinophilia. No apparent interactive effects between methanol and gasoline were observed.

Attachment O – Methanol

Title: Recent developments in methanol toxicity.
Author(s): Medinsky MA, Dorman DC.
Source: Toxicol Lett. 1995 Dec;82-83:707-11.
Abstract: The disposition of methanol and its putative toxic metabolite formate has been studied in humans, non-human primates, and rodents after exposure to high, neurotoxic doses. The rate at which rodents detoxify formate is more rapid than that of primates. Formate, an endogenous biological substrate, is detoxified by metabolism to CO2 via a tetrahydrofolate-(THF) dependent pathway. Species with high hepatic THF levels, such as rodents, are less sensitive to the neurotoxic effects of large methanol doses compared with species with low THF levels, such as primates. Data on the capacity of primates to detoxify formate derived from inhalation of low levels of methanol are critical for assessing human risk from methanol fuels. Female cynomolg us monkeys exposed to low concentrations of [14C]methanol (10-200 ppm) for 2 h have blood levels of methanol-derived formate that are 100- to 1000-fold lower than endogenous levels of formate. Healthy human volunteers exposed at rest or during exercise to 200 ppm methanol for 6 h or exposed to 20 mg/kg orally have elevated blood levels of methanol, but blood formate concentrations are not significantly increased above endogenous concentrations. Deficiencies in THF may prolong blood levels of formate and increase the likelihood of toxic effects. Limited studies in non-human primates with low THF levels exposed to 900 ppm methanol for 2 h have shown that concentrations of methanol-derived formate in blood remain below endogenous levels. Thus human populations may not be at added risk of neurotoxic effects resulting from exposure to low levels of methanol.

Title: Comparative Toxicokinetics of Inhaled Methanol in the Female CD-1 Mouse and Sprague-Dawley Rat
Author(s): Robert A. Perkins, Keith W. Ward and Gary M. Pollack
Source: Fundamental and Applied Toxicology Volume 28, Issue 2, December 1995, Pages 245-254
Abstract: Female CD-1 mice were exposed for 8 hr, both individually and in groups of eight to nine, to 2500, 5000, and 10,000 ppm methanol vapor in a flowthrough exposure chamber. The ventilation of individually exposed mice and the absorption of methanol from the chamber airstream were measured. The extraction of methanol from the airstream and the blood methanol concentration at various time points during and following exposure were determined for the group-exposed mice. The similarity of systemic kinetic parameters (volume of distribution; Michaelis-Menten elimination parameters, V_{max} and K_M) between inhalation exposure and iv and po routes of administration was verified. Total 8-hr ventilation decreased slightly with increasing exposure concentration. The

Attachment O – Methanol

fraction of inhaled methanol absorbed (0.85 ± 0.14) did not vary statistically with exposure concentration. Measured ventilation, fractional absorption, and systemic kinetic parameters were combined in a semiphysiologic pharmacokinetic model that yielded accurate predictions of blood methanol concentrations during and after an 8-hr exposure. Model predictions for the mouse were compared to a previously developed inhalation toxicokinetic model for the rat. The comparison demonstrated that at similar methanol vapor concentrations, mice evidenced a two- to threefold higher blood methanol concentration than rats, despite the fact that the apparent Vmax for methanol elimination in the mouse is twofold larger than that in the rat. These data may have significant implications in understanding species differences in methanol-induced teratogenic effects.

Title: Comparative toxicokinetics of methanol in the female mouse and rat.
Author(s): Ward KW, Perkins RA, Kawagoe JL, Pollack GM.
Source: Fundam Appl Toxicol. 1995 Jul;26(2):258-64.
Abstract: The toxicokinetics of methanol in female CD-1 mice and Sprague-Dawley rats were examined to explore the possibility of species differences in the disposition of the compound. Mice received a single dose of 2.5 g/kg methanol either po (by gavage) or i.v. (as a 1-min infusion). Rats received a single oral dose of 2.5 g/kg methanol. As expected, the disposition of methanol was nonlinear in both species. Data obtained after i.v. administration of methanol to mice were well described by a one-compartment model with Michaelis-Menten elimination. Blood methanol concentration--time data after oral administration could be described by a one-compartment (mice) or two-compartment (rats) model with Michaelis-Menten elimination from the central compartment and biphasic absorption from the gastrointestinal tract. Kinetic parameters (Vmax for elimination, apparent volume of the central compartment [Vc], first-order rate constants for intercompartmental transfer [k12 and k21], and first-order absorption rate constants for fast [kAF] and slow [kAS] absorption processes) were compared between species. When normalized for body weight, mice evidenced a higher maximal elimination rate than rats (Vmax = 117 +/- 3 mg/hr/kg vs 60.7 +/- 1.4 mg/hr/kg for rats). The contribution of the fast absorption process to overall methanol absorption also larger in the mouse than in the rat.

Title: Time-resolved cutaneous absorption and permeation rates of methanol in human volunteers.
Author(s): Batterman SA, Franzblau A.
Source: Int Arch Occup Environ Health. 1997;70(5):341-51.
Abstract: This paper reports on an experimental study of dermal exposure to neat methanol in human volunteers for the purposes of estimating percutaneous

Attachment O – Methanol

absorption rates, permeation kinetics, baseline (pre-exposure) levels of methanol in blood, and inter- and intrasubject variability. A total of 12 volunteers (seven men and five women) were exposed to methanol via one hand for durations of 0 to 16 min in a total of 65 sessions, making this the largest controlled study of percutaneous absorption for this common solvent. In each session, 14 blood samples were collected sequentially and analyzed for methanol. These data were used to derive absorption rates and delivery kinetics using a two compartment model that accounts for elimination and pre-exposure levels. The pre-exposure methanol concentration in blood was 1.7 +/- 0.9 mg 1(-1), and subjects had statistically different mean concentrations. The maximum methanol concentration in blood was reached 1.9 +/- 1.0 h after exposure. Delivery rates from skin into blood lagged exposure by 0.5 h, and methanol continued to enter the systemic circulation for 4 h following exposure. While in vitro studies have reported comparable lag times, the prolonged permeation or epidermal reservoir effect for such miscible solvents has not been previously measured. The mean derived absorption rate, 8.1 +/- 3.7 mg cm-2 h-1, is compatible with that found in the other in vivo study of methanol absorption. Both in vivo absorption rate estimates considerably exceed in vitro estimates. The maximum concentration of methanol in blood following an exposure to one hand lasting approximately 20 min is comparable to that reached following inhalational exposures at a methanol concentration of 200 ppm, the threshold limit value-time weighted average (TLV-TWA). While variability in blood concentrations and absorption rates approached a factor of two, differences between individuals were not statistically significant. The derived absorption and permeation rates provide information regarding kinetics and absorbed dose that can help to interpret biological monitoring data and confirm mathematical models of chemical permeation.

Title: Mutagenic effect of methanol in gas station operators from Sao Paulo/Brazil

Author(s): Gattas, GJF, Cardoso, LA, Faria, MM [Ethics and Occupational Medicine-FMUSP, Sao Paulo (Brazil)] [and others]

Source: Environmental and Molecular Mutagenesis; 1997, Volume 29; Issue: Suppl.28; Conference: 28.

Abstract: The frequency of micronucleus (MN) in oral scamous cells of 76 gas station operators was investigated. Subjects were exposed to a fuel called MEG a mix of 33% methanol, 60% ethanol and 7% gasoline. In Brazil the ethyl alcohol, extracted from sugar cane have been used as a fuel since 70's. In November 1991, for economical reasons, the MEG fuel was introduced in big cities. The MN frequency was evaluated in three different periods: before MEG introduction (1989), and twice after MEG utilization (1992 and 1995). Some individuals were analyzed more than one time. For each individual the frequency of MN was performed in 2000 oral cells. Statistical analysis through non parametric tests

Attachment O – Methanol

revealed a highly significant increase (P=0.001) in the frequency of MN before (1.38/2000 cells) and immediately after methanol introduction (3.0/2000 cells). The frequency of MN returned to be normal (1.4/2000 cells), in the third evaluation when the exposition to methanol decreased. It should represent genetic risk for individuals under occupational exposure and for the population as a whole when methanol has been introduced as a large-scale fuel.

Title: Critical periods of sensitivity to the developmental toxicity of inhaled methanol in the CD-1 mouse.

Author(s): Rogers JM, Mole ML.

Source: Teratology. 1997 Jun;55(6):364-72.

Abstract: Exposure of pregnant CD-1 mice to methanol (MeOH) by inhalation on gestation days (gd) 6-15 results in dose-related increases in fetal cleft palate, exencephaly, and skeletal defects. Here, critical periods for the developmental toxicity of Me0H were assessed in pregnant CD-1 mice exposed to 10,000 ppm Me0H or filtered air for 7 hr/day on 2 consecutive days during gd 6-13, or to single day (7 hr) exposures to 10,000 ppm Me0H during gd 5-9. Mice received water but not food during exposure. Maternal blood Me0H was determined at times during, at the end of, and subsequent to a single 7 hr exposure on gd 7. On gd 17, remaining mice were weighed, killed, and gravid uteri removed. Live, dead, and resorbed fetuses were counted, and live fetuses were examined, weighed, and preserved in 70% ethanol. All fetuses were examined externally and for cleft palate, eviscerated, and stained with Alizarin red for skeletal examination. Pregnant mice lost an average of 0.3-2.9 g during 7 hr exposure to either filtered air or Me0H, but a Me0H treatment effect was evident only with 2-day exposure on gd 7-8. Peak maternal blood Me0H concentration (at the end of exposure) was approximately 4 mg/ml, and Me0H was cleared from maternal blood within 24 hr. Some fully resorbed litters were observed with 2-day Me0H exposures on gd 6-7 or 7-8, or 1-day exposure on gd 7. With 1-day Me0H exposure on gd 7, the number live was lower than with exposure on any other day. As previously reported, cleft palate, exencephaly, and skeletal defects were the fetal anomalies observed in this mouse strain. Cleft palate occurred with 2-day exposures on gd 6-7 through gd 11-12 (peak on gd 7-8), and with 1-day exposure on gd 5 through gd 9 (peak on gd 7). Exencephaly occurred with 2-day exposures on gd 6-7 through gd 8-9 (peak gd 6-7) or 1-day exposure on gd 5 through gd 8 (peak on gd 7). Skeletal elements malformed included the exoccipital (peak gd 6-7, gd 5), atlas (peak gd 6-7, gd 5,6), axis (peak gd 6-7, gd 7), cervical vertebra 7 with a rib (peak gd 6-7, gd 7), and lumbar vertebra 1 with a rib (peak gd 7-8, gd 7). An increased incidence of fetuses with 25 presacral vertebrae (normal = 26) was observed with methanol exposure on gd 5, whereas an increased incidence of fetuses with 27 presacral vertebrae was observed with Me0H exposure on gd 7.

Attachment O – Methanol

These results indicate that gastrulation and early organogenesis represent a period of increased embryonal sensitivity to methanol.

Title: Preparation, antimicrobial evaluation, and mutagenicity of [2- hydroxyary1]-[1-methyl-5- nitro-1H-2-imidazolyl] methanols, [5-tert-Buty1- 2-methylaminopheny1]41- methy1-5-nitro-1H-2-imidazolyl]methanol, and [2-Hydroxyary1]-[1-methyl-5-nitro-1H-2-imidazolyl] ketones

Author(s): Y. Arredondo, M. Moreno-Marias, R. Pleixats, C. Palacin, M. M. Raga, J. M. Castello and J. A. Ortiz

Source: Bioorganic & Medicinal Chemistry Volume 5, Issue 10, October 1997, Pages 1959-1968

Abstract: Efficient preparations of the titled compounds are described, their antimicrobial activity and mutagenic properties being evaluated. Some of the studied compounds are nonmutagenic and present a MIC as low as some of the usual standards in the field.

Title: Inhalation toxicity of methanol/gasoline in rats: effects of 13-week exposure.

Author(s): Poon R, Park G Viau C Chu I, Potvin M, Vincent R, Valli V.

Source: Toxicol Ind Health. 1998 Jul-Aug;14(4):501-20.

Abstract: The subchronic inhalation toxicity of a methanol/gasoline blend (85% methanol, 15% gasoline, v/v) was studied in rats. Sprague Dawley rats (10 animals per group) of both sexes were exposed to vapours of methanol/gasoline at 50/3, 500/30 and 5000/300ppm for 6 hours per day, 5 days per week, for 13 weeks. Control animals inhaled filtered room air only. Control recovery and high dose recovery groups were also included which inhaled room air for an extra 4 weeks following the treatment period. No clinical signs of toxicity were observed in the treatment group and their growth curves were not significantly different from the control. Except for decreased forelimb grip strength in high dose females, no treatment-related neurobehavioural effects (4-6 hours post inhalation) were observed using screening tests which included cage-side observations, righting reflex, open field activities, and forelimb and hindlimb grip strength. At necropsy, the organ to body weight ratios for the liver, spleen, testes, thymus and lungs were not significantly different from the control group. There were no treatment-related effects in the hematological endpoints and no elevation in serum formate levels. Minimal serum biochemical changes were observed with the only treatment-related change being the decreased creatinine in the females. A dose-related increase in urinary ascorbic acid was detected in males after 2, 4 and 8 weeks of exposure, but not after the 12th week, and in females only at week-2. Increased urinary albumin was observed in treated males starting at the lowest dose and at all exposure periods, but not in females. A treatment-related increase in urinary beta 2-microglobulin was detected in males at week-2 only.

Attachment O – Methanol

Except for mild to moderate mucous cell metaplasia in nasal septum B, which occurred more often and with a slightly higher degree of severity in the low dose groups of both sexes, and presence of a minimal degree of interstitial lymphocyte infiltration in the prostate glands in the high dose males. No other significant microscopic changes were observed in the tissues of treated animals. Based on the marked increase in urinary ascorbic acid and albumin in the high dose males and the decreased forelimb grip strength in the high dose females, we concluded that the no-observed adverse effect level (NOAEL) of methanol/gasoline vapour is 500/30 ppm.

Title: Comparison of three in vitro assays at evaluation of IC50 of acetylsalicylic acid, ferrous sulfate, amitriptyline, methanol, isopropanol and ethylene glycol in human cancer cells HeLa.

Author(s): Ruppová K, Wsólová L, Urbanciková M, Slamenová D.

Source: Neoplasma. 2000;47(3):172-6.

Abstract: Evaluation of the 50% inhibitory concentration (IC50) of acetylsalicylic acid, ferrous sulfate, amitriptyline, methanol, isopropanol and ethylene glycol was done on human cancer cells cultured in in vitro conditions. Three different in vitro assays were used in this study: the plating efficiency test, the microprotein test and the neutral red uptake test. Obtained results were evaluated by statistical methods. All used methods seem to be useful for screening a cytotoxic potential of the tested chemicals. The knowledge of cytotoxic effects of frequently used chemicals on mammalian cells is important not only for necessary in vitro genotoxicity and carcinogenicity studies but also for assessing the toxicity of chemicals to find out possible hazards to the human health. Results presented in this paper underline the usefulness of the wider methodological approach for the comparison of the different endpoints as well as a necessity for selection of a battery of in vitro cytotoxicity tests allowing to estimate the possible harmful effects of xenobiotics.

Title: Frequency of oral mucosa micronuclei in gas station operators after introducing methanol.

Author(s): Gattas GJ Cardoso Lde A, Medrado-Faria Mde A, Saldanha PH.

Source: Occup Med (Lond). 2001 Mar;51(2):107-13.

Abstract: Methanol has been proposed in different countries as an alternative automotive fuel to be used as an additive to, or replacement for, gasoline or ethanol. Utilization of methanol is increasing exposure to low levels of methanol vapors in the environment and more specifically in occupational settings such as gas stations. Pump operators are exposed to relatively high levels of fuel vapors, the consequences of which have not been fully examined. In this study, the micronucleus assay in squamous oral cells was performed on pump operators of

Attachment O – Methanol

28 gas stations in three different periods in the city of S5o Paulo, Brazil. The frequency of micronuclei (MN) was evaluated before and 1 year after a mixed fuel called MEG, which contains 33% methanol, 60% ethanol and 7% gasoline, was introduced. The third evaluation, 3 years later, represents a period where the number of cars using alcohol fuel had decreased drastically and the pump operator exposure to MEG became very low. The frequency of MN observed in 76 employees in 1992 (mean = 3.62 +/- 0.39) was significantly increased (P < 0.001) as compared with 76 operators exposed in 1989 (mean = 1.41 +/- 0.26) and 129 exposed in 1995 (mean = 1.20 +/- 0.15). These differences were also significant when compared with control groups not exposed professionally to motor fuel. These findings could indicate a mutagenic hazard of the MEG occurring in those with occupational exposure.

Title: (14)C methanol incorporation into DNA and proteins of organogenesis stage mouse embryos in vitro.

Author(s): Huang YS, Held GA, Andrews JE, Rogers JM.

Source: Reprod Toxicol. 2001 Jul-Aug;15(4):429-35.

Abstract: Methanol (MeOH), a widely used industrial solvent and alternative motor fuel, has been shown to be mutagenic and teratogenic. We have demonstrated that methanol is teratogenic in mice in vivo and causes dysmorphogenesis in cultured organogenesis stage mouse embryos. Although Me0H is a product of endogenous metabolism in the gut and can be found in humans following consumption of various foods, elevated levels of methanol could lead to methylation of cellular macromolecules. DNA methylation has been demonstrated to suppress transcription of fetal genes and may also play an important role in genetic imprinting. Embryonal proteins are also potential targets for methanol-induced methylation. We investigated the potential of administered methanol to incorporate into and/or alter the methylation of embryonal DNA or to affect specific protein methylation. Gestational day 8 CD-1 mouse embryos were grown for 24 h in culture medium (CM) with 0, 4, or 8 mg Me0H + 20 microCi (14)C-Me0H/mL. At the end of the culture period, yolk sacs and embryos were separated for each treatment group. The DNA was purified by cesium chloride gradient centrifugation in the presence of ethidium bromide and (14)C incorporation was determined. Methylation of a selected gene, Hoxc-8, was assessed by using methylation-specific restriction enzymes. The (14)C activity was found superimposed over the DNA-containing fraction, indicating incorporation. DNA from embryos treated with 4 mg Me0H/mL CM gave the highest incorporation of (14)C-MeOH (8 mg/mL was growth inhibiting). Methylation of Hoxc-8 appeared to be increased in embryos treated with 4 mg Me0H/mL CM, but not in embryos treated with 8 mg Me0H/mL. Lack of incorporation of methylation at the higher concentration may be due to the failure

Attachment O – Methanol

of embryos to grow at this concentration of Me0H. The incorporation of (14)C-MeOH into embryo proteins was investigated by polyacrylamide gel electrophoresis (PAGE) and autoradiography. Incorporation of (14)C-MeOH into specific proteins was observed but the labeling specificity was not methanol dose-related. These results indicate that methyl groups from (14)C-MeOH are incorporated into mouse embryo DNA and protein. Our results further suggest that methanol exposure may increase genomic methylation under certain conditions which could lead to altered gene expression.

Title: A Biologically Based Dynamic Model for Predicting the Disposition of Methanol and Its Metabolites in Animals and Humans

Author(s): Michele Bouchard, Robert C. Brunet, Pierre-Olivier Droz and Gaetan Carrier

Source: Oxford Journals, Life Sciences & Medicine, Toxicological Sciences, 2001, Volume 64, Issue 2 Pp. 169-184.

Abstract: A multicompartment biologically based dynamic model was developed to describe the time evolution of methanol and its metabolites in the whole body and in accessible biological matrices of rats, monkeys, and humans following different exposure scenarios. The dynamic of intercompartment exchanges was described mathematically by a mass balance differential equation system. The model's conceptual and functional representation was the same for rats, monkeys, and humans, but relevant published data specific to the species of interest served to determine the critical parameters of the kinetics. Simulations provided a close approximation to kinetic data available in the published literature. The average pulmonary absorption fraction of methanol was estimated to be 0.60 in rats, 0.69 in monkeys, and 0.58-0.82 in human volunteers. The corresponding average elimination half-life of absorbed methanol through metabolism to formaldehyde was estimated to be 1.3, 0.7-3.2, and 1.7 h. Saturation of methanol metabolism appeared to occur at a lower exposure in rats than in monkeys and humans. Also, the main species difference in the kinetics was attributed to a metabolism rate constant of whole body formaldehyde to formate estimated to be twice as high in rats as in monkeys. Inversely, in monkeys and in humans, a larger fraction of body burden of formaldehyde is rapidly transferred to a long-term component. The latter represents the formaldehyde that (directly or after oxidation to formate) binds to various endogenous molecules or is taken up by the tetrahydrofolic-acid-dependent one-carbon pathway to become the building block of synthetic pathways. This model can be used to quantitatively relate methanol or its metabolites in biological matrices to the absorbed dose and tissue burden at any point in time in rats, monkeys, and humans for different exposures, thus reducing uncertainties in the dose-response relationship, and animal-to-human and exposure scenario comparisons. The model, adapted to kinetic data in human volunteers exposed

Attachment O – Methanol

acutely to methanol vapors, predicts that 8-h inhalation exposures ranging from 500 to 2000 ppm, without physical activities, are needed to increase concentrations of blood formate and urinary formic acid above mean background values reported by various authors (4.9-10.3 and 6.3-13 mg/liter, respectively). This leaves blood and urinary methanol concentrations as the most sensitive biomarkers of absorbed methanol.

Title: Effects of methanol-containing additive on emission characteristics from a heavy-duty diesel engine

Author(s): Mu-Rong Chao, Ta-Chang Lin, How-Ran Chao, Feng-Hsiang Chang and Chung-Bang Chen

Source: The Science of The Total Environment, Volume 279, Issues 1-3, 12 November 2001, Pages 167-179

Abstract: This study was aimed to investigate the effect of methanol-containing additive (MCA) on the regulated emissions of hydrocarbons (HC), carbon monoxide (CO), nitrogen oxides ($N0_x$), particulate matter (PM), as well as the unregulated carbon dioxide (CO_2) and polycyclic aromatic hydrocarbons (PAHs) from a diesel engine. The engine was tested on a series of diesel fuels blended with five additive levels (0, 5, 8, 10 and 15% of MCA by volume). Emissions tests were performed under both cold- and hot-start transient heavy-duty federal test procedure (HD-FTP) cycles and two selected steady- state modes. Results show that MCA addition slightly decreases PM emissions but generally increases both THC and CO emissions. Decrease in NO_x emissions was found common in all MCA blends. As for unregulated emissions, CO_2 emissions did not change significantly for all MCA blends, while vapor-phase and particle-associated PAHs emissions in high load and transient cycle tests were relatively low compared to the base diesel when either 5 or 8% MCA was used. This may be attributed to the lower PAHs levels in MCA blends. Finally, the particle-associated PAHs emissions also showed trends quite similar to that of the PM emissions in this study.

Title: [Effect of ethanol on methanol immunotoxicity]. [Article in Russian]

Author(s): Zabrodskii PF, Germanchuk VG.

Source: Eksp Klin Farmakol. 2001 Sep-Oct;64(5):40-2.

Abstract: The results of experiments on Wistar rats under acute methanol poisoning conditions (1 LD 50) showed that ethanol serves as an antidote of methanol. Ethanol reduces nonspecific resistance of the organism and decreases activity of the native killer cells, antibody production mainly with respect to the T-dependent antigen, antibody-mediated cell cytotoxicity, and the formation of delayed type hypersensitivity.

Title: Assessing the influence of methanol-containing additive on biological

Attachment O – Methanol

characteristics of diesel exhaust emissions using microtox and mutatox assays.

Author(s): Lin TC, Chao MR.

Source: Sci Total Environ. 2002 Feb 4;284(1-3):61-74.

Abstract: Here we investigate the effect of the methanol-containing additive (MCA) on the biological characteristics of diesel exhaust emissions. Microtox and Mutatox assays, respectively, were used to evaluate the acute toxicity and genotoxicity of crude extracts from diesel engine exhaust. The engine was tested on a series of diesel fuels blended with five additive levels (0, 5, 8, 10 and 15% of MCA by volume). Emission tests were performed over the hot start portion of the transient Heavy-Duty-Federal Test Procedure (HD-FTP) and two selected steady-state modes. Microtox results show that MCA additive moderately lowers the toxicity levels of particle-associated (SOF) samples, but generally increase the vapor-phase (XOC) associated toxicity. A strong correlation was found between XOC-associated toxicity and total hydrocarbon (THC) concentrations, while only a slight link was found between SOF-associated toxicity and particulate matter (PM) concentrations. For Mutatox test results, when either 5 or 8% MCA used, XOC and SOF-associated genotoxicity in both steady-state and hot-start transient cycle tests were relatively lower compared to those of the base diesel. The genotoxic potential of XOC samples was significantly increased after treatment with an exogenous metabolic activation system (59). On the contrary, the genotoxic potential of SOF samples without S9 metabolic activation was generally higher than those with S9. It is noteworthy that the total particle-associated (SOF) PAHs emissions showed trends quite similar to that of the genotoxic potential. As expected, the total particle-associated (SOF) PAHs correlated moderately with direct mutagenicity, and fairly well with indirect mutagenicity. Finally, the genotoxicity data did not parallel the Microtox results in this study, indicating that potentially long-term genotoxic agents may not be revealed by short-term toxicity assays.

Title: Post-mortem analysis of formic acid disposition in acute methanol intoxication.

Author(s): Luis A Ferrari, Miriam G Arado, Cesar A Nardo, Leda Giannuzzi

Source: Forensic Science International (2003) Volume: 133, Issue: 1-2, Pages: 152-158

Abstract: Fifteen cases of falai massive methanol intoxication have been investigated. Victims received either no treatment or ethanol therapeutic treatment. Methanol poisoning cases were classified in three groups according to survival time: more than 3 days (group 1), up to 3 days (group 2) and few hours (group 3). Body distribution of methanol and formic acid, as the main metabolite, was analyzed in blood and in different organs (brain, kidney, lung and liver). Relationships between formic add concentration in the different tissues, survival time and type of treatment applied to victims were studied. Formic acid in blood and tissues was analyzed by head space gas chromatography (head space-GC) with FID

Attachment O – Methanol

detector, previous transformation in methyl formate, essentially as described by Abolin. Formic acid concentration was between 0.03 and 1.10g11 in the samples under study. A good correlation between blood and brain, but poor between blood and the remaining tissues was found. Obtained data suggested that the use of blood and brain could help to improve the analysis of formic acid intoxication. The best correlation among organs was found between lung and kidney for all groups (r(2)=0.91, 0.84 and 0.87, corresponding to groups 1, 2 and 3, respectively). Lethality index was defined as LI = (concentration of formic acid in blood in (g/0/0.5) x 100, taking into account that 0.5g/l is the concentration reported by Mahieu in severe methanol poisoning. LI parameter was used to estimate formic acid incidence on the lethality of methanol poisoning cases. LI showed a good correlation with total formic acid concentration of the different tissues analyzed (r(2)=0.80). Furthermore, LI allowed us to discriminate between individuals that received therapeutic treatment and survived different periods. L1,100 indicated a severe intoxication and shod survival time if the victim was assisted with ethanol therapy and hemodialysis was not applied. With regard to victims who received no therapeutic treatment and died in few hours, LI was in the range 40-100. LI was below 40 for individuals that survived more than 3 days and hemodialysis was not performed. Results showed the importance of performing formic acid analysis to diagnose severe methanol intoxication in post-mortem cases.

Title: Rethinking the toxic methanol level.
Author(s): Kostic MA, Dart RC.
Source: J Toxicol Clin Toxicol 2003;41(6):793-800.
Abstract: INTRODUCTION: Treatment thresholds for methanol poisoning are based on case reports and published opinion. Most guidelines recommend treatment for a methanol level > or = 20 mg/dL in a nonacidotic patient. No supportive data have been offered nor has the time of the exposure been addressed. For instance, no distinction has been drawn between a methanol level drawn 1 hr vs. 24 hr from ingestion. We analyzed all published cases of methanol poisoning to determine the applicability of the 20 mg/dL threshold in a nonacidotic patient, specifically those arriving early for care (within 6 hr) with a peak or near-peak blood methanol concentration.
METHODS: Using predefined search criteria, a systematic review of the world literature was performed using MEDLINE and EMBASE. In addition, each article's references were hand searched for pre-1966 articles, as were fatality abstracts from all U.S. poison centers. Human cases were included if they reported a known time of a single methanol exposure, acid-base data, blood methanol, and blood ethanol (if not acidotic).
RESULTS: Dating to 1879, 372 articles in 18 languages were abstracted using a

Attachment O – Methanol

standard format; 329 articles (2433 patients) involved methanol poisoning, and 70 articles (173 patients) met inclusion criteria. Only 22 of these patients presented for care within 6hr of ingestion with an early methanol level. All but 1 patient was treated with an inhibitor of alcohol dehydrogenase (ADH). A clear acidosis developed only with a methanol level > or = 126 mg/dL. The patient that did not receive an ADH inhibitor was an infant with an elevated early methanol level (46 mg/dL) that was given folate alone and never became acidotic. Intra and inter-rater reliability were 0.95.
CONCLUSIONS: Nearly all reports of methanol poisoning involve acidotic patients far removed from ingestion. The small amount of data regarding patients arriving early show that 126 mg/dL is the lowest early blood methanol level ever clearly associated with acidosis. Contrary to conventional teaching, there are case reports of acidosis after only a few hours of ingestion. The data are insufficient to apply 20 mg/dL as a treatment threshold in a nonacidotic patient arriving early for care. Prospective studies are necessary to determine if such patients may be managed without antidotal therapy or dialysis.

Title: Conventional and diffusion-weighted MRI in the evaluation of methanol poisoning.
Author(s): Server A Hovda KE, Nakstad PH, Jacobsen D, Dullerud R, Haakonsen M.
Source: Acta Radiol. 2003 Nov;44(6):691-5.
Abstract: Cerebral lesions were studied in 2 methanol-poisoned patients using conventional magnetic resonance imaging (MRI). In 1 patient, diffusion-weighted MRI (DWI) was also performed. In this patient, conventional MRI showed symmetrical, bilateral increased signal in the lentiform nuclei, involving predominantly putamina, but also extending into the corona radiata, centrum semiovale and subcortical white matter. DWI showed decreased diffusion, which most probably reflects cytotoxic edema. In the other patient, fluid attenuated-inversion recovery (FLAIR) and T2-weighted images showed hyperintensity in the putamina, characteristic of post-necrotic changes.

Title: Methanol toxicity in a newborn.
Author(s): Martin Belson, Brent W Morgan
Source: J Toxicol Ciro Toxicol. 2004 42 (5):673-7 15462162 Cit:1
Abstract: Methanol poisoning during human pregnancy rarely has been described. We report the first human newborn with a documented methanol concentration resulting from maternal exposure. CASE REPORT: A 26-year-old pregnant woman EGA 30 weeks with HIV infection and asthma presented to the emergency department in respiratory distress. She was acidotic (pH 7.17) with an anion gap of 26, and fetal bradycardia was noted. Her son was delivered by

Attachment O – Methanol

emergent C-section (birthweight 950 g, Apgars 1 and 3) and required aggressive resuscitation. During his hospital course, acidosis (initial pH 6.9) persisted despite fluid, blood, and bicarbonate administration. His mother also had persistent metabolic acidosis despite fluids, bicarbonate, and dopamine. Results of other laboratory tests on the mother included undetectable ethanol and salicylates and an osmolar gap of 41. An ethanol drip was initiated for the mother 36 h after admission when a methanol level of 54 mg/dL was reported. When consulted on hospital day 3, our regional poison center recommended hemodialysis for the mother and administering fomepizole and testing the methanol level of the newborn (61.6 mg/dL). Because the infant developed a grade 4 intraventricular bleed, no further therapy was offered, and he died on day 4. His mother died on day 10.

Title: Chronic maternal methanol inhalation in nonhuman primates (Macaca fascicularis): Exposure and toxicokinetics prior to and during pregnancy

Author(s): Thomas M. Burbacher, Danny D. Shen, Bojan Lalovic, Kimberly S. Grant, Lianne Sheppard, Doris Damian, Stephen Ellis and Noelle Liberato

Source: Neurotoxicology and Teratology Volume 26, Issue 2, March-April 2004, Pages 201-221

Abstract: Toxicokinetic studies were conducted following daily inhalation exposure to methanol vapor prior to and throughout pregnancy in adult female Macaca fascicularis monkeys. They were part of a larger study to investigate the effects of chronic methanol exposure on maternal reproductive performance and early offspring effects. In a two-cohort study design, 48 females (24/cohort) were assigned to parallel exposure groups at 0 (control), 200, 600, or 1800 ppm methanol vapor for approximately 2.5 h/day, 7 days/week throughout breeding and pregnancy. Blood methanol at 30 min postexposure was monitored biweekly. The time course for the clearance of blood Me0H concentrations following exposure was characterized on four occasions: twice during the prebreeding period and during mid- and late pregnancy. Average blood methanol concentrations at 30 min postexposure were 5, 11, and 35 pg/ml across all four toxicokinetic studies in the 200, 600 and 1800 ppm groups, respectively. Blood concentrations in the 200 ppm group were barely above basal (preexposure) blood methanol concentrations or those observed in the control group (approximately 3 pg/ml). Nonlinear elimination kinetics were observed in most of the 1800 ppm group females. There was a decrease in elimination half- life (7-20%) and an increase in clearance (30%) after 3-months of daily Me0H exposure compared to the initial exposure. There were no statistically significant changes in the first-order blood methanol half-life or clearance during pregnancy, but the mean distribution volume per kilogram body weight decreased by 22% and 17% in the 600 and 1800 ppm groups. Plasma formate levels did not differ between

Attachment O – Methanol

the methanol and control exposure groups. Plasma formate and serum folate concentrations increased slightly over the course of this study in both the exposed and control groups but these increases were not related to methanol exposure.

Title: Acute renal injury following methanol poisoning: analysis of a case series.
Author(s): Verhelst D, Moulin P, Haufroid V, Wittebole X, Jadoul M, Hantson P.
Source: Int J Toxicol. 2004 Jul-Aug;23(4):267-73.
Abstract: The objective of this paper is to document the prevalence of indicators of acute renal injury in a series of methanol- poisoned patients treated in an intensive care unit and to discuss the possible mechanisms. This is a retrospective analysis of the medical records of 25 consecutive patients admitted to the intensive care unit after severe intentional methanol poisoning. Acute renal impairment was defined as a serum creatinine concentration higher than 177 micro mol/L and/or a urinary output on admission and for the first 24 h below 0.5 ml/kg/h. Clinical pathological signs of acute renal injury were found in 15 patients. In comparison with the 10 other patients taken as control group, the patients who developed renal injury had a lower blood pH value on admission, a higher serum osmolality, and a higher peak formate concentration. Two factors contributing to renal injury could be identified: hemolysis and myoglobinuria. The role of osmotic changes (osmotic nephrosis) or of a direct cytotoxic effect of formic acid remains speculative. Analysis of proteinuria suggests that proximal tubular cells may be preferentially affected. Results of histopathological evaluation of the kidney on a limited sample size (n = 5) were inconclusive but suggestive of possible hydropic changes in the proximal tubule secondary to methanol toxicity. Acute renal injury may be associated with other signs of severity in methanol poisoning, but it is almost always reversible in survivors. Indicators of acute renal injury were identified. The pathophysiology of this acute renal injury is multifactorial and far more complex than shock-related tubular necrosis.

Title: Uptake and Disposition of Inhaled Methanol Vapor in Humans
Author(s): Lena Ernstgard, Eiji Shibata and GunnarJohanson
Source: Oxford Journals Life Sciences & Medicine Toxicological Sciences, 2005, Volume 88, Issue 1 Pp. 30-38.
Abstract: Methanol is a widely used solvent and a potential fuel for motor vehicles. Human kinetic data of methanol are sparse. As a basis for biological exposure monitoring and risk assessment, we studied the inhalation toxicokinetics of methanol vapor in four female and four male human volunteers during light physical exercise (50^{1}M in an exposure chamber. The relative uptake of methanol was about 50% (range 47-53%). Methanol in blood increased from a background level of about 20 to 116 and 244 pM after 2 h exposure at 0, 100 ppm (131 mg/m^3) and 200

Attachment O – Methanol

ppm (262 mg/m^3), respectively. Saliva showed substantially higher levels than blood immediately after exposure. This difference disappeared in a few minutes; thereafter the concentrations and time courses in blood, urine, and saliva were similar, with half times of 1.4, 1.7, and 1.3 h, respectively. The postexposure decrease of methanol in exhaled air was faster, with a half time of 0.8 h. The methanol concentrations were approximately twice as high in all four types of biological samples at 200 compared to 100 ppm. No increase in urinary formic acid was seen in exposed subjects. Our study indicates nonsaturated, dose-proportional kinetics of methanol up to 200 ppm for 2 h. No gender differences were detected. Similar, parallel patterns were seen with regard to the methanol time courses in blood, urine, and saliva, whereas the concentration in exhaled air decreased markedly faster. Thus, apart from blood and urine, saliva also seems suitable for biomonitoring of methanol exposure.

[Genotoxicity comparison between gasoline- and methanol-fueled exhaust by TK gene mutation assay].

Author(s): Llano Y Zhan L, Zhanq Z, Zhanq H, Zeng X, Gou X, Lin C Cai C, Shao X, Shao G, Wu D.

Source: Shenq Wu Yi Xue Goon Chenq Xue Za Zhi. 2005 Apr;22(2):347-50.

Abstract: Methanol fuel is a most promising substitute for gasoline. It is scarcely reported about methanol-fueled exhaust on the health effect, neither about genotoxicity research between methanol- and gasoline-fueled exhaust. In the present study, the two kinds of exhaust were sampled directly from tailpipe at the same type bus, the same state, L5178Y thymidine kinase (TK) gene mutation assay was used to investigate their genotoxicity at the same dose range, and compared with micronucleus and comet assay. The results showed that the genotoxicity of gasoline-fueled exhaust is stronger than that of methanol-fueled exhaust, while the cytotoxicity of methanol-fueled exhaust is stronger than that of gasoline-fueled exhaust at dose range. The study demonstrated that L5178Y TK gene mutation assay is more sensitive than micronucleus and comet assay.

Title: [Comparative study on the genotoxicity of gasoline-fueled vehicle exhaust and methanol-fueled vehicle exhaust]. [Article in Chinese]

Author(s): Zhanq ZZ, Zhanq Q, Lianq Y, Li CQ, Lai CC, Huang WB, Wu DS.

Source: Sichuan Da Xue Xue Bao Yi Xue Ban. 2005 Mar;36(2):249-52.

Abstract: OBJECTIVE—Comparing the genotoxicity of gasoline-fueled vehicle exhaust (gasoline exhaust for short) with that of methanol-fueled vehicle exhaust (methanol exhaust for short) so as to provide a scientific basis for replacement of gasoline by methanol as fuel in vehicle.
METHODS—The MTT method was used first to evaluate the cytotoxicity of the two kinds of vehicle exhausts, and the concentration that had no obvious

Attachment O – Methanol

cytotoxicity would be used as the highest dose in the experiments. The A549 cells micronucleus test and single cell 6e1 electrophoresis (SCGE) assay (comet assay) in vitro were applied to compare the genotoxicity of gasoline exhaust and methanol exhaust.
RESULTS—The MTT results showed that the gasoline-fueled vehicle exhaust exerted stronger cytotoxicity to A549 cells in both 2 h and 24 h exposure times, compared with the methanol exhaust. In A549 cells micronucleus test in vitro, at doses 0.025, 0.05, 0.1 and 0.2 L/ml, the micronucleated cell (MNC) rates were 2.65%, 4.35%, 4.95% and 5.85% respectively, which were higher than those (1.30% and 1.35%) of controls ($P<0.01$). For the methanol-fueled vehicle exhaust, there was no significant difference in the rate of A549 cells micronucleus between the test groups and control group. In the comet assay, gasoline-fueled vehicle exhaust could induce A549 cells DNA damage. The rate of caudate cell and the length of DNA migration increased with the escalation of dosing level. However, the methanol-fueled vehicle exhaust did not show any DNA damage to A549 cells.
CONCLUSION—The results demonstrate that gasoline-fueled vehicle exhaust can induce DNA and chromosome damage, it has a distinct genotoxicity, whereas the methanol-fueled vehicle exhaust does not show any potential genotoxicity in both tests.

Title: [Comparative study on the mutagenicity of the gasoline-fueled vehicle exhaust and methanol-fueled vehicle exhaust].

Author(s): Zhanq ZZ Lianq Y Ran Y, Gou XJ, Lin C Cai C Wu D.

Source: Wei Sheno Yan Jiu. 2005 Mar;34(2):163-6.

Abstract: OBJECTIVE: In order to compare the mutagenicity of the gasoline-fueled vehicle exhaust (gasoline exhaust for short) and the methanol-fueled vehicle exhaust (methanol exhaust for short), provide a scientific basis for replacement gasoline by methanol as fuel in vehicle.
METHODS: The Ames assay and A549 cell micronucleus test in vitro were applied to compare the mutagenicity of gasoline-fueled vehicle exhaust and methanol-fueled vehicle exhaust.
RESULTS: The results showed that the gasoline-fueled vehicle exhaust expressed evident mutagenicity in Ames assay (TA98 strain) with or without rat liver-derived metabolic activation system (S9). The mutagenicity increased obviously when S9 was used and showed a good dose-response relationship. However, the methanol-fueled vehicle exhaust did not show any mutagenic potential in Ames assay (TA98, TA100 strains) with or without rat liver-derived metabolic activation system (S9). In A549 cells micronucleus test in vitro, between 0.025 - 0.2JL/ml, the rate of A549 cells micronucleus could be induced by the gasoline-fueled vehicle exhaust. For the methanol-fueled vehicle exhaust,

Attachment O – Methanol

there was no significant differences in the rate of A549 cells micronucleus between the tested groups and control.
CONCLUSION: The results strongly suggested that the gasoline-fueled vehicle exhaust had potential mutagenicity and the methanol-fueled vehicle exhaust did not show any potential mutagenicity. The results also provided a scientific basis for replacement gasoline by methanol as fuel in vehicle.

Title: 266: Inhalation of Methanol-Containing Products: A Significant Source of Possible Toxicity
Author(s): M.L. Givens, K. Kalbfleisch, and S. Bryson
Source: Annals of Emergency Medicine, Volume 48, Issue 4, Supplement 1, October 2006, Pages 80-81
Abstract: None provided.

Title: Inhalational abuse of methanol products: elevated methanol and formate levels without vision loss
Author(s): Vikhvat S. Bebarta MD, Kennon Heard MD, Richard C. Dart. MD, PhD
Source: American Journal of Emergency Medicine, Volume 24 Issue 6 , Pages 725-728, October 2006
Abstract: Inhalant abuse of methanol-containing products has increased over the last decade. We performed a prospective observational study of 7 subjects who presented to an ED after inhalant abuse of methanol-containing hydrocarbon products. Four patients had a methanol level greater than 24 mgidt. and 2 had an anion gap greater than 17 mEq/L. The mean formic acid level was 71 pg/n)L, and 1 patient had a level considered high enough to induce retinal toxicity (>200 pg/mL). No patient had an abnormal ophthalmologic examination. All patients were treated with intravenous folate, 2 received alcohol dehydrogenase blockade, and no patient received hemodialysis or intravenous bicarbonate. All patients' acidosis resolved within 4 hours. The methanol and formic acid levels are lower than those reported after methanol ingestion. These preliminary data suggest that inhalant abusers of methanol products may have significantly elevated methanol and formic acid levels, but are at low risk for methanol induced complications of visual dysfunction and refractory acidosis.

Title: Health effects of subchronic exposure to diesel-water-methanol emulsion emission.
Author(s): Reed MD, Blair LF, Burling K, Daly I, Gigliotti AP, Gudi R, Mercieca MD, McDonald JD, O'Callaghan JP, Seilkop SK, Ronskoh NL, Wagner VO, Kraska RC.
Source: Toxicol Ind Health. 2006 Mar;22(2):65-85.
Abstract: The U.S. Environmental Protection Agency's National Ambient Air Quality

Attachment O – Methanol

Standards for ozone and particulate matter (PM) require urban non-attainment areas to implement pollution-reduction strategies for anthropogenic source emissions. The type of fuel shown to decrease combustion emissions components versus traditional diesel fuel, is the diesel emulsion. The Lubrizol Corporation, in conjunction with Lovelace Respiratory Research Institute and several subcontracting laboratories, recently conducted a health assessment of the combustion emissions of PuriNOx diesel fuel emulsion (diesel-water-methanol) in rodents. Combustion emissions from either of two, 2002 model Cummins 5.9L ISB engines, were diluted with charcoal-filtered air to exposure concentrations of 125, 250 and 500 microg total PM/m3. The engines were operated on a continuous, repeating, heavy-duty certification cycle (U.S. Code of Federal Regulations, Title 40, Chapter I) using Rotella-T 15W-40 engine oil. Nitrogen oxide (NO) and PM were reduced when engines were operated on PuriNOx versus California Air Resources Board diesel fuel under these conditions. Male and female F344 rats were housed in Hazleton H2000 exposure chambers and exposed to exhaust atmospheres 6 h/day, five days/week for the first 11 weeks and seven days/week thereafter. Exposures ranged from 61 to 73 days depending on the treatment group. Indicators of general toxicity (body weight, organ weight, clinical pathology and histopathology), neurotoxicity (glial fibrillary acidic protein assay), genotoxicity (Ames assay, micronucleus, sister chromatid exchange), and reproduction and development were measured. Overall, effects observed were mild. Emulsion combustion emissions were not associated with neurotoxicity, reproductive/developmental toxicity, or in vivo genotoxicity. Small decreases in serum cholesterol in the 500-microg/m3 exposure group were observed. PM accumulation within alveolar macrophages was evident in all exposure groups. The latter findings are consistent with normal physiological responses to particle inhalation. Other statistically significant effects were present in some measured parameters of other exposed groups, but were not clearly attributed to emissions exposure. Positive mutagenic responses in several strains of Salmonella typhimurium were observed subsequent to treatment with emulsion emissions subfractions. Based on the cholesterol results, it can be concluded that the 250-microg/m3 exposure level was the no observed effect level. In general, biological findings in exposed rats and bacteria were consistent with exposure to petroleum diesel exhaust in the F344 rat and Ames assays.

Title: Two cases of rapid onset Parkinson's syndrome following toxic ingestion of ethylene glycol and methanol.

Author(s): Reddy NJ, Lewis LD Gardner TB, Osterlinq W, Eskey CJ Nierenberq DW.

Source: Clin Pharmacol Ther. 2007 Jan;81(1):114-21.

Abstract: Ethylene glycol and methanol are toxic alcohols commonly found in a variety of

Attachment O – Methanol

commercial products. We report two cases, one associated with ethylene glycol and one with methanol poisoning, which both led to acute hemorrhagic necrosis of the basal ganglia and resulted in acute Parkinson's syndrome. It is unlikely that oxalate crystal deposition is the only mechanism for such basal ganglia necrosis, because similar findings were seen following methanol intoxication. We discuss other possible mechanisms that may contribute towards this unusual neurotoxicity. Both of our patients survived their toxic ingestions, but then developed acute Parkinson's syndrome within 10 days of the ingestion. However, the patient who ingested methanol developed respiratory muscle stiffness/weakness, which responded poorly to anti-Parkinsonian drug therapy. Treatment with carbidopa/levodopa improved cogwheel rigidity and bradykinesia in both patients. We conclude that acute Parkinsonism is one of the lesser-recognized devastating complications of both ethylene glycol and methanol poisoning.

Title: Methanol poisoning: acute MR and CT findings in nine patients
Author(s): S. Sefidbakht, A. R. Rasekhi, K. Karnali, A. Borhani Haghighi, A. Salooti, A. Meshksar, H. R. Abbasi, M. Moghadami and S. A. Nabavizadeh
Source: Neuroradiology, 2007, Volume 49, Number 5, 427-435
Abstract: Introduction—Methanol poisoning is an uncommon but potent central nervous system toxin. We describe here the CT and MR findings in nine patients following an outbreak of methanol poisoning.
Methods—Five patients with a typical clinical presentation and elevated anion and osmolar gaps underwent conventional brain MRI with a 1.5-T Gyroscan Interna scanner. In addition nonenhanced CT was performed in another three patients with more severe toxicity.
Results—Bilateral hemorrhagic or nonhemorrhagic necrosis of the putamina, diffuse white matter necrosis, and subarachnoid hemorrhage were among the radiological findings. Various patterns of enhancement of basal ganglia' lesions were found including no enhancement, strong enhancement and rim enhancement.
Conclusion—A good knowledge of the radiological findings in methanol poisoning seems to be necessary for radiologists. The present study is unique in that it enables us to include in a single report most of the radiological findings that have been reported previously.

Title: Comparison of cytotoxicity and genotoxicity induced by the extracts of methanol and gasoline engine exhausts.
Author(s): Zhanci Z Che W, Liana Y, Wu M, Li N, Shu Y, Liu F, Wu D.
Source: Toxicol In Vitro. 2007 Sep;21(6):1058-65.
Abstract: Gasoline engine exhaust has been considered a major source of air pollution in

Attachment O – Methanol

China, and methanol is considered as a potential substitute for gasoline fuel. In this study, the genotoxicity and cytotoxicity of organic extracts of condensate, particulate matters (PM) and semivolatile organic compounds (SVOC) of gasoline and absolute methanol engine exhaust were examined by using MTT assay, micronucleus assay, comet assay and Ames test. The results have showed that gasoline engine exhaust exhibited stronger cytotoxicity to human lung carcinoma cell lines (A549 cell) than methanol engine exhaust. Furthermore, gasoline engine exhaust increased micronucleus formation, induced DNA damage in A549 cells and increased TA98 revertants in the presence of metabolic activating enzymes in a concentration-dependent manner. In contrast, methanol engine exhaust failed to exhibit these adverse effects. The results suggest methanol may be used as a cleaner fuel for automobile.

Title: Formic acid and methanol concentrations in death investigations.
Author(s): H Rachelle Wallage, James H Watterson
Source: J Anal Toxicol 2008 Apr ,32 (3).241-7
Abstract: Methanol ingestion results in the formation of formic acid, a toxic metabolite that can cause metabolic acidosis. Methanol toxicity is therefore dependent on the amount of methanol ingested, the nature of treatment received, elapsed time since ingestion, and the accumulation of formic acid. Both methanol and formic acid concentrations are determined at this laboratory using headspace gas chromatography. An examination of 12 fatalities attributed to methanol poisoning is presented. Six individuals were found deceased, and their postmortem methanol and formic acid concentrations ranged from 84 to 543 mg/dL and 64 to 110 mg/dL, respectively. In the other six individuals, hospital treatment such as bicarbonate, ethanol infusion, and hemodialysis was administered. Antemortem methanol and formic acid concentrations ranged from 68 to 427 mg/dL and 37 to 91 mg/dL, respectively, whereas corresponding postmortem methanol and formic acid levels ranged from undetectable to 49 mg/dL and undetectable to 48 mg/dL, respectively. Hospital treatment of formic acid toxicity resulted in significantly reduced postmortem methanol and formic acid concentrations. Furthermore, the toxicological relevance of nine methanol-positive cases where postmortem methanol concentrations ranged from 3 to 142 mg/dL, with corresponding formic acid levels of less than 10 mg/dL, is discussed.

Title: Medical toxicology and public health—Update on research and activities at the centers for disease control and prevention, and the agency for toxic substances and disease registry
Inhalational methanol toxicity
Author(s): Richard Kleiman, Richard Nickle and Michael Schwartz
Source: Journal of Medical Toxicology, 2009, Volume 5, Number 3, 158-164

Attachment O – Methanol

Abstract: Methanol toxicity is still a relatively common poisoning worldwide. Exposure typically occurs through ingestion, but there is potential for occupational inhalational exposures as well. People with potential exposure to methanol in any form need to wear the appropriate personal protective equipment, and they need to be familiar with proper detection methods. Medical professionals need to understand the metabolism of methanol and why knowledge of this metabolism is important for the diagnosis and management of this unique poison, Several methods of treatment exist for methanol toxicity. Through development of an under-standing of the pathophysiology behind the toxin, the appropriate measures can be taken.

Title: Assessment of the cancer potential of methanol.
Author(s): Cruzan G.
Source: Crit Rev Toxicol. 2009;39(4):347-63.
Abstract: There are no published cancer studies of methanol-exposed cohorts. Genotoxicity studies do not suggest carcinogenic activity from methanol exposure. Oncogenicity studies of methanol were conducted by inhalation for approximately 20 hrs/day at up to 1000 ppm in F344 rats and B6C3F1 mice (NEDO), and by incorporation into drinking water at up to 20,000 ppm in Sprague-Dawley rats (Ramazzini Foundation, by Soffritti et al.). No increased neoplasms were found in the NEDO rat and mouse inhalation studies, even at air levels (up to 1000 ppm for >19 hours/day, 7 days/week) that caused 10-fold increased blood methanol levels. The maximum dose level was 600 mg/kg/day. The breakdown of methanol to formaldehyde in rats is saturated at doses above 600 mg/kg according to Horton et al. Thus, higher inhalation exposure concentrations are not expected to lead to tumors in rats or mice. In the Soffritti et al. study there was excessive early mortality, and lung pathology (inflammation, dysplasia, or neoplasm) was present in 87-94% of those dying anytime in the study. Soffritti et al. reported lympho-immunoblastic lymphoma. There are no historical control data to which this study can be compared because this diagnosis is not used by any other pathologist in animal studies. Lung infections probably played a role in formation of the lesions called lympho-immunoblastic lymphoma in the Ramazzini methanol study. The data from genotoxicity studies, the inhalation and drinking water oncogenicity studies of methanol in rats and mice, and mode of action considerations support a conclusion that methanol is not likely to be carcinogenic in humans.

Title: Methanol toxicity secondary to inhalant abuse in adult men.
Author(s): Wallace EA, Green AS.
Source: Olin Toxicol (Phila). 2009 Mar;47(3):239-42.
Abstract: BACKGROUND: The purpose of this report is to evaluate the presentation,

Attachment O – Methanol

treatment, and outcomes of adults with methanol toxicity from inhalation of carburetor cleaning fluid fumes.
METHODS: Retrospective chart review of adults with positive serum volatile screen for methanol and history of carburetor cleaning fluid fume inhalation.
RESULTS: Sixteen patients were admitted 68 times. Eleven Native American patients accounted for 90% of admissions. Sixty-five cases presented with nausea/vomiting; 27 with intoxication or altered mental status; 21 with specific visual complaints. About 93% had a pH <7.35, 96% had serum bicarbonate <20 mEq/L, 81% had osmolal gap >or=10 mOsmlL, and 69% had anion gap >16. Ten had an initial serum methanol level <20 mg/dL, 29 cases 20-49 mg/dL, 19 cases >or=50 mg/dL. Six patients had a measurable serum ethanol level. Of the 29 patients with a methanol level of 20-49 mg/dL, 20 received intravenous antidote (ethanol or fomepizole); three received an antidote and hemodialysis. All who presented with a serum methanol level >or=50 mg/dL received intravenous ethanol or fomepizole. All visual symptoms resolved before discharge and all patients survived without sequelae. Discussion. This is the largest reported number of cases of methanol toxicity from the inhalation of carburetor cleaning fluid fumes and demonstrates a problem with recurrent abuse among some older Native American men.
CONCLUSION: Intentional inhalation of methanol fumes may produce toxicity. Clinicians need to question patients, especially older Native American men, regarding the possible inhalation of carburetor cleaning fluid fumes in those who present with an unexplained metabolic anion gap acidosis.

Title: Methanol exposure does not produce oxidatively damaged DNA in lung, liver or kidney of adult mice, rabbits or primates.

Author(s): McCallum GP Siu M, Sweetinci JN Wells PG.

Source: Toxicol Appl Pharmacol. 2011 Jan 15;250(2):147-53.

Abstract: In vitro and in vivo genotoxicity tests indicate methanol (MeOH) is not mutagenic, but carcinogenic potential has been claimed in one controversial long-term rodent cancer bioassay that has not been replicated. To determine whether Me0H could indirectly damage DNA via reactive oxygen species (ROS)-mediated mechanisms, we treated male CD-1 mice, New Zealand white rabbits and cynomolgus monkeys with Me0H (2.0 g/kg ip) and 6h later assessed oxidative damage to DNA, measured as 8-oxo-2'-deoxyguanosine (8-oxodG) by HPLC with electrochemical detection. We found no Me0Hdependent increases in 8-oxodG in lung, liver or kidney of any species. Chronic treatment of CD-1 mice with Me0H (2.0 g/kg ip) daily for 15 days also did not increase 8-oxodG levels in these organs. These results were corroborated in DNA repair-deficient oxoguanine glycosylase 1 (Ogg1) knockout (KO) mice, which accumulated 8-oxodG in lung, kidney and liver with age, but exhibited no increase following

Attachment O – Methanol

Me0H, despite a 2-fold increase in renal 8-oxodG in Ogg1 KO mice following treatment with a ROS-initiating positive control, the renal carcinogen potassium bromate ($KBrO_3$; 100 mg/kg ip). These observations suggest that Me0H exposure does not promote the accumulation of oxidatively damaged DNA in lung, kidney or liver, and that environmental exposure to Me0H is unlikely to initiate carcinogenesis in these organs by DNA oxidation.

Title: Metabolic mechanisms of methanol/formaldehyde in isolated rat hepatocytes: Carbonyl-metabolizing enzymes versus oxidative stress.

Author(s): Macallister SL, Choi J, Dedina L, O'Brien PJ.

Source: Chem Biol Interact. 2011 May 30;191(1-3):308-14. Epub 2011 Jan 26.

Abstract: Methanol (CH(3)OH), a common industrial solvent, is metabolized to toxic compounds by several enzymatic as well as free radical pathways. Identifying which process best enhances or prevents CH(3)OH-induced cytotoxicity could provide insight into the molecular basis for acute CH(3)OH-induced hepatoxicity. Metabolic pathways studied include those found in 1) an isolated hepatocyte system and 2) cell-free systems. Accelerated Cytotoxicity Mechanism Screening (ALMS) techniques demonstrated that CH(3)OH had little toxicity towards rat hepatocytes in 95% 0(2), even at 2M concentration, whereas 50mM was the estimated LC(50) (2h) in 1% 0(2), estimated to be the physiological concentration in the centrilobular region of the liver and also the target region for ethanol toxicity. Cytotoxicity was attributed to increased NADH levels caused by CH(3)0H metabolism, catalyzed by ADH1, resulting in reductive stress, which reduced and released ferrous iron from Ferritin causing oxygen activation. A similar cytotoxic mechanism at 1% 0(2) was previous found for ethanol. With 95% 0(2), the addition of Fe(II)/H(2)O(2), at non-toxic concentrations were the most effective agents for increasing hepatocyte toxicity induced by 1M CH(3)OH, with a 3-fold increase in cytotoxicity and ROS formation. Iron chelators, desferoxamine, and NADH oxidizers and ATP generators, e.g. fructose, also protected hepatocytes and decreased ROS formation and cytotoxicity. Hepatocyte protein carbonylation induced by formaldehyde (HCHO) formation was also increased about 4-fold, when CH(3)OH was oxidized by the Fenton-like system, Fe(II)/H(2)0 (2), and correlated with increased cytotoxicity. In a cell-free bovine serum albumin system, Fe(II)/H(2)0(2) also increased CH(3)OH oxidation as well as HCHO protein carbonylation. Nontoxic ferrous iron and a H(2)0(2) generating system increased HCHO-induced cytotoxicity and hepatocyte protein carbonylation. In addition, HCHO cytotoxicity was markedly increased by ADH1 and ALDH2 inhibitors or GSH-depleted hepatocytes. Increased HCHO concentration levels correlated with increased HCHO-induced protein carbonylation in hepatocytes. These results suggest that CH(3)OH at 1% 0(2) involves activation of the Fenton system to form HCHO. However, at higher 0(2) levels, radicals generated

Attachment O – Methanol

through Fe(II)/H(2)0(2) can oxidize CH(3)0H/HCHO to form pro-oxidant radicals and lead to increased oxidative stress through protein carbonylation and ROS formation which ultimately causes cell death.

Title: Methanol exposure does not lead to accumulation of oxidative DNA damage in bone marrow and spleen of mice, rabbits or primates.

Author(s): McCallum GP Siu M, Ondovcik SL, Sweeting JN, Wells PG.

Source: Mol Carcinog. 2011 Mar;50(3):163-72.

Abstract: Genotoxicity tests indicate methanol (MeOH) is not mutagenic, but a rodent study has suggested carcinogenic potential, which could result from free radical-initiated oxidative DNA damage. To investigate this possibility we treated male CD-1 mice, New Zealand white rabbits, and cynomolgus monkeys with Me0H (2.0 g/kg ip) and assessed tissue oxidative DNA damage 6 h post-dose, measured as 8-hydroxy-2'-deoxyguanosine (8-oxodG). We found no Me0H-dependent increases in 8-oxodG in bone marrow or spleen of any species. Chronic treatment of CD-1 mice with Me0H (2.0 g/kg ip) daily for 15 d also did not increase 8-oxodG levels in these organs. Further studies in the DNA repair deficient oxoguanine glycosylase 1 (Oggl) knockout (KO) mice supported these findings. Fibroblasts from Ogg1 KO mice accumulated 8oxodG following acute exposure to the renal carcinogen potassium bromate (KBrO(3) ; 2.0 mM) but did not accumulate 8oxodG following exposure to 125 mM Me0H 6 h post-treatment. Oggl KO mice accumulated 8-oxodG in bone marrow and spleen with age but not following exposure to Me0H. In addition, free radical-mediated hydroxynonenal-histidine protein adducts were not enhanced by Me0H in primate bone marrow or spleen, or in rabbit bone marrow or mouse spleen, although modest increases were observed in rabbit spleen and mouse bone marrow. Taken together these observations suggest that Me0H exposure does not promote the accumulation of oxidative DNA damage in bone marrow and spleen, and it is unlikely that human environmental exposure to Me0H would lead to lymphomas via this mechanism.

Attachment O – Methanol

Welfare Effects Studies (toxicity in aquatic organisms, toxicity in terrestrial species, toxicity to plants/bacteria, effects on visibility/weather/climate, effects on materials, economic effects, effects on personal comfort and well-being)

Title: Comparative Toxicity of Methanol and N,N-Dimethylformamide to Freshwater Fish and Invertebrates
Author(s): S. H. Poirier, M. L. Knuth, C. D. Anderson-Buchou, L. T. Brooke, A. R. Lima and P. J. Shubat
Source: Bulletin of Environmental Contamination and Toxicology, 1986, Volume 37, Number 1, 615-621
Abstract: None provided.

Title: Acute Toxicity of Methanol to *Mytilus edulis*
Author(s): A. Helmstetter, A. P. Gamerdinger and R. J. Pruett
Source: Bulletin of Environmental Contamination and Toxicology, 1996, Volume 57, Number 4, 675-681
Abstract: None provided.

Title: Permeation and toxicity of ethylene glycol and methanol in larvae of Anopheles gambiae.
Author(s): Liu XH, Pan H, Mazur P.
Source: J Exp Biol. 2003 Jul;206(Pt 13):2221-8.
Abstract: In this study, we applied proton NMR to measure the permeation of two cryoprotective agents (CPAs), ethylene glycol (EG) and methanol, into 1st instar Anopheles larvae. Calibration with standard solutions of EG or methanol (0-10 moll(- 1)) confirmed the reliability of the NMR measurements for determining the concentration of these solutes. To assess permeation, larvae were immersed in 1.5 mol l(-1) EG or 1.5 mol l(-1) methanol for different periods of time at 22 degrees C. The concentration of both CPAs in the larvae was then measured as a function of exposure time using (1)H-NMR spectroscopy. Results show that after a 6 h exposure to 1.5 mol l(-1) EG, the larval concentration of EG reaches a maximum value of 1.44 mol 1(-1), which is 96% of the theoretical maximum. By contrast, after just 1 h exposure to 1.5 mol 1(-1) methanol, the larval methanol concentration reaches its maximum, which, however, is only 75% of the theoretical maximum. Toxicity data show that larval survival remains 91% and 95% after 4 h and 1 h exposure to 1.5 mol l(-1) EG and 1.5 mol l(-1) methanol, respectively, at which time the larval concentration of EG and methanol has risen to 1.21 mol l (-1) and 1.13 mol l(-1), respectively. These results suggest that CPAs such as EG and methanol do permeate Anopheles larvae to up to 81% and 75% of equilibrium, respectively, before the exposure becomes toxic.
Title: Toxicity of methanol to fish, crustacean, oligochaete worm, and aquatic

Attachment O – Methanol

ecosystem.

Author(s): Kavirai A, Bhunia F, Saha NC.

Source: Int J Toxicol. 2004 Jan-Feb;23(1):55-63.

Abstract: Static renewal bioassays were conducted in the laboratory and in outdoor artificial enclosures to evaluate toxic effects of methanol to one teleost fish and two aquatic invertebrates and to limnological variables of aquatic ecosystem. Ninety-six- hour acute toxicity tests revealed cladoceran crustacea Moina micrura as the most sensitive to methanol (LC50, 4.82 g/L), followed by freshwater teleost Oreochromis mossambicus (LC50, 15.32 g/L) and oligochaete worm Branchiura sowerbyi (LC50, 54.89 g/L). The fish, when exposed to lethal concentrations of methanol, showed difficulties in respiration and swimming. The oligochaete body wrinkled and fragmented under lethal exposure of methanol. Effects of five sublethal concentrations of methanol (0, 23.75, 47.49, 736.10, and 1527.60 mg/L) on the feeding rate of the fish and on its growth and reproduction were evaluated by separate bioassays. Ninety-six-hour bioassays in the laboratory showed significant reduction in the appetite of fish when exposed to 736.10 mg/L or higher concentrations of methanol. Chronic toxicity bioassays (90 days) in outdoor enclosures showed a reduction in growth, maturity index and fecundity of fish at 47.49 mg/L or higher concentrations of methanol. Primary productivity, phytoplankton population, and alkalinity of water were also reduced at these concentrations. Chronic exposure to 1527.60 mg/L methanol resulted in damages of the epithelium of primary and secondary gill lamellae of the fish. The results revealed 23.75 mg/L as the no-observed-effect concentration (NOEC) of methanol to freshwater aquatic ecosystem.

Title: Toxicity and sublethal effects of methanol on swimming performance of juvenile Florida pompano

Author(s): Baltz DM, Chesney EJ, Tarr MA, Kolok AS, Bradley MJ

Source: Transactions of the American Fisheries Society, 2005, Vol. 134/3, pp. 730-740

Abstract: Deepwater petroleum production requires that a number of additives, such as methanol, be transported offshore in large quantities, posing the risk of spills. Although these additives may not be highly toxic per se, it is important to evaluate and understand the risks to marine organisms, especially fishes that are often strongly associated with oil production facilities. We evaluated the toxicity and sublethal effects of methanol on the swimming performance of juvenile Florida pompano Trachinotus carolinus. A 24-h static exposure test identified the median lethal concentration as 1.28% (volume per volume) at 30 practical salinity units and 25°C. The mean critical swimming speed (U_{crit}) of juveniles (20.50 ' 4.59 g; mean ' SE) was evaluated before and after exposure to a 1.07% concentration and showed that U_{crit} was significantly reduced ($P < 0.0002$) from 90.10 ' 1.35 cm/s to 84.20 ' 1.36 cm/s in postexposure trials. After exposure and

Attachment O – Methanol

a 17-h recovery period in clean seawater, the mean 6.5% decline in performance of the treatment group contrasted sharply with a mean increase of 4.0% in the control group, indicating that conditioning, training effects, or both were significantly surpassed by the negative sublethal effects of methanol exposure.

Attachment P – Styrene

Health Effects Studies (acute toxicity, subchronic toxicity, chronic toxicity – e.g., including reproductive/developmental toxicity, neurotoxicity, immunotoxicity, organ system effects, mutagenicity/genotoxicity, carcinogenicity)

Title: Mutagenic potential of styrene in man.
Author(s): Watanabe T, Endo A, Sato K, Ohtsuki T Mivasaka M, Koizumi A, Ikeda M.
Source: Ind Health 1981;19(1):37-45.
Abstract: Not available.

Title: Styrene causes SCE in mice...:
Author(s): Conner, MK, Alarie, Y & Dombroske RL (1980)
Source: Food and Cosmetics Toxicology Volume 19, 1981, Page 516
Abstract: Sister chromatid exchange in murine alveolar macrophages, bone marrow, and regenerating liver cells induced by styrene inhalation.

Title: Genetic toxicity of styrene and some of its derivatives.
Author(s): Norpoa H, Vainio H.
Source: Scand J Work Environ Health. 1983 Apr;9(2 Spec No):108-14.
Abstract: Styrene, an important plastic monomer, is mutagenic after metabolic activation in several test systems. Probably because of an unfavorable activation : inactivation ratio, some mutagenicity assays have not, however, found styrene mutagenic. Styrene is converted by microsomal monooxygenases in vivo to styrene-7,8-oxide, which is a well-known mutagen. Arene oxides have also been proposed as the reactive metabolites of styrene, but the significance of these compounds is not yet fully understood. Only few derivatives of styrene have been tested for mutagenicity. The results are characterized by difficulties in metabolic activation. Many styrene-7,8-oxide analogues substituted at the phenyl ring are electrophilic reactants and mutagenic in vitro. Human whole-blood lymphocyte cultures have a peculiar feature, le, styrene and many of its analogues substituted at the ring or vinyl chain induce sister chromatid exchanges in the cultured cells without exogenous metabolizing systems. This activation is brought about by erythrocytes present in the cultures and probably results from the conversion of styrenes to styrene-7,8-oxides.

Title: A physiologically-based description of the inhalation pharmacokinetics of styrene monomer in rats and humans
Author(s): Andersen ME, Ramsey JC.
Source: Toxicology Letters, Volume 18, Supplement 1, 28 August-3 September 1983, Page 140
Abstract: Not available.

Attachment P – Styrene

Title: A physiologically based description of the inhalation pharmacokinetics of styrene in rats and humans.

Author(s): Ramsey JC, Andersen ME.

Source: Toxicol Appl Pharmacol. 1984 Mar 30;73(1)159-75.

Abstract: A physiologically based pharmacokinetic model which describes the behavior of inhaled styrene in rats accurately predicts the behavior of inhaled styrene in humans. The model consists of a series of mass-balance differential equations which quantify the time course of styrene concentration within four tissue groups representing (1) highly perfused organs, (2) moderately perfused tissues such as muscle, (3) slowly perfused fat tissue, and (4) organs with high capacity to metabolize styrene (principally liver). The pulmonary compartment of the model incorporates uptake of styrene controlled by ventilation and perfusion rates and the blood:air partition coefficient. The metabolizing tissue group incorporates saturable Michaelis-Menten metabolism controlled by the biochemical constants Vmax and Km. With a single set of physiological and biochemical constants, the model adequately simulates styrene concentrations in blood and fat of rats exposed to 80, 200, 600, or 1200 ppm styrene (data from previously published studies). The simulated behavior of styrene is particularly sensitive to changes in the constants describing the fat tissue group, and to the maximum metabolic rate described by Vmax. The constants used to simulate the fate of styrene in rats were scaled up to represent humans. Simulated styrene concentrations in blood and exhaled air of humans are in good agreement with previously published data. Model simulations show that styrene metabolism is saturated at inhaled concentrations above approximately 200 ppm in mice, rats, and humans. At inhaled concentrations below 200 ppm, the ratio of styrene concentration in blood to inhaled air is controlled by perfusion limited metabolism. At inhaled concentrations above 200 ppm, this ratio is controlled by the blood:air partition coefficient and is not linearly related to the ratio attained at lower (nonsaturating) exposure concentrations. These results show that physiologically based pharmacokinetic models provide a rational basis with which (1) to explain the relationship between blood concentration and air concentration of an inhaled chemical, and (2) to extrapolate this relationship from experimental animals to humans.

Title: Inhalation pharmacokinetics: evaluating systemic extraction, total in vivo metabolism, and the time course of enzyme induction for inhaled styrene in rats based on arterial blood:inhaled air concentration ratios.

Author(s): Andersen ME, Gams ML, Ramsey JC.

Source: Toxicol Appl Pharmacol. 1984 Mar 30;73(1):176-87

Abstract: A method is described for evaluating systemic extraction of soluble vapors during inhalation exposures. The physiological basis of the method is the inability to

Attachment P – Styrene

achieve complete equilibrium of vapor between arterial blood and inhaled air whenever there is substantial extraction of the soluble vapor during a single pass through the systemic circulation. The technique was applied to estimate styrene extraction ratios at the end of 6-hr exposures in male rats exposed to various concentrations of inhaled styrene. From extraction ratios and several physiological constants, metabolic constants were evaluated for styrene metabolism in vivo. In naive rats, the maximum velocity of metabolism was 10.0 mg/kg/hr, and Km was of the order of 0.2 mg/liter. Pretreatment with pyrazole (320 mg/kg, 1/2 hr before exposure) essentially abolished in vivo styrene metabolism, while pretreatment with phenobarbital (80 mg/kg/day for the 4 days before styrene exposure) increased Vmax about sixfold. Prior exposure to styrene (1000 ppm for 6 hr/day on each of 4 days before experimentation) increased Vmax by a factor of 2. Significant induction of styrene metabolism in vivo was observed in 24-hr continuous exposure to 400, 600, or 1200 ppm. A curve fitting routine was employed with a physiological model of styrene inhalation kinetics to estimate the dynamics of the induction process in the 24-hr exposures. At 400 ppm, induction began after a lag of 15.5 hr, had a half-life of 3.5 hr, and reached 2.7 times the Vmax in naive rats. At 600 ppm, it began after 10.6 hr, proceeded with a half-life of 2.2 hr, and increased Vmax by 3.4 times. At 1200 ppm, induction began earlier, 4.6 hr, and reached a greater value, 4.4 times Vmax, but had a half-life similar to that at 600 ppm. No induction occurred in 48-hr exposure to 200 ppm. Induction complicates kinetic modeling of continuous inhalation with soluble, well-metabolized vapors because it is time and concentration dependent. These methods should prove useful for studying the in vivo metabolism of other soluble, well-metabolized vapors and for examining the time course of induction of the metabolizing enzymes for these chemicals.

Title: Evaluation of low exposure to styrene. I. Absorption of styrene vapours by inhalation under experimental conditions.

Author(s): Wieczorek H, Piotrowski JK.

Source: Int Arch Occup Environ Health. 1985;57(1):57-69.

Abstract: Volunteers (six men and one woman) were exposed by inhalation to styrene within the concentration range of 20 to 200 mg/m3. The average retention of styrene vapours in the respiratory tract was 71%. The yield of styrene metabolism measured within 24 h was 39 and 17% for mandelic acid and phenylglyoxylic acid, respectively. The determination of mandelic acid in urine collected immediately after the exposure was applied as exposure test. The excretion rate of this metabolite assured the best correlation with the absorbed dose. The relative standard deviations of the test related to actual dose level vary, depending on the analysed concentration range, from 0.21 to 0.33. Quantitative interpretation of the test is possible for styrene concentrations in the

Attachment P – Styrene

air exceeding 20 mg/m3. The concentration amounting to 100 mg/m3 (TLV) corresponds with the mandelic acid excretion rate of 15 mg per hour.

Title: Evaluation of low exposure to styrene. II. Dermal absorption of styrene vapours in humans under experimental conditions.
Author(s): Wieczorek H.
Source: Int Arch Occurs Environ Health. 1985;57(1):71-5.
Abstract: Four volunteers were exposed dermally to styrene vapours within the concentration range of 1300 to 3200 mg/m3. The increase in the levels of mandelic and phenylglyoxylic acids in urine after exposure was strongly noticeable. The dermal vapour absorption coefficient (alpha) was calculated: for styrene it was ca. 0.022 m3/h. It was calculated that the dermal absorption of the styrene vapours contributed about 5% to the amount absorbed in the respiratory tract under the same conditions.

Title: Mutagenic action of styrene and its metabolites. II. Genotoxic activity of styrene, styrene oxide, styrene glycol and benzoic acid tested with the SOS chromotest.
Author(s): Glośnicka R, Dziadziuszko H.
Source: Bull Inst Marit Trop Med Gdynia. 1986;37(3-4):295-302.
Abstract: Not available.

Title: A pharmacokinetic model of styrene inhalation with the fugacity approach.
Author(s): Paterson S, Mackay D.
Source: Toxicol Appl Pharmacol. 1986 Mar 15;82(3):444-53.
Abstract: The physiologically based pharmacokinetic model of J. C. Ramsey and M. E. Andersen (1984, Toxicol. Appl. Pharmacol. 73, 159-175) of styrene inhalation in rats, with extrapolation to humans, was reformulated with the chemical equilibrium criterion of fugacity instead of concentration to describe compartment partitioning. Fugacity models have been used successfully to describe environmental partitioning processes which are similar in principle to pharmacokinetic processes. The fugacity and concentration models are mathematically equivalent and produce identical results. The use of fugacity provides direct insights into the relative chemical equilibrium partitioning status of compartments, thus facilitating interpretation of experimental and model data. It can help to elucidate dominant processes of transfer, reaction and accumulation, and the direction of diffusion. Certain model simplifications become apparent in which compartments which remain close to equilibrium may be grouped. Maximum steady-state tissue concentrations for a known exposure may be calculated readily. It is suggested that pharmacokinetic fugacity models can complement conventional concentration models and may facilitate linkage to fugacity models describing environmental sources, pathways, and exposure

Attachment P – Styrene

routes.

Title: Reproductive toxicology of inhaled styrene oxide in rats and rabbits.
Author(s): Sikov MR, Cannon WC, Carr DB, Miller RA, Niemeier RW, Hardin BD.
Source: J Appl Toxicol. 1986 Jun;6(3):155-64.
Abstract: Experiments were performed to evaluate reproductive and developmental toxicology in rats and rabbits exposed to styrene oxide by inhalation. Female rats were exposed to 100 or 300 ppm styrene oxide or to filtered air for 7 h/day, 5 days/week for 3 weeks. Extensive mortality occurred in rats that received prolonged exposure to 100 ppm styrene oxide while 300 ppm was rapidly lethal. As a result exposures were terminated in this latter group and the group was eliminated from further study. The rats of the 0 and 100 ppm groups were then mated and exposed to 0 or 100 ppm styrene oxide daily through 18 days of gestation (dg). Female rabbits were artificially inseminated and exposed for 7 h daily to 0, 15, or 50 ppm styrene oxide through 24 dg. Both of these lower concentrations used for exposure of the rabbits produced mortality of does. The rats were killed at 20 dg and the rabbits at 30 dg. Pregnant animals were examined for toxic changes including altered tissue weights and histopathologic effects. Litters were evaluated using several measures of embryotoxicity, and live fetuses were examined for external, visceral, and skeletal malformations. Exposure during gestation appeared to increase preimplantation loss in rats, and tended to increase the incidence of resorptions in rabbits. In both species, fetal weights and crown-rump lengths were reduced by gestational exposure. The incidences of ossification defects of the sternebrae aned occipital bones were increased by gestational exposure of rats to styrene oxide. These results indicate that inhalation exposures at these concentrations produce reproductive and development toxicity, as well as maternal toxicity.

Title: The effect of aerosol on estimates of inhalation exposure to airborne styrene.
Author(s): Malek RF, Daisey JM, Cohen BS.
Source: Am Ind Hyg Assoc J. 1986 Sep;47(9):524-9.
Abstract: Exposure to volatile organic contaminants usually is attributed to vapors alone; samples are collected on charcoal tubes or by passive dosimeters. This study demonstrated that aerosols, generated during the spraying of polyester resin solution, can contribute significantly to the exposure to volatile organic contaminants. Four spraying experiments were performed during which 64 samples were collected and analyzed to determine the styrene air concentrations. The results from the four spraying experiments showed that aerosols represented 30% +/- 3% of the total air concentration of styrene. The contribution of aerosols to inhalation exposure needs to be considered in other industrial situations where spray processes are used.

Attachment P – Styrene

Title: The effect of aerosol on estimates of inhalation exposure to airborne styrene.
Author(s): Murphy JH.
Source: Am Ind Hyg Assoc J. 1987 Jan;48(1):A14, A16.
Abstract: Not available.

Title: Hearing loss in rats caused by inhalation of mixed xylenes and styrene.
Author(s): Pryor GT, Rebell CS, Howd RA.
Source: J Appl Toxicol. 1987 Feb;7(1):55-61.
Abstract: We have reported that inhalation exposure of rats to toluene causes permanent hearing loss, e.g. Pryor et al. Neurobehay. Toxicol. Teratol. 5, 53-62 (1983). Therefore, it was of considerable interest to examine the ototoxic potential of two structurally related solvents--mixed xylenes and styrene--compared with that of toluene. Male, weanling Fischer-344 rats were exposed to clean air or solvents in four identical 62.5 l Plexiglas chambers. Exposures to 800, 1000, and 1200 ppm were daily for 14 hours/day and lasted 6 weeks for mixed xylenes, 3 weeks for styrene. An additional experiment with xylenes examined the effect of exposure for only 4 (1700 ppm) or 8 (1450 ppm) hours or for 8 (1450 ppm) hours on three consecutive days to compare the results with those obtained with toluene under comparable exposure schedules. Both xylenes and styrene caused marked hearing loss as assessed by behavioral (conditioned avoidance) and electrophysiologic (brainstem auditory-evoked response) methods. Moreover, both solvents appeared to be more potent ototoxicants than toluene, as indicated by effective concentration, effective durations of exposure, and the range of frequencies affected.

Title: Long-term carcinogenicity bioassays on styrene administered by inhalation, ingestion and injection and styrene oxide administered by ingestion in Sprague-Dawley rats, and para-methyistyrene administered by ingestion in Sprague-Dawley rats and Swiss mice.
Author(s): Conti B, Maltoni C, Perino G, Ciliberti A.
Source: Ann N Y Aced Sci. 1988;534:203-34.
Abstract: Styrene was administered to Sprague-Dawley rats by inhalation (300, 100, 50, 25, 10 and 0 ppm, 4 hours daily, 5 days weekly, for 52 weeks); by gavage (250, 50 and 0 mg/kg b.w. in olive oil, once daily, 4-5 days weekly, for 52 weeks), by intraperitoneal injection (50 and 0 mg in olive oil, four times at 2-month intervals), by subcutaneous injection (50 and 0 mg in olive oil, once). Styrene oxide was administered to Sprague-Dawley rats by gavage as styrene (250, 50 and 0 mg/kg b.w. in olive oil, once daily, 4-5 days weekly, for 52 weeks). The animals were kept under observation until spontaneous death. Para-methylstyrene was also administered by gavage to Sprague-Dawley rats at 500, 250, 50, 10 and 0 mg/kg

Attachment P – Styrene

b.w., and to Swiss mice at 250, 50, 10 and 0 mg/kg b.w., in olive oil, once daily, 5 days weekly, for 108 weeks and 78 weeks, respectively. The study was terminated when the survival rate reached 50% in at least one experimental group. Styrene, when given by inhalation, was found to cause an increase in total (benign and malignant) and malignant mammary tumors. Styrene oxide produced a high incidence of tumors in the forestomach (papillomas, acanthomas, and in situ and invasive squamous cell carcinomas). Para-methylstyrene was not shown to be carcinogenic.

Title: Styrene-induced immunomodulation in mice
Author(s): R.K.S. Dogra, S. Khanna, S.N. Srivastava, L.J. Shukla and R. Shanker
Source: International Journal of Immunopharmacology, Volume 11, Issue 5, 1989, Pages 577-586
Abstract: Male mice given different oral doses (0.05, 0.03 or 0.02 a LD_{50}/animal/day) of styrene (LD_{50} = 1 g/kg) daily for 5 days did not incite any overt toxicity in lymphoid organs or on hematologic parameters. At the tested dose levels styrene produced a mild reduction in the organ weight of adrenal and spleen and slight reduction in the cellular viability of lymph nodes. There was a dose-dependent suppression in the humoral immune response (IgM-producing PFC5 of spleen and serum anti-SRBC HA titre) to SRBC. The proliferative response to the B-cell mitogen, LPS however revealed a significant increase in the incorporation of ^{3}HT with middle and lowest doses of styrene. The results of cell- mediated immunity appeared somewhat unexpected and more complex as exposure resulted in a dose-dependent enhancement in the cutaneous DTH reaction to SRBC together with increased blastogenic response of splenic lymphocytes to phytohaemagglutinin (PHA). Additionally, there was significant impairment in the functional activity (NBT reduction, attachment and phagocytic indices) of nonadherent and adherent peritoneal exudate cells. Based on the present data the study identifies the immunotoxic potential of styrene and which acts differently on various arms of the rodent's immune system.

Title: Urinary excretion of mandelic, phenylglyoxylic, and specific mercapturic acids in rats exposed repeatedly by inhalation to various concentrations of styrene vapors.
Author(s): Truchon G, Gerin M Brodeur J.
Source: Can J Physiol Pharmacol. 1990 May;68(5):556-61.
Abstract: Adult male Sprague-Dawley rats were exposed by inhalation to various concentrations of styrene vapors (25, 50, 100, or 200 ppm) 6 h/day, 5 days/week, for 4 consecutive weeks. The concentrations were varied from day to day according to a random pattern allowing treated animals to be exposed five times to each concentration of styrene. Each day, the following urinary metabolites

Attachment P – Styrene

were analysed from samples collected during exposure (0-6 h) and after exposure (6-24 h): mandelic acid; phenylglyoxylic acid; and two mercapturic acids, N-acetyl-S-(1-phenyl-2-hydroxyethyl)-L-cysteine (M1) and N-acetyl-S(2-phenyl-2-hydroxyethyl)-L-cysteine (M2). Various parameters of renal toxicity and hepatic microsomal and cytosolic enzyme activities were also measured. The results show that there is a very good relationship between the excretion of all four styrene metabolites and the degree of daily exposure to styrene over the entire period of urine collection, with correlation coefficients ranging from 0.82 to 0.98. The correlation was poor for mandelic acid during the 0-6 h period. There was no evidence that repeated exposure to styrene caused renal toxicity, nor induced hepatic microsomal enzyme activities; cytosolic glutathione S-transferase activity was increased moderately by 1.5 times. Thus, under conditions of exposure to styrene likely to be found in the workplace, all four metabolites measured were good indicators of styrene exposure throughout the length of the experiment. Since mercapturic acids result from the conjugation of styrene oxide with glutathione, the data suggest that measurement of these metabolites offers the possibility to monitor internal exposure to a toxic electrophilic compound more directly.

Title: Pulmonary toxicity of inhaled styrene in acetone-, phenobarbital- and 3-methylcholanthrene-treated rats.

Author(s): Elovaara E Vainio H, Aitio A.

Source: Arch Toxicol. 1990;64(5):365-9.

Abstract: Pulmonary changes in glutathione (GSH) indicated by the concentration of non-protein sulphydryls showed a decrease of 43% in rats exposed for 5 h per day three times to 500 cm3/m3 (2100 mg/m3) styrene vapour. In these rats, only a marginal decrease was observed in the pulmonary cytochrome P450 oxidative metabolism. Following a single 24-h inhalation exposure to 500 cm3/m3 styrene, the decreases in GSH were 66% in lung but only 16% in liver. On the other hand, a multifold increase in the disposition of thioether compounds was found in urine. Pulmonary cytochrome P450-dependent metabolism was decreased, shown by low residual activities of 7-ethoxyresorufin (less than 20%), 7-ethoxycoumarin (53%) and 7-pentoxyresorufin 0-dealkylases (76%). Epoxide hydrolase and GSH S-transferase enzyme activities which catalyze styrene detoxification were not decreased. Styrene exposure (24 h) of acetone-, phenobarbital- or 3-methylcholanthrenepretreated rats resulted in pulmonary effects different from each other and from those of styrene alone. Acetone potentiated the lung effect and elevated 1.5-fold urine thioether output. Inducer pretreatment seemed to be a factor aggravating styrene toxicity; in effect this was clearest in acetone-induced rats. In general, GSH depletion accompanied by inhibition of cytochrome P450-dependent oxidative drug metabolism were the earliest biochemical lesions

Attachment P – Styrene

manifested in styrene-exposed lung.

Title: Metabolism of inhaled styrene in acetone-, phenobarbital- and 3-methylcholanthrene-pretreated rats: stimulation and stereochemical effects by induction of cytochromes P45011E1, P4501IB and P4501A.
Author(s): Elovaara E Engstrom K, Nakaiima T, Park SS, Gelboin HV, Vainio H.
Source: Xenobiotica. 1991 May;21(5):651-61.
Abstract: 1. The effect of various cytochrome P-450 inducers, namely acetone, phenobarbital (PB) and 3-methylcholanthrene (MC), on the pharmacokinetics of styrene metabolism was studied. 2. Styrene metabolism in vivo was studied measuring phenylglyoxylic acid (PGA), the enantiomers of mandelic acid (MA), and total thioethers excreted in the urine during a 24 h period of airborne exposure to styrene at 500 cm3/m3 (2100 mg/m3). In acetone-pretreated rats, PGA and MA and thioether formation were elevated 30-50%. The R/S ratio of MA enantiomers was about two in all styrene-exposed groups except PB-pretreated rats, which showed a ratio of four. 3. Styrene metabolism in liver microsomes measured in vitro was increased by styrene 140%, acetone plus styrene by 190%, methylcholanthrene plus styrene by 180% and phenobarbital plus styrene by 250%. 4. N-Nitrosodimethylamine demethylation (NDMAD) and 7-pentoxyresorufin dealkylation (PROD) in liver microsomes were enhanced 100-150% by styrene inhalation. The metabolism of 7-ethoxyresorufin was not significantly enhanced. 5. Monoclonal antibodies to P-450 1A1, IA2, I1B1 and 11E1 were utilized to identify cytochrome P- 450s by Western blot analysis. These studies showed clearly that styrene inhalation induced principally cytochrome P4501E1, whereas styrene given by gavage at a high narcotic dosage induced both P45011E1 (NDMAD, 60%) and P4501IB (PROD, 3000%). 6. Our conclusions are that styrene metabolism in vivo in both autoinduced and induced by other foreign compounds, that cytochrome P45011E1 induction has a major impact on styrene metabolism and that P45011B1 induction yields an altered MA metabolite enantiomer ratio.

Title: Cytogenetic studies of mice exposed to styrene by inhalation
Author(s): Kligerman AD, J Bryant, JA Campbell, BW Collins, CL Doerr, GL Erexson, P Kwanyuen and DL Morgan
Source: Mutation Research/Environmental Mutagenesis and Related Subjects Volume 271, Issue 2, 1992, Page 173
Abstract: European environmental mutagen society selected poster abstract of the 21st annual meeting of the European environmental mutagen society, 25-31 August 1991, Prague (Czechoslovakia)

Title: Cytotoxic and genotoxic effects of styrene-7,8-oxide in neuroadrenergic Pc 12

Attachment P – Styrene

cells.

Author(s): Dypbukt JM Costa LG, Manzo L, Orrenius S, Nicotera P.

Source: Carcinooenesis. 1992 Mar;13(3):417-24.

Abstract: Exposure of Pc 12 cells to styrene-7,8-oxide (SO) (0.5-1 mM) caused a rapid increase in cytosolic Ca2+, depletion of intracellular glutathione and ATP, DNA damage and loss of cell viability. Lower SO concentrations (less than or equal to 100 microM), did not cause loss of cell viability or affect cell growth rate. However, at 30 and 100 microM, SO stimulated the formation of alkali-sensitive, DNA single-strand breaks (SSB). DNA SSB were fully repaired when cells exposed to 30 microM SO were subsequently incubated for 3 h in fresh medium, whereas DNA repair was only partial after exposure to 100 microM SO. When cells exposed to 30 or 100 microM SO were incubated with the inhibitors of repair synthesis 1-beta-D-arabinofuranosyl-cytosine (AraC) and hydroxyurea (HU), SSB accumulated, indicating the involvement of the excision- repair system in the removal of DNA lesions. A SO adduct with guanine at the N7 position was detected in the DNA extracted from treated cells. SO did not induce the formation of double-strand breaks, interstrand cross-links, or DNA- protein cross-links. Although cells exposed to 30 or 100 microM SO underwent normal cell division, latent DNA damage was retained for up to 14 subsequent replicative cycles. In addition, SO-treated cells partially lost their normal ability to differentiate in response to nerve growth factor (NGF) stimulation. NGF failed to induce differentiation in cells that had replicated for 20 generations after exposure to 100 microM SO. Spontaneous differentiation stimulated by high-density culture was also inhibited in SO-treated cells. These results indicate that non-lethal concentrations of SO can cause modifications that compromise the ability of Pc 12 cells to respond to NGF and differentiate.

Title: Cytogenetic studies of mice exposed to styrene by inhalation.

Author(s): Kligerman AD, Allen JW, Bryant MF, Campbell JA, Collins BW, Doerr CL, Erexson GL, Kwanyuen P, Morgan DL.

Source: Mutat Res. 1992 Jul;280(1):35-43.

Abstract: The data for the in vivo genotoxicity of styrene (STY) are equivocal. To evaluate the clastogenicity and sister-chromatid exchange (SCE)-inducing potential of STY in vivo under carefully controlled conditions, B6C3F1 female mice were exposed by inhalation for 6 h/day for 14 consecutive days to either 0, 125, 250 or 500 ppm STY. One day after the final exposure, peripheral blood, spleen, and lungs were removed and cells were cultured for the analysis of micronucleus (MN) induction using the cytochalasin B-block method, chromosome breakage, and SCE induction. Peripheral blood smears were also made for scoring MN in erythrocytes. There was a significant concentration-related elevation of SCE frequency in lymphocytes from the spleen and the peripheral blood as well as in

Attachment P – Styrene

cells from the lung. However, no statistically significant concentration-related increases were found in the frequency of chromosome aberrations in the cultured splenocytes or lung cells, and no significant increases in MN frequencies were observed in binucleated splenocytes or normochromatic erythrocytes in peripheral blood smears.

Title: Peripheral markers of neurochemical function among workers exposed to styrene.
Author(s): Checkoway H, Costa LG, Camp J, Coccini T, Daniell WE, Dills RL
Source: Br J Ind Med. 1992 Aug;49(8):560-5.
Abstract: A cross sectional study of biological markers of neurochemical function in peripheral blood cells, and self reported nervous system symptoms, was conducted among 60 workers exposed to styrene in three reinforced plastics plants and 18 reference workers not exposed to styrene or other solvents. Concentrations of styrene in the air at the plants ranged from less than 1 to 160 ppm. Biomarkers of neurochemical function measured were: sigma receptor binding in lymphocytes, monoamine oxidase type B (MAO-B) activity in platelets, and serotonin uptake by platelets. Blood styrene concentration was used as the exposure index to take account of the use of protective equipment and dermal uptake. Four blood styrene exposure groups were defined as: non-exposed (reference) and exposed to less than 0.05, 0.05-0.19, and greater than or equal to 0.20 micrograms/ml. The prevalences of headache, dizziness, light headedness, fatigue, irritability, memory loss, and feeling "drunk" at work increased with increasing blood styrene concentration. No effect on sigma receptor binding was seen. A slight positive correlation was found for uptake of serotonin, which has been used as an exposure related effect indicator in previous studies of workers exposed to solvents. The MAO-B activity decreased with increasing blood styrene concentration; the mean (SE) MAO-B values for the four groups were 34.2 (3.0), 28.1 (5.3), 20.1 (4.8), and 16.9 (7.7) pmol/10(7) cells/min. The MAO-B activity also correlated negatively with the number of reported nervous system symptoms, whereas no associations were seen between prevalence of symptoms and either serotonin uptake or sigma receptor binding. The findings for MAO-B activity are consistent with previously reported experimental data, and suggest that MAO-B may be a useful marker of styrene neurotoxicity.

Title: Studies of the induction of chromosomal aberration and sister chromatid exchange in rats exposed to styrene by inhalation.
Author(s): Preston RJ, Abernethy DJ.
Source: IARC Sci Publ. 1993;(127):225-33.
Abstract: A large number of studies have been reported on the genotoxicity of styrene in

Attachment P – Styrene

vitro and in vivo and the potential effects on humans of occupational exposure. Because of a variety of technical problems and difficulties in data interpretation, it has not been clearly established whether styrene can induce chromosomal aberrations and/or sister chromatid exchange (SCE) in vivo in animals or humans. The importance of clarifying this situation led to the development of the study described in this paper. Male Fischer 344 rats were exposed to styrene at concentrations of 150, 500 or 1000 ppm for 6 h/day on 5 days/week for 4 weeks. A negative control (air) was included. An additional control (ethylene oxide, 150 ppm) group was included in an attempt to establish the usefulness of rat lymphocytes for cytogenetic analysis in this protocol of long-term exposure by inhalation. The choice of agent and of exposure was based on the expectation that they would produce a positive response for SCE and/or chromosomal aberrations under the assay conditions used. Peripheral blood samples were drawn at 1, 2, 3 and 4 weeks of exposure and at 4 weeks after the end of exposure. Cultures were established, and SCE (second mitosis) and chromosomal aberrations (first mitosis) were analysed. The frequency of chromosomal aberrations was not increased over that in the air controls in the animals exposed to styrene or ethylene oxide at any of the sampling times. Styrene did not induce SCE at any of the concentrations or sampling times; however, the frequency of SCE was increased following exposure to ethylene oxide at all sampling times, with a positive exposure-response relationship with time of exposure as the variable. The data are compared with other, similar sets reported in the literature, and their significance for predicting responses in people occupationally exposed to styrene is discussed.

Title: Genetic toxicity of 1,3-butadiene and styrene.
Author(s): Norppa H, Sorsa M.
Source: IARC Sci Publ. 1993;(127):185-93.
Abstract: 1,3-Butadiene and styrene (vinyl benzene) are indirect genotoxins, which require metabolic activation to an epoxide form in order to bind covalently to DNA. Styrene 7,8-oxide, the active metabolite of styrene, is a carcinogen in rodents and has been shown to be genotoxic in most in-vitro test systems and at various genetic endpoints. The few studies available on the genotoxicity of styrene 7,8-oxide in vivo have yielded negative or (in mice) weakly positive results. Styrene is not usually genotoxic in vitro in assays employing a microsomal preparation from rat liver for metabolic activation, but positive effects have been obtained when other sources of metabolic activation, such as human erythrocytes, were provided. In vivo, styrene has been found repeatedly to be weakly genotoxic in the assay for sister chromatid exchange, especially in mice. Cytogenetic damage (usually chromosomal aberrations) has been reported in many studies of workers, mainly from the reinforced plastics industry where ambient

Attachment P – Styrene

concentrations of styrene may be high (50-100 ppm), while most negative findings are associated with exposure to lower levels. Butadiene is metabolized to two reactive forms, 1,2-epoxy-3-butene and further to 1,2:3,4-diepoxybutane, both of which are genotoxic in various test systems in vitro. The lowest effective dose of the latter is 1-2 orders of magnitude higher than that of the respective monoepoxide. Butadiene itself has not been tested extensively for genotoxicity in vitro. A species-specific difference in the responses of mice and rats at various cytogenetic end-points is seen in vivo, the lowest effective concentrations in rats being clearly higher than those in mice.(ABSTRACT TRUNCATED AT 250 WORDS)

Title: Dose-dependent genotoxic effects of styrene on human blood lymphocytes and the relationship to its oxidative and metabolic effects.

Author(s): Chakrabarti S Duhr MA, Senecal-Quevillon M, Richer CL.

Source: Environ Mol Mutagen, 1993;22(2):85-92.

Abstract: Although the genotoxic potential of styrene is known, very limited information is available regarding its dose-dependent genotoxic response to human blood lymphocytes and how such response correlates with different metabolic events in whole blood lymphocytes. The present study was therefore carried out to study such a relationship using in vitro human blood lymphocytes from healthy volunteers. To study genotoxic response to styrene, sister chromatid exchanges (SCEs), cell cycle, and cell survival were analyzed. Lymphocytes were cultured for 72 hr in the presence of different concentrations of styrene (0-1,000 microM). Twenty-four hr before harvest, BrdU (5 micrograms/m1) was added to assess the increase in SCEs and cell cycle delay. Both the SCE frequency and the cell cycle length were increased linearly with increasing concentrations of styrene up to 200 microM, without addition of any exogenous metabolizing system. Above 200 microM, no further increase in genotoxic response occurred. The range of concentrations (10-200 microM) at which increase of cell cycle length due to styrene was observed did not impair the viability of the cells, suggesting that such cell cycle delay is a genotoxic-related event and not caused by cytotoxicity. In vitro metabolic transformation of styrene in whole-blood lymphocyte cultures without the presence of any exogenous metabolic activation system showed the formation of a reactive intermediate, styrene 7,8-oxide, to be capacity-limited, as verified from a nonlinear increase in the formation of styrene glycol. The value of such metabolic parameter reached a plateau above 200 microM styrene. The same phenomenon of saturation has also been observed with regard to other metabolic effects due to styrene in whole blood lymphocytes in culture, such as dose-dependent increase in lipid peroxidation and depletion of blood lymphocyte glutathione, Based on the relationship between the formation of different metabolic events and the genotoxicity of styrene, it may be possible that the

Attachment P – Styrene

genotoxic properties of styrene in human blood lymphocytes may be mediated initially not only by the formation of the presumably reactive styrene 7,8-oxide, but also by that of a reactive oxygen species as well. However, the present data are not sufficient enough to definitely identify the role of reactive oxygen species in such toxicity and therefore it warrants further study.

Title: Study of the neurobehavioural toxicity of styrene at low levels of exposure.
Author(s): Jégaden D, Amann D, Simon JF, Habault M, Leoctux B, Galopin P.
Source: Int Arch Occup Environ Health. 1993;64(7):527-31.
Abstract: Thirty workers in a dockyard exposed to concentrations of styrene lower than the TLV-TWA of 50 ppm and 30 control workers not subject to exposure but employed by the same company were subjected to three psychometric tests on one Monday morning and evening. The results were usually better in the evening than in the morning in both groups, which proves the lack of acute intoxication at the end of the day at this level of exposure. On the other hand, all of the tests conducted on the exposed subjects are significantly less good than those on the controls. The results suggest the existence of minor but significant organic mental disorders in the subjects exposed to a mean dose of 30 ppm in this study. These results are inconsistent with those of several recent studies. The advisability of lowering the TLV of 50 ppm in discussed.

Title: Dose-dependent kinetics of inhaled styrene in man.
Author(s): Löf A, Johanson G.
Source: IARC Sci Publ. 1993;(127):89-99.
Abstract: One female and one male volunteer were exposed to styrene vapour (2 h; 50 W) at four different concentrations (26, 77, 201 and 386 ppm). Styrene levels were measured in arterialized capillary blood during and after exposure by head-space gas chromatography, and the levels of mandelic acid in urine were analysed by high-performance liquid chromatography. Non-linear relationships between the level of exposure to styrene and the concentration of styrene in arterial blood and 0-5 h cumulative excretion of mandelic acid indicated metabolic saturation. A physiologically based pharmacokinetic model was used to estimate the maximum metabolic rate (Vmax) of styrene from data on blood styrene. According to the model, the Vmax is 2.9 mmol/h, and metabolic saturation occurs at concentrations of 100-200 ppm styrene, depending on the level of physical activity. To our knowledge, this is the first time that dose-dependent kinetics of styrene has been shown in humans.

Title: Cytogenetic studies of rodents exposed to styrene by inhalation.
Author(s): Kligerman AD, Allen JW, Erexson GL, Morgan DL.
Source: IARC Sci Publ. 1993;(127):217-24.

Attachment P – Styrene

Abstract: Female B6C3F1 mice and Fischer 344 rats were exposed to styrene at nominal concentrations of 125, 250 and 500 ppm by inhalation for 6 h per day for 14 consecutive days. One day after the final exposure, murine peripheral blood lymphocytes, spleen and lungs were removed, and the cells were cultured for analysis of chromosomal aberrations, micronucleus induction (using the cytochalasin B-block method) and sister chromatid exchange. Peripheral blood smears were scored for micronucleus induction in normochromatic erythrocytes. For the rats, peripheral blood lymphocytes were cultured for analyses of sister chromatid exchange, chromosomal aberrations and micronuclei in cytochalasin B-induced binucleated cells and were also examined in the single-cell gel assay for analysis of DNA strand breakage under alkaline conditions. Bone-marrow smears were made from femurs of rats for analysis of micronucleus induction in normochromatic erythrocytes. Small but statistically significant concentration-related increases in the frequency of sister chromatid exchange were seen in both mice and rats in all cell types examined. No statistically significant concentration-related increase in chromosomal aberration or micronucleus induction frequencies were observed in either species, and there was no significant increase in DNA strand breakage in peripheral blood lymphocytes from exposed rats. These results indicate that styrene is a weak inducer of sister chromatid exchange in vivo when administration to rodents by inhalation.

Title: Combined effects of solvents on the rat's auditory system: Styrene and trichloroethylene

Author(s): Charles S. Rebert, William K. Boyes, Gordon T. Pryor, David J. Svensgaard, Kara M. Kassay, G. Ross Gordon and Natasha Shinsky

Source: International Journal of Psychophysiology, Volume 14, Issue 1, January 1993, Pages 49-59

Abstract: Because exposures to toxic agents typically involve more than one substance, it is necessary to know if combined exposures pose different risks than those to single agents. Many solvents have been implicated on central nervous disorders and some of them are known to produce hearing loss, probably mediated by damage to cochlear hair cells. Hearing loss was studied by recording the brainstem auditory evoked response (BAER) in male Long Evans rats exposed 8 h/day for 5 days to mixtures of styrene (STY) and trichloroethylene (TCE). Dose groups included air or solvent pairs (STY/TCE) in the following concentrations (ppm): (0: 3000), (250 : 2250), (500: 1500), (750: 750) and (1000 : 0). Decreased BAER amplitude, indicative of hearing loss, was correlated with blood levels of total solvent. The effects were as predicted by a linear dose- addition model, indicating neither synergistic nor antagonistic interactions at the concentrations studied.

Attachment P – Styrene

Title: Styrene inhalation toxicity studies in mice. I. Hepatotoxicity in B6C3F1 mice.
Author(s): Morgan DL, Mahler JF, O'Connor RW, Price HC Jr, Adkins B Jr.
Source: Fundam Appl Toxicol. 1993 Apr;20(3):325-35.
Abstract: Studies were conducted to evaluate the toxic effects of short-term repeated styrene inhalation in B6C3F1 mice. Male and female mice were exposed to 0, 125, 250, or 500 ppm styrene, 6 hr/day, for up to 14 days. Styrene toxicity was characterized by severe centrilobular hepatic necrosis and deaths after one exposure to 500 ppm or two exposures to 250 ppm. Mortality and hepatotoxicity were not increased by additional exposures, and in surviving mice, regeneration and repair of initial hepatic injury occurred in spite of continued exposure for 14 days. A marked sex difference was observed, with male mice significantly more susceptible to styrene toxicity than females. A nonlinear dose response was observed where mortality in male and female mice was greater in the 250 ppm dose group than that in the 500 ppm dose group. Severe congestion and necrosis of the liver was present in moribund mice; hepatic congestion and serum alanine aminotransferase and sorbitol dehydrogenase were significantly greater in moribund animals.

Title: Styrene inhalation toxicity studies in mice. II. Sex differences in susceptibility of B6C3F1 mice.
Author(s): Morgan DL, Mahler JP, Dill JA, Price HC Jr, O'Connor RW, Adkins B Jr.
Source: Fundam Appl Toxicol. 1993 Oct; 21(3):317-25.
Abstract: Styrene is a commercially important chemical used in the production of plastics and resins. In initial short-term styrene inhalation studies, toxicity was significantly greater in male B6C3F1 mice than in females, suggesting that males may metabolize styrene more extensively and/or may be less able to detoxify reactive metabolites. In addition, a nonlinear dose- response was observed where toxicity and mortality were greater in mice exposed to 250 ppm than in those exposed to 500 ppm. These studies were conducted to investigate potential mechanism(s) for sex differences and the nonlinear dose- response in styrene toxicity by evaluating the effects of repeated styrene exposure on styrene oxide production, hepatic GSH availability, and hepatotoxicity in male and female B6C3F1 mice. Mice (36/sex/dose) were exposed to 0, 125, 250, or 500 ppm styrene 6 hr/day for up to 3 days. Styrene exposure caused increased mortality and hepatotoxicity (centrilobular necrosis, increased serum liver enzymes) in males and females after one or two exposures to 250 and 500 ppm. Hepatic GSH levels were decreased in a dose-dependent manner in males and females. After one exposure, GSH levels in males rebounded above controls in all dose groups. After three exposures to 125 or 250 ppm males appeared to maintain GSH levels; GSH was still decreased in the 500 ppm group. GSH levels in females were decreased after each exposure in all dose groups to lower levels

Attachment P – Styrene

than in males, and did not rebound above controls.(ABSTRACT TRUNCATED AT 250 WORDS)

Title: Styrene inhalation toxicity studies in mice. III. Strain differences in susceptibility.
Author(s): Morgan DL, Mahler JF, Dill JA, Price HC Jr, O'Connor RW, Adkins B Jr.
Source: Fundam Appl Toxicol. 1993 Oct;21(3):326-33.
Abstract: Inhalation toxicity studies were conducted to evaluate mouse strain differences in the susceptibility to styrene vapors. Male and female B6C3F1, C57BL/6, Swiss, and DBA/2 mice (8 weeks old) were exposed to 0, 125, 250, or 500 ppm styrene 6 hr/day, for 4 days (20/sex/dose). Histopathological changes and changes in liver weights were evaluated as a measure of hepatotoxicity. Styrene uptake and styrene-7,8-oxide (SO) formation were estimated by measuring levels of styrene and SO in blood. An estimate of SO detoxification by conjugation with GSH was obtained by measuring hepatic GSH depletion. In general, mortality, increased liver weights, and hepatocellular necrosis were observed in the 250 and 500 ppm dose groups for all strains and both sexes. Considerable sex and strain differences were observed. Mortality, increased liver weights, and hepatocellular necrosis were greatest in B6C3F1 and C57BL/6 mice in the 250 ppm dose group and in males; hepatotoxicity was similar in both strains. Swiss mice exhibited dose-dependent increases in mortality, liver weights, and in hepatocellular necrosis, with only slight sex differences at early time points. Hepatotoxicity in DBA/2, B6C3F1, and C57BL/6 strains was greater at 250 than 500 ppm; however, toxicity was less severe in DBA/2 than in other strains based on absence of mortality in either sex and less extensive liver necrosis at both 250 and 500 ppm. Blood styrene and SO levels did not correlate well with strain differences in toxicity.(ABSTRACT TRUNCATED AT 250 WORDS)

Title: Review of styrene and styrene oxide long-term animal studies.
Author(s): McConnell EE, Swenberq JA.
Source: Crit Rev Toxicol. 1994;24 Suppl:S49-55.
Abstract: Eleven long-term toxicity studies were reviewed on styrene and five on styrene oxide in an effort to evaluate the potential carcinogenic activity of these chemicals in animals. The styrene studies included inhalation exposure (rats, mice, guinea pigs, and rabbits), intragastric gavage (rats and mice), drinking water (rats), and intraperitoneal injection (rats), while styrene oxide exposure was via intragastric gavage (rats and mice) or skin painting (mice). Each study was reviewed and evaluated for details and adequacy of design, adequacy of reported data, and interpretation. The results of this review are 1. There was no convincing evidence of carcinogenic activity of styrene in animals, although many of the studies were considered inadequate. 2. Styrene oxide was carcinogenic to the forestomach of both sexes of rats and mice after gavage exposure and was

Attachment P – Styrene

associated with an increase in liver neoplasms in male mice in one study. No carcinogenic activity was observed in mice after dermal exposure (skin paint). 3. None of the studies of styrene or styrene oxide reported here are well suited for extrapolating potential carcinogenic activity of either compound to humans because all have deficiencies in design, conduct, interpretation, or utilized a less than ideal route of exposure. A chronic state-of-the-art inhalation study is needed to evaluate this aspect of hazard assessment.

Title: Determination of low level exposure to volatile aromatic hydrocarbons and genotoxic effects in workers at a styrene plant.

Author(s): Holz O, Scherer G, Brodtmeier S, Koops F, Warncke K, Krause T, Austen A, Ancierer J, Tricker AR, Adikofer F et al.

Source: Occup Environ Med. 1995 Jun;52(6):420-8.

Abstract: OBJECTIVES: Low exposures to volatile aromatic hydrocarbons and cytogenetic effects in peripheral white blood cells were determined in 25 healthy workers employed in different areas of a styrene production plant in the former German Democratic Republic. The results were compared with 25 healthy unexposed controls (matched for age and sex) employed in the same company. METHODS: The concentrations of aromatic hydrocarbons determined from active air sampling in all areas of the factory (styrene: 73-3540 micrograms/m3 (< 0.01-0.83 ppm); ethylbenzene 365-2340 micrograms/m3 (0.08-0.53 ppm); benzene 73 -3540 micrograms/m3 (< 0.02-1.11 ppm); toluene 54-2960 micrograms/m3 (0.01-0.78 ppm); xylenes 12-94 micrograms/m3 (< 0.01-0.02 ppm)) were considerably lower than in the pump house (> 4000 micrograms/m3 styrene, ethylbenzene, benzene, and toluene; > 500 micrograms/m3 xylenes), which was only intermittently occupied for short periods. Passive personal monitoring, biomonitoring of exhaled air and metabolites (mandelic, phenyiglyoxylic, trans, trans-muconic, hippuric, o-, m- and p-methylhippuric acids, and phenol) in urine samples collected before and after an eight hour working shift was used to assess individual exposure. Questionnaires and examination of company records showed that the historical exposure was far higher than that measured. Genotoxic monitoring was performed by nuclease P1-enhanced 32Ppostlabelling of DNA adducts in peripheral blood monocytes, and DNA single strand breaks, sister chromatid exchange, and micronuclei in lymphocytes. The content of kinetochores in the micronuclei was determined by immunofluorescence with specific antibodies from the serum of CREST patients. RESULTS: No genotoxic effects related to exposure were detected by DNA adducts or DNA single strand breaks and sister chromatid exchange. The only effect related to exposure was an increase in kinetochore positive micronuclei in peripheral lymphocytes; the frequency of total micronuclei in peripheral lymphocytes did not change. Smoking was confirmed by measurement of plasma

Attachment P – Styrene

cotinine, and no confounding effect was found on any of the cytogenetic variables.
CONCLUSIONS: Low occupational exposure to styrene, benzene, and ethylbenzene did not induce alterations of genotoxicological variables except kinetochore positive micronuclei. This is the first reported use of the CREST technique for an in vivo study in occupational toxicology, which thus could serve as a valuable and sensitive technique for toxicogenic monitoring.

Title: Mental health deterioration in workers exposed to styrene
Author(s): Sassine MP, Meroler D, Larribe F, Bélanger S.
Source: Rev Epidemiol Sante Publique. 1996 Jan;44(1):14-24.
Abstract: Emotional instability which might be an early symptom of more severe disorders, is one of the first manifestations of chronic exposure to organic solvents. The present study measures the association between exposure to styrene and mood states of active workers. A total of 128 workers (85% of the total population) from 3 factories where styrene is used, participated on a voluntary basis. They filled out the following self-administered questionnaires: Profile of Mood States (POMS), Psychiatric Symptom Index and Well-being Index. The results indicate a significant relationship between post work-shift urinary mandelic acid (biological indicator of styrene exposure) and the scores obtained on the POMS scales of tension-anxiety (Spearman's rank correlation rho = 0.30; $p < 0.01$), anger-hostility (rho = 0.29; $p < 0.01$), fatigue-inertie (rho = 0.34; $p < 0.01$), and confusion-bewilderment (rho = 0.23; $p = 0.04$), as well as the Psychological Distress Index (rho = 0.30; $p < 0.01$). All scores were adjusted for the effects of 4 potentially confounding variables: age, schooling, alcohol and cigarette consumption. These indicators of mood states do not constitute a diagnosis of mental disease but reveal progressive deterioration of well being associated with neurotoxic exposure in the workplace.

Title: Subchronic Inhalation Studies of Styrene in CD Rats and CD-1 Mice
Author(s): George Cruzan, Janette R. Cushman, Larry S. Andrews, Geoffrey C. Granville, Roland R. Miller, Colin J. Hardy, Derek W. Coombs and Pamela A. Mullins
Source: Fundamental and Applied Toxicology Volume 35, Issue 2, February 1997, Pages 152-165
Abstract: Groups of 10 male and 10 female Charles River (CRL) CD (Sprague—Dawley-derived) rats were exposed to styrene vapor at 0, 200, 500, 1000, or 1500 ppm 6 hr per day 5 days per week for 13 weeks. Styrene had no effect on survival, hematology, or clinical chemistry. Males at 1500 ppm weighed 10% less after 13 weeks and males and females at 1000 and 1500 ppm consumed more water than controls. Histopathologic changes were confined to the olfactory epithelium of the nasal mucosa. Groups of 20 male and 20 female CRL CD-1 and B6C3F1

Attachment P – Styrene

mice were exposed to styrene vapor at 0, 15, 60, 250, or 500 ppm 6 hr per day 5 days per week for 2 weeks. Mortality was observed in both CD-1 and B6C3F1 mice exposed to 250 or 500 ppm ; more female mice, but not males, died from exposure to 250 ppm than from 500 ppm. Groups of 10 male and 10 female CRL CD-1 mice were exposed to styrene vapors at 0, 50, 100, 150, or 200 ppm 6 hr per day 5 days per week for 13 weeks Two females exposed to 200 ppm died during the first week. Liver toxicity was evident in the decedents and i n some female survivors at 200 ppm. Changes were observed in the lungs of mice exposed to 100, 150, or 200 ppm and in the nasal passages of all treatment groups, those exposed to 50 ppm being less affected. Satellite groups of 15 male rats and 30 male mice were exposed as described above for 2, 5, or 13 weeks for measurement of cell proliferation (BrdU labeling). No increase in cell proliferation was found in liver of rats or mice or in cells of the bronchiolar or al veolar region of the lung of rats. No increase in labeling index of type II pneumocytes was seen in mouse lungs, while at 150 and 200 ppm, an increased labeling index of Clara cells was seen of ter 2 weeks and in occasional mice after 5 weeks. Large variations in the labeling index among animals emphasize the need for large group sizes. For nasal tract effects, a NOAEL was not found in CD-1 mice, but in CD rats, the NOAEL was 200 ppm. For other effects, the NOAEL was 500 ppm in rats and 50 ppm in mice.

Title: Effects of various pretreatments on the hepatotoxicity of inhaled styrene in the B6C3F1 mouse.

Author(s): Morgan DL, Mahler JF, Wilson RE, Moorman MP, Price HC Jr, Patrick KR, Richards JH, O'Connor RW.

Source: Xenobiotica. 1997 Apr;27(4):401-11.

Abstract: 1. The roles of cytochrome P450 monooxygenases (P450) and glutathione (GSH) in styrene hepatotoxicity were investigated in mice by pretreating with either phenobarbital (PB; P450 inducer), SKF 525A (P450 inhibitor), N-acetylcysteine (NAC; GSH precursor), or saline (vehicle control) prior to a 6-h exposure to either 500 ppm styrene on air.
2. Styrene caused hepatocellular degeneration or necrosis in all groups; these changes were more extensive and severe in mice pretreated with PB. Styrene significantly increased relative liver weights and serum ALT and SDH levels only in mice pretreated with PB. NAC did not prevent GSH depletion or hepatotoxicity.
3. In the fat of SKF 525A-pretreated mice a slight but statistically significant increase in styrene levels was observed, suggesting that metabolism was decreased; the SO/styrene ratio in the fat of PB-pretreated mice showed a slight, but statistically significant, increase indicating a slight increase in styrene metabolism. Neither SKF 525A nor PB caused changes in microsomal enzyme activity in vitro.

Attachment P – Styrene

4. These results suggest that styrene may be activated by a pathway not totally dependent upon P450 enzyme activity, or more likely that PB and SKF 525A are not specific for the P450 enzymes involved in activation and detoxification of styrene.

Title: Evaluation of the metabolism and hepatotoxicity of styrene in F344 rats, B6C3F1 mice, and CD-I mice following single and repeated inhalation exposures

Author(s): Susan C.J Sumner, Russell C Cattley, Bahman Asgharian, Derek B Janszen and Timothy R Fennell

Source: Chemico-Biological Interactions Volume 106, Issue 1, 29 August 1997, Pages 47-65

Abstract: Styrene is used for the manufacture of plastics and polymers. The metabolism and hepatotoxicity (mice only) of styrene was compared in male B6C3F1 mice, CD-1 mice, and F344 rats to evaluate biochemical mechanisms of toxicity. Rats and mice were exposed to 250 ppm styrene for 6 h/ day for 1 to 5 days, and liver (mice only) and blood were collected following each day of exposure. Mortality and increased serum alanine aminotransferase (ALT) activity were observed in mice but not in rats. Hepatotoxicity in B6C3F1 mice was characterized by severe centrilobular congestion after one exposure followed by acute centrilobular necrosis. Hepatotoxicity was delayed by 1 day in CD-1 mice, and the increase in ALT and degree of necrosis was less than observed for B6C3F1 mice. Following exposure to unlabeled styrene for 0, 2, or 4 days, rats and mice were exposed to [7„14—i-k... styrene (60 pCi/mmol) for 6 h. Urine, feces, and expired air were collected for up to 48 h. Most styrene-derived radioactivity was excreted in urine, The time-course of urinary excretion indicates that rats and CD-1 mice eliminated radioactivity at a faster rate than B6C3F1 mice following a single 250 ppm exposure, consistent with a greater extent of liver injury for B6C3F1 mice. The elimination rate following 3 or 5 days of exposure was similar for rats and both mouse strains. Following three exposures, the total radioactivity eliminated in excreta was elevated over that measured for one exposure for both mouse strains. An increased excretion of metabolites on multiple exposure is consistent with the absence of ongoing acute necrosis following 4 to 5 daily exposures. These data indicate that an induction in styrene metabolism occurs after multiple exposures, resulting in an increased uptake and/or clearance for styrene.

Title: Evaluation of genotoxic potential of styrene in furniture workers using unsaturated polyester resins.

Author(s): Karakava AE, Karahalil B, Yilmazer M, Aygtin N, Sardas S, Burgaz S.

Source: Mutat Res. 1997 Aug 14;392(3):261-8.

Abstract: Styrene is a widely used chemical, mostly in making synthetic rubber, resins, polyesters, plastics and insulators. Increasing attention has been focused on this

Attachment P – Styrene

compound since experiments using cytogenetic end-points have implicated styrene as a potential carcinogen and mutagen. In order to perform biological monitoring of genotoxic exposure to styrene monomer, we evaluated the urinary thioether (UT) excretion, and sister chromatid exchanges (SCEs) and micronuclei (MN) in peripheral lymphocytes from 53 furniture workers employed in small workplaces where polyester resin lamination processings were done and from 41 matched control subjects. The mean air concentration of styrene in the breathing zone of workers was 30.3 ppm. As a metabolic marker for styrene exposure, mandelic acid + phenylglyoxylic acid was measured in the urine and the mean value was 207 mg/g creatinine. The mean +/- SD value of UT excretions of workers was 4.43 +/- 3.42 mmol SH/mol creatinine and also mean UT for controls was found to be a 2.75 +/- 1.78 mmol SH-/mol creatinine. The mean +l-SD/cell values of SCE frequency in peripheral lymphocytes from the workers and controls were 6.20 +/- 1.56 and 5.23 +/1.23, respectively. The mean +/- SD frequencies (%o) of MN in the exposed and control groups were 1.98 +/- 0.50 and 2.09 +/- 0.35, respectively. Significant effects of work-related exposure were detected in the UT excretion and SCEs analyzed in peripheral blood lymphocytes ($p < 0.05$ and $p < 0.01$, respectively). The MN frequency in lymphocytes from the styrene- exposed group did not differ from that in the controls ($p > 0.05$). Effect of smoking, age and duration of exposure on the genotoxicity parameters analyzed were also evaluated. In conclusion, although our data do not demonstrate a dose- response relationship, they do suggest that styrene exposure was evident and that this styrene exposure may contribute to the observed genotoxic damage in furniture workers.

Title: Styrene-induced alterations in the respiratory tract of rats treated by inhalation or intraperitoneally.

Author(s): Coccini T, Fenoglio C, Nano R, De Piceis Polver P, Moscato G, Manzo L.

Source: J Toxicol Environ Health. 1997 Sep:52(1):63-77.

Abstract: Although exposure to styrene occurs primarily via inhalation, the action of this agent on the respiratory tract has scarcely been investigated. This article describes morphological and biochemical changes occurring in the respiratory tract of rats after either inhalation of styrene vapors (300 ppm, 6 hid, 5 d/wk, for 2 wk) or systemic (ip) treatment with 40 or 400 mg/kg styrene for 3 consecutive days. Electron microscopy analysis showed diffuse cell damage involving the tracheal, bronchiolar, and alveolar epithelium. In the tracheal epithelium, several cell types were affected. Ciliated cells presented vacuolation, detachment of cilia, blebbing of the apical cytoplasm, and compound cilia. Most secretory cells showed scant secretory granules and blebbings. Dense bodies and fibrillary inclusions were seen in intermediate and basal cells. Styrene also caused alterations of cytoplasmic components in type II pneumocytes and bronchiolar

Attachment P – Styrene

cells as well as thickness of the alveolar wall. These abnormalities were accompanied by depletion of glutathione (GSH) in the lung tissue. Pneumotoxic effects of systemic administration of styrene were dose dependent and tended to be more severe than those seen in the animals exposed for longer periods to styrene by inhalation. Metabolic activation of styrene and subsequent cell damage induced by the reactive metabolite styrene oxide may be involved in the sequence of events culminating in the toxic insult to the respiratory tract.

Title: Chronic toxicity/oncogenicity study of styrene in CD rats by inhalation exposure for 104 weeks.

Author(s): Cruzan G, Cushman JR, Andrews LS, Granville GC, Johnson KA, Hardy CJ, Coombs DW, Mullins PA, Brown WR.

Source: Toxicol Sci. 1998 Dec;46(2):266-81.

Abstract: Groups of 70 male and 70 female Charles River CD (Sprague-Dawley-derived) rats were exposed whole body to styrene vapor at 0, 50, 200, 500, or 1000 ppm 6 h/day 5 days/week for 104 weeks. The rats were observed daily, body weights and food and water consumption were measured periodically, and a battery of hematologic and clinical pathology examinations was conducted at weeks 13, 26, 52, 78, and 104. Nine or 10 rats per sex per group were necropsied after 52 weeks of exposure and the remaining survivors were necropsied after 104 weeks. Control and high-exposure rats received a complete histopathologic examination, while target organs, gross lesions, and all masses were examined in the lower exposure groups. Styrene had no effect on survival in males, but females exposed to 500 or 1000 ppm had a dose-related increase in survival. Levels of styrene in the blood at the end of a 6-h exposure during week 95 were proportional to exposure concentration. Levels of styrene oxide in the blood of rats exposed to 200 ppm or greater styrene were proportional to styrene exposure concentration. There were no changes of toxicologic significance in hematology, clinical chemistry, urinalysis, or organ weights. Males exposed to 500 or 1000 ppm gained less weight than the controls during the first year and maintained the difference during the second year. Females exposed to 200, 500, or 1000 ppm gained less weight during the first year; those exposed to 500 or 1000 ppm continued to gain less during months 13-18. Styrene-related non-neoplastic histopathologic changes were confined to the olfactory epithelium of the nasal mucosa. There was no evidence that styrene exposure caused treatment-related increases of any tumor type in males or females or in the number of tumor-bearing rats in the exposed groups compared to controls. In females, there were treatment-related decreases in pituitary adenomas and mammary adenocarcinomas. Based on an overall evaluation of eight oncogenicity studies, there is clear evidence that styrene does not induce cancer in rats.

Attachment P – Styrene

Title: Characterization of hepatocellular resistance and susceptibility to styrene toxicity in B6C3F1 mice.

Author(s): Mahler JF, Price HC Jr, O'Connor RW, Wilson RF, Eldridge SR, Moorman MP, Morgan DL.

Source: Toxicol Sci. 1999 Mar;48(1):123-33.

Abstract: Short-term inhalation exposure of B6C3F1 mice to styrene causes necrosis of centrilobular (CL) hepatocytes. However, in spite of continued exposure, the necrotic parenchyma is rapidly regenerated, indicating resistance by regenerated cells to styrene toxicity. These studies were conducted to test the hypothesis that resistance to repeated styrene exposure is due to sustained cell proliferation, with production of hepatocytes that have reduced metabolic capacity. Male mice were exposed to air or 500 ppm styrene (6 h/day); hepatotoxicity was evaluated by microscopic examination, serum liver enzyme levels, and bromodeoxyuridine (BrdU)-labeling index (LI). Metabolism was assessed by measurement of blood styrene and styrene oxide. Both single and repeated exposures to styrene resulted in mortality by Day 2; in mice that survived, there was CL necrosis with elevated BrdU LI at Day 6, and complete restoration of the necrotic parenchyma by Day 15. The BrdU LI in mice given a single exposure had returned to control levels by Day 15. Re-exposure of these mice on Day 15 resulted in additional mortality and hepatocellular necrosis, indicating that regenerated CL cells were again susceptible to the cytolethal effect of styrene following a 14-day recovery. However, in mice repeatedly exposed to styrene for 14 days, the BrdU LI remained significantly increased on Day 15, with preferential labeling of CL hepatocytes with enlarged nuclei (karyomegaly). If repeated exposures were followed by a 10-day recovery period, CL karyomegaly persisted, but the BrdU LI returned to control level and CL hepatocytes became susceptible again to styrene toxicity as demonstrated by additional mortality and acute necrosis after a challenge exposure. These findings indicated a requirement for continued styrene exposure and DNA synthesis in order to maintain this resistant phenotype. Analyses of proliferating-cell nuclear-antigen (PCNA) labeling were conducted to further characterize the cell cycle kinetics of these hepatocytes. The proportion of cells in S-phase was increased by repeated exposure. However, PCNA analysis also revealed an even larger increase in the G1 cell compartment with repeated exposures, without a concurrent increase in G2 phase or in mitotic cell numbers. These data indicate that resistance to styrene-induced necrosis under conditions of repeated exposure is not due to sustained cell turnover and production of new, metabolically inactive cells, but rather is due to some other, as yet unknown, protective phenotype of the regenerated cells.

Title: New aspects in genotoxic risk assessment of styrene exposure--a working

Attachment P – Styrene

hypothesis.

Author(s): Marczynski B, Peel M, Baur X.

Source: Med Hypotheses. 2000 Apr;54(4):619-23.

Abstract: Styrene is one of the most important plastic monomers worldwide. Styrene-7,8-oxide (SO), the major in-vivo metabolite of styrene, is classified as probably carcinogenic to humans and carcinogenic in rodents. Biological monitoring of exposure to styrene is usually carried out by determination of mandelic acid and phenylglyoxylic acid, the two main styrene metabolites in urine. SO binds covalently to human plasma protein and haemoglobin. The ability of SO to induce DNA adducts and DNA strand-breaks has been well documented. Recently in-vitro results showed that SO may disrupt the pre-existing oxidative status in white blood cells. This disruption would alter the balance between oxidants and antioxidants in cells. Styrene exposure can also result in oxidative DNA damage. A significant increase of 8-hydroxy-2;-deoxyguanosine (8-0HdG) has been found in white blood cells of styrene-exposed workers. According to these findings we propose a new hypothesis for the genotoxic risk assessment of styrene. Depletion of glutathione and increase in lipid peroxidation, similarity in the decrease of high molecular weight (HMV\ DNA fragments after SO exposure compared to hydrogen peroxide (H(2)O(2)) exposure, oxidative DNA damage (increased amounts of 8-OHdG and an increased level of DNA strand-breaks) following styrene or SO exposure are due to oxidative stress which can be a result of the imbalance between oxidants and antioxidants. Formation of protein-, RNA- and DNA-adducts, changes in DNA repair capacity and styrene metabolism following styrene exposure could cause this imbalance between oxidants and antioxidants. Oxidative stress seems to be the basis for genotoxic risk assessment of styrene.

Title: Styrene-induced changes in amacrine retinal cells: an experimental study in the rat.

Author(s): Vettori MV Corradi D, Coccini T, Carta A, Cavazzini S, Manzo L, Mutti A.

Source: Neurotoxicoloov. 2000 Aug;21(4):607-14.

Abstract: Dopamine (DA) is synthesized in amacrine cells and released upon membrane depolarization in a calcium-dependent way. Thus, it is recognized to function as a major neurotransmitter or modulator in vertebrate retina. Owing to DA modulating activity on cone-horizontal cells transmission, depletion or dysfunction of amacrine cells could interfere with chromatic processing, accounting for the acquired dyschromatopsia described among styrene-exposed workers. The present study has been designed to test the hypothesis that amacrine cells represent a vulnerable target of styrene in subchronically exposed rats. Ten female Sprague-Dawley rats were exposed to 300 ppm styrene 6 h/day, 5 days/week, for 12 weeks; ten rats exposed to fresh air served as a control group.

Attachment P – Styrene

Whole mounted retinas were used for the morphometry of tyrosine hydroxylase (TH) immunoreactive cells (IR). DA content and TH activity were measured by HPLC and electrochemical detection and glutathione (GSH) was measured by HPLC tandem mass spectrometry (LC-MS/MS). In treated rats, morphometric analysis showed a loss of TH-IR amacrine cells (6.2/mm2 vs. 8.7/mm2 recorded in controls, p = 0.002), without any peripheral-central variation in cell loss. DA content was also lower in exposed, as compared to control animals (208.64 vs. 267.98 microg/g w.w., p = 0.004). The activity of TH in the whole retina was similar in styrene-exposed and control rats when expressed as a function of the wet weight, whereas it was much higher in styrene-exposed rats (+64%) when expressed as a function of the number of TH-IR amacrine cells (p < 0.001). Finally, retinal GSH was reduced by 30% in exposed as compared to control rats (p = 0.01). In summary, retinal TH-IR cells were sensitive to styrene exposure, which seems to cause both structural and functional changes, represented by cell loss and DA depletion, respectively. These findings confirm the vulnerability of dopaminergic systems to styrene toxicity, providing some insights on the possible mechanism of loss in chromatic discrimination recorded among workers occupationally-exposed to styrene.

Title: Quantification of DNA adducts formed in liver, lungs, and isolated lung cells of rats and mice exposed to (14)C-styrene by nose-only inhalation.

Author(s): Boogaard PJ, de Kloe KP, Wong BA, Sumner SC, Watson WP, van Sittert NJ.

Source: Toxicol Sci. 2000 Oct;57(2):203-16.

Abstract: Bronchiolo-alveolar tumors were observed in mice exposed chronically to 160 ppm styrene, whereas no tumors were seen in rats up to concentrations of 1000 ppm. Clara cells, which are predominant in the bronchiolo-alveolar region in mouse lungs but less numerous in rat and human lung, contain various cytochrome P450s, which may oxidize styrene to the rodent carcinogen styrene-7,8-oxide (SO) and other reactive metabolites. Reactive metabolites may form specific DNA adducts and induce the tumors observed in mice. To determine DNA adducts in specific tissues and cell types, rats and mice were exposed to 160 ppm [ring-U-(14)C]styrene by nose-only inhalation for 6 h in a recirculating exposure system. Liver and lungs were isolated 0 and 42 h after exposure. Fractions enriched in Type II cells and Clara cells were isolated from rat and mouse lung, respectively. DNA adduct profiles differed quantitatively and qualitatively in liver, total lung, and enriched lung cell fractions. At 0 and 42 h after exposure, the two isomeric N:7-guanine adducts of SO (measured together, HPEG) were present in liver at 3.0 +/- 0.2 and 1.9 +/- 0.3 (rat) and 1.2 +/- 0.2 and 3.2 +/- 0.5 (mouse) per 10(8) bases. Several other, unidentified adducts were present at two to three times higher concentrations in mouse, but not in rat liver. In both rat and mouse lung, HPEG was the major adduct at approximately 1 per

Attachment P – Styrene

10(8) bases at 0 h, and these levels halved at 42 h. In both rat Type II and non-Type H cells, HPEG was the major adduct and was about three times higher in Type II cells than in total lung. For mice, DNA adduct levels in Clara cells and non-Clara cells were similar to total lung. The hepatic covalent binding index (CBI) at 0 and 42 h was 0.19 +/- 0.06 and 0.14 +/- 0.03 (rat) and 0. 25 +/- 0.11 and 0.44 +/- 0.23 (mouse), respectively. The pulmonary CB's, based on tissues combined for 0 and 42 h, were 0.17 +/- 0.04 (rat) and 0.24 +/- 0.04 (mouse). Compared with CBIs for other genotoxicants, these values indicate that styrene has only very weak adduct- forming potency. The overall results of this study indicate that DNA adduct formation does not play an important role in styrene tumorigenicity in chronically exposed mice.

Title: A review of the developmental and reproductive toxicity of styrene.
Author(s): Brown NA, Lamb JC, Brown SM, Neal BH.
Source: Redd Toxicol Pharmacol. 2000 Dec;32(3):228-47.
Abstract: The reproductive and developmental toxicity of styrene has been studied in animals and humans. The animal studies on styrene have diverse study designs and conclusions. Developmental or reproductive toxicity studies have been conducted in rats, mice, rabbits, and hamsters. In most cases, high doses are required to elicit effects, and the effects are not unique to reproduction or development. In a number of the reports, either the experimental designs are limited or the descriptions of the designs and the endpoints measured are insufficient to draw conclusions about the toxicity of styrene. The more complete and better-reported studies show that styrene does not cause developmental toxicity at dose levels that are not maternally toxic. Some neurochemical or neurobehavioral effects have been reported at high exposures. Styrene does not affect fertility or reproductive function. Considerable animal toxicity data on styrene support the conclusion that styrene is neither an endocrine-active substance nor an endocrine disrupter. Human studies often suffer from either inadequate exposure data or exposure to a wide variety of materials, so that attribution of effects to styrene exposure is impossible. Furthermore, investigators often have failed to account for other exposures in the workplace or for other potentially confounding factors in their studies. Menstrual cycle irregularities and congenital abnormalities were initially reported; however, the better and more recent reports do not show that styrene causes developmental or reproductive effects in humans. Human studies also support the conclusion that styrene is not an endocrine disrupter. Although some study authors have concluded that styrene is either a human or an animal reproductive or developmental toxicant, careful review demonstrates that such conclusions are not justified.

Attachment P – Styrene

Title: Genotoxic effects of styrene-7,8-oxide in human white blood cells: comet assay in relation to the induction of sister-chromatid exchanges and micronuclei.

Author(s): Laffon B, Pásaro E, Méndez J.

Source: Mutat Res, 2001 Apr 5;491(1-2):163-72.

Abstract: Styrene is used in the production of plastics, resins and rubber. The highest human exposures to styrene take place by inhalation during the production of fiberglass reinforced plastics. Styrene is metabolized mainly in the liver to styrene-7,8-oxide (SO), its principal in vivo mutagenic metabolite. In this study, human peripheral white blood cells were exposed to several SO concentrations (10-200 microM) in order to evaluate its genotoxic properties by means of comet assay, sister- chromatid exchanges (SCE) and cytokinesis-blocked micronucleus (MN) test, in addition to determine its clastogenic or aneugenic properties by combining MN with fluorescence in situ hybridization (FISH) procedures. Our results show that SO induces DNA damage, SCE and MN in human leukocytes in vitro at concentrations above 50 microM, and that there is a strong relationship between DNA damage, as measured by the comet assay, and cytogenetic damage induced by SO at the doses employed. SO shows preferentially a clastogenic activity and produces a cytostatic effect at high doses, reflected by the significant decrease of the calculated proliferation indices. A good dose-effect relationship is obtained in the three tests performed at the concentration range assayed.

Title: Chronic toxicity/oncogenicity study of styrene in CD-1 mice by inhalation exposure for 104 weeks.

Author(s): Cruzan G, Cushman JR, Andrews LS, Granville GC, Johnson KA, Bevan C, Hardy CJ, Coombs DW, Mullins PA, Brown WR.

Source: J Appl Toxicol. 2001 May-Jun;21(3):185-98.

Abstract: Groups of 70 male and 70 female Charles River CD-1 mice were exposed whole body to styrene vapor at 0, 20, 40, 80 or 160 ppm 6 h per day 5 days per week for 98 weeks (females) or 104 weeks (males). The mice were observed daily; body weights, food and water consumption were measured periodically, a battery of hematological and clinical pathology examinations were conducted at weeks 13, 26, 52, 78 and 98 (females)/104 (males). Ten mice of each gender per group were pre-selected for necropsy after 52 and 78 weeks of exposure and the survivors of the remaining 50 of each gender per group were necropsied after 98 or 104 weeks. An extensive set of organs from the control and high-exposure mice were examined histopathologically, whereas target organs, gross lesions and all masses were examined in all other groups. Styrene had no effect on survival in males. Two high-dose females died (acute liver toxicity) during the first 2 weeks; the remaining exposed females had a slightly higher survival than control mice. Levels of styrene and styrene oxide (SO) in the blood at the end of

Attachment P – Styrene

a 6 h exposure during week 74 were proportional to exposure concentration, except that at 20 ppm the SO level was below the limit of detection. There were no changes of toxicological significance in hematology, clinical chemistry, urinalysis or organ weights. Mice exposed to 80 or 160 ppm gained slightly less weight than the controls. Styrene -related non-neoplastic histopathological changes were found only in the nasal passages and lungs. In the nasal passages of males and females at all exposure concentrations, the changes included respiratory metaplasia of the olfactory epithelium with changes in the underlying Bowman's gland; the severity increased with styrene concentration and duration of exposure. Loss of olfactory nerve fibers was seen in mice exposed to 40, 80 or 160 ppm. In the lungs, there was decreased eosinophilia of Clara cells in the terminal bronchioles and bronchiolar epithelial hyperplasia extending into alveolar ducts. Increased tumor incidence occurred only in the lung. The incidence of bronchioloalveolar adenomas was significantly increased in males exposed to 40, 80 or 160 ppm and in females exposed to 20, 40 and 160 ppm. The increase was seen only after 24 months. In females exposed to 160 ppm, the incidence of bronchiolo-alveolar carcinomas after 24 months was significantly greater than in the controls. No difference in lung tumors between control and styrene-exposed mice was seen in the intensity or degree of immunostaining, the location of tumors relative to bronchioles or histological type (papillary, solid or mixed). It appears that styrene induces an increase in the number of lung tumors seen spontaneously in CD-1 mice.

Title: Leukemia and exposure to 1,3-butadiene, styrene and dimethyldithiocarbamate among workers in the synthetic rubber industry

Author(s): Elizabeth Delzell, Maurizio Macaluso, Nalini Sathiakumar and Robert Matthews

Source: Chemico-Biological Interactions, Volumes 135-136,1 June 2001, Pages 515-534

Abstract: This study evaluated relations between exposure to 1,3-butadiene (BD), styrene (STY) and dimethyldithiocarbamate (DMDTC) and mortality from leukemia among synthetic rubber industry workers. Subjects were 13 130 men employed for at least 1 year during 1943-1991 at any of six plants that manufactured synthetic rubber. Death certificates and medical records identified workers with leukemia. Cumulative exposure estimates were based on plant- and time period-specific process and task characteristics, linked to subjects' work histories. Poisson regression estimated relative rates (RRs) for workers exposed to each agent compared to unexposed workers. Leukemia (N=59) was positively associated with BD ppm-years (RRs of 1.0, 1.2, 2.0 and 3.8, for exposures of 0, >0—<86.3, 86.3—<362.2 and 362.2+ ppm-years; only the RR for the highest exposure category was statistically significant), STY ppm-years (RRs of 1.0, 1.2, 2.3 and 3.2, for exposures of 0, >0—<20.6, 20.6—<60.4 and 60.4+ ppm-years; only the RR for the highest exposure category was statistically significant) and

Attachment P – Styrene

DMDTC mg-years/cm (RRs of 1.0, 2.3, 4.9 and 2.9, for 0, >0—<566.6, 566.6—<1395.1 and 1395.1+ mg-years/cm; the RR for each non-zero exposure category was statistically significant) after adjusting for age and years since hire. After further adjusting each agent-specific set of RRs for the other two agents, a positive but imprecise relation remained for BD and DM DTC but not for STY. The association with BD was stronger for ppm-years due to exposure intensities >100 ppm than for ppm-years due to lower concentrations. BD and DMDTC, but not STY, were positively associated with leukemia in multivariable analyses. The independent effect of each agent was difficult to evaluate because of correlations with other agents and imprecision.

Title: The toxicity of styrene to the nasal epithelium of mice and rats: studies on the mode of action and relevance to humans.

Author(s): Green T, Lee R, Toqhill A, Meadowcroft S, Lund V, Foster J.

Source: Chem Biol Interact. 2001 Aug 31;137(2):185-202.

Abstract: Inhaled styrene is known to be toxic to the nasal olfactory epithelium of both mice and rats, although mice are markedly more sensitive. In this study, the nasal tissues of mice exposed to 40 and 160 ppm styrene 6 h/day for 3 days had a number of degenerative changes including atrophy of the olfactory mucosa and loss of normal cellular organisation. Pretreatment of mice with 5-phenyl-1-pentyne, an inhibitor of both CYP2F2 and CYP2E1 completely prevented the development of a nasal lesion on exposure to styrene establishing that a metabolite of styrene, probably styrene oxide, is responsible for the observed nasal toxicity. Comparisons of the cytochrome P-450 mediated metabolism of styrene to its oxide, and subsequent metabolism of the oxide by epoxide hydrolases and glutathione S-transferases in nasal tissues in vitro, have provided an explanation for the increased sensitivity of the mouse to styrene. Whereas cytochrome P-450 metabolism of styrene is similar in rats and mice, the rat is able to metabolise styrene oxide at higher rates than the mouse thus rapidly detoxifying this electrophilic metabolite. Metabolism of styrene to its oxide could not be detected in human nasal tissues in vitro, but the same tissues did have epoxide hydrolase and glutathione S-transferase activities, and were able to metabolise styrene oxide efficiently, indicating that styrene is unlikely to be toxic to the human nasal epithelium.

Title: DNA adducts, strand breaks and micronuclei in mice exposed to styrene by inhalation

Author(s): Pavel Vodicka, Mikko Koskinen, Ludmila Vodickova, Rudolf S tetina, Petr Smerak, Ivo Barta and Kari Hemminki

Source: Chemico-Biological interactions, Volume 137, Issue 3, 28 September 2001, Pages 213-227

Attachment P – Styrene

Abstract: Genotoxic and clastogenic effects of styrene were studied in mice. Male NMRI mice were exposed by inhalation to styrene in concentrations of 750 and 1500 mg/m^3 for 21, 7, 3 and 1 days (6 h/day, 7 days/week). Followed parameters included sty rene in blood, specific styrene oxide (SO) induced DNA adducts, DNA strand breaks and micronuclei. The formation of SO induced 7-SO-guanines and 1-SO-adenines in DNA was analysed from lung tissues by two versions of the ^{32}Ppostlabell ing technique. In lungs after 21 days of exposure to 1500 mg/m^3 the level of 7-SOguanine was 23.0±11.9 adducts/10^8 normal nucleotides, while 1-SO-adenine was detected at the levels of 0.6±0.2 adducts/10^8 normal nucleotides. Both 7-SO-guanines and 1-SOadenines strongly correlated with exposure parameters, particularly with styrene concentration in blood (r=0.875, P=0.0002 and r=0.793, P=0.002, respectively). DNA breaks were measured in peripheral lymphocytes, bone marrow cells and l iver cells using comet assay. To discern oxidative damage and abasic sites, endonuclease III was used. In bone marrow of exposed mice slight increase of strand breaks can be detected after 7 days of inhalation. A significant increase was rev ealed in the endonuclease III-sensitive sites after 21 days of inhalation in bone marrow. In the liver cells inhalation exposure to both concentrations of styrene did not virtually affect either levels of DNA single-strand breaks or endonuclease III- sensitive sites. The inhalation of 1500 mg/m^3 of styrene induced significant increase of micronuclei after 7 days of exposure (10.4±2.5/1000 cells, i.e. twice higher micronuclei frequency than in controls). After 21 days of inhalation no significant difference between the control group and the two exposed groups was observed. Whether the decrease of micronuclei after 21 days of inhalation was due to the inhibition of cell proliferation caused by styrene or due to the natural eliminaton of chromatide fragments, remains to be clarified. An interesting link has been found bet ween DNA single-strand breaks in bone marrow and frequencies of micronuclei (r=0.721, P=0.028).

Title: Physiological modeling of the relative contributions of styrene-7,8-oxide derived from direct inhalation and from styrene metabolism to the systemic dose in humans.

Author(s): Tornero-Velez R, Rappaport SM.

Source: Toxicol Sci. 2001 Dec:64(2):151-61.

Abstract: Workers in the reinforced plastics industry are exposed to large quantities of styrene and to small amounts of the carcinogen, styrene-7,8-oxide (SO), in air. Since SO is also the primary metabolite of styrene, we modified a published physiologically based pharmacokinetic (PBPK) model to investigate the relative contributions of inhaled SO and metabolically derived SO to the systemic levels of SO in humans. The model was tested against air and blood measurements of styrene and SO from 252 reinforced plastics workers. Results suggest that the

Attachment P – Styrene

highly efficient first-pass hydrolysis of SO via epoxide hydrolase in the liver greatly reduces the systemic availability of SO formed in situ from styrene. In contrast, airborne SO, absorbed via inhalation, is distributed to the systemic circulation, thereby avoiding such privileged-access metabolism. The best fit to the model was obtained when the relative systemic availability (the ratio of metabolic SO to absorbed SO per unit exposure) equaled 2.75 x 10(-4), indicating that absorbed SO contributed 3640 times more SO to the blood than an equivalent amount of inhaled styrene. Since the ratio of airborne styrene to SO rarely exceeds 1500 in the reinforced plastics industry, this indicates that inhalation of SO presents a greater hazard of cytogenetic damage than inhalation of styrene. We conclude that future studies should assess exposures to airborne SO as well as styrene.

Title: Evaluation of genotoxic effects in a group of workers exposed to low levels of styrene.
Author(s): Laffon B, Pásaro E, Méndez J.
Source: Toxicology. 2002 Feb 28;171(2-3):175-86.
Abstract: Occupational exposure to styrene was studied in a group of workers engaged in the production of fiberglass-reinforced plastics. Sister-chromatid exchanges (SCE), micronuclei (MN), and DNA damage (evaluated by means of comet assay) were measured in peripheral blood cells from the exposed workers and from a control population. Mandelic acid concentration, an indicator of styrene exposure level, was measured in urine samples collected at the end of the work shift. Average estimated values for styrene exposure were slightly below the threshold limit value (TLV) of 20 ppm recommended by the American Conference of Governmental Industrial Hygienists. Significant increases (P< or =0.01) have been found for SCE and MN frequencies and comet tail length among exposed individuals, as well as significant decreases (P< or =0.01) in the proliferation indices, as compared with control population. High correlation has been obtained between endpoints evaluated and exposure length, and increased values of SCE and MN frequencies and comet tail length have been found among smokers only in the exposed population. The high correlation obtained among SCE and MN frequencies and comet tail length, and the increase of these parameters in the exposed group with regard to control group justify the use of these three biomarkers in the evaluation of genotoxic effects in human populations exposed to styrene.

Title: Physiologically based modeling of the inhalation kinetics of styrene in humans using a bayesian population approach.
Author(s): Jonsson F, Johanson G.
Source: Toxicol Aopl Pharmacol. 2002 Feb 15;179(1):35-49.

Attachment P – Styrene

Abstract: Animal studies have implicated styrene as toxic to the central nervous system and its major metabolite styrene-7,8-oxide as a carcinogen. Therefore, a reliable estimate of the metabolic capacity for styrene in humans is of interest. However, the available models describing styrene kinetics in humans lack rigorous statistical validation and also ignore the population variability in metabolism. The population variability may be estimated by the use of population models. Furthermore, the statistical validation of pharmacokinetic models may be improved by use of Bayesian methods. These two approaches may be combined and recently have been gaining interest in the toxicology literature. A population-based physiologically based pharmacokinetic (PBPK) model for styrene was developed. The model was calibrated to extensive human toxicokinetic data from three previous studies in which 24 volunteers were exposed to 50-386 ppm of styrene at rest and various levels of exercise. Model fitting was performed in a Bayesian framework using Markov chain Monte Carlo simulation. The uncertainty around the partition coefficients and metabolic parameters for styrene was reduced. The metabolic capacity for styrene in humans was estimated to be 0.92 micromolll kg(-1), with a lognormal standard deviation of 1.66. The estimated Vmax is 40% higher than previously estimated, whereas the population standard deviation is estimated for the first time.

Title: DNA adducts of styrene-7,8-oxide in target and non-target organs for tumor induction in rat and mouse after repeated inhalation exposure to styrene.

Author(s): Otteneder M, Lutz U, Lutz WK.

Source: Mutat Res. 2002 Mar 20;500(1-2):111-6.

Abstract: Styrene by inhalation had been shown to increase the lung tumor incidence in mice at 20 ppm and higher, but was not carcinogenic in rats at up to 1000 ppm. Styrene-7,8-oxide, the major metabolic intermediate, has weak electrophilic reactivity. Therefore, DNA adduct formation was expected at a low level and a 32P-postlabeling method for a determination of the two regioisomeric 2'-deoxyguanosyl-06-adducts at the alpha(7)- and beta(8)-positions had been established. The first question was whether DNA adducts could be measured in the rat at the end of the 2 years exposure of a bioassay for carcinogenicity, even though tumor incidence was not increased. Liver samples of male and female CD rats were available for DNA adduct analysis. Adducts were above the limit of detection only in the highest dose group (1000 ppm), with median levels of 9 and 8 adducts per 10(7) nucleotides in males and females, respectively (sum of alpha- and beta-adducts). The result indicates that the rat liver tolerated a relatively high steady-state level of styrene-induced DNA adducts without detectable increase in tumor formation. The second question was whether different DNA adduct levels in the lung of rats and mice could account for the species difference in tumor incidence. Groups of female CD-1 mice were

Attachment P – Styrene

exposed for 2 weeks to 0, 40, and 160 ppm styrene (6h per day; 5 days per week), female CD rats were exposed to 0 and 500 ppm. In none of the lung DNA samples were adducts above a limit of detection of 1 adduct per 10(7) DNA nucleotides. The data indicate that species- and organ-specific tumor induction by styrene is not reflected by DNA adduct levels determined in tissue homogenate. The particular susceptibility of the mouse lung might have to be based on other reactive metabolites and DNA adducts, indirect DNA damage and/or cell-type specific toxicity and tumor promotion.

Title: A study on toxic organic emissions from batch combustion of styrene.
Author(s): Westblad C Levendis YA, Richter H, Howard JB Carlson J.
Source: Chemosphere. 2002 Oct;49(4):395-412.
Abstract: Results from a laboratory-scale investigation on batch combustion of styrene are reported herein. Limited quantities of waste styrene monomer are incinerated, however this monomer is, also, the primary pyrolyzate during combustion of waste polystyrene, the second most abundant polymer produced worldwide. Thus, its combustion-generated emissions are of importance to the operation of hazardous waste incinerators and municipal waste-to-energy powerplants. This work focuses on emissions of polycyclic aromatic hydrocarbons (PAHs), particulates, as well as carbon monoxide. To investigate methods for minimizing such emissions, batch combustion of the monomer was conducted in a two-stage muffle furnace. An additional air mixing chamber was installed between the two stages. Small quantities of the liquid monomer were inserted in the primary furnace which served as a gasifier/burner. The furnace temperature was in the range of 300-1000 degrees C and diffusion flames were formed under most conditions. Upon mixing with additional air, combustion of unburned gaseous fuel and primary reaction products continued in the secondary furnace (afterburner), which was kept at a constant temperature of either 1000 or 800 degrees C. Using this technique, conditions that minimize emissions were explored and theoretical investigations on the fate of pollutants in the secondary furnace were undertaken. Results revealed that combustion of styrene, which is a highly volatile fuel, occurred with the formation of flames that were often non- anchored, unsteady and unstable. Emissions of organic pollutants, soot and CO were more intense than in the case of the polystyrene combustion, studied previously under identical conditions, due to the additional depolymerization/pyrolysis steps therein. The emissions from the secondary furnace exceeded those of the primary furnace, consistent with the fact that a very significant fraction of the fuel conversion occurred in the secondary chamber. Clear trends in the emissions of PAHs and soot, products of incomplete combustion, with the temperature of the primary furnace (gasifier) were observed. Emissions were drastically reduced with lowering the gasifier temperature. While final cumulative emissions of PAHs and

Attachment P – Styrene

soot accounted for more than one third of the mass of the fuel at high temperatures, their concentrations at the exit of the afterburner were negligible when the primary furnace was operated at 300 degrees C under pyrolytic conditions. In the latter case air was added to the afterburner. Numerical modeling based on a complex reaction network was used for the description of the primary furnace as well as of the afterburner. Kinetic analysis showed acetylene and benzene to be key species in the growth of PAHs. Formation of PAHs in the afterburner, found experimentally, was reproduced by the model using a plug-flow assumption.

Title: Effects of 5-day styrene inhalation on serum prolactin and dopamine levels and on hypothalamic and striatal catecholamine concentrations in male rats.

Author(s): Jerry H, Metten M, Gamer AO, Wuttke W.

Source: Arch Toxicol. 2002 Nov;76(11):657-63.

Abstract: In several studies a hypersecretion of the pituitary hormone prolactin (PRL) in styrene-exposed workers has been described. This should cause reproductive problems like oligomenorrhea, secondary amenorrhea and reduced fertility [Arfini et al. (1987) J Occup Med 29:826-830, Bergamaschi et al. (1996) Neurotoxicology 17:753-760, Mutti and Smargiassi (1998) Toxicol Ind Health 14:311-323]. Secretion of PRL is tonically inhibited by the catecholamine dopamine (DA), which is released from hypothalamic neurons. It has been suggested that the activity of the enzyme dopamine-beta-hydroxylase (DBH) in the serum is a peripheral marker of central dopaminergic function. A slight reduction of such enzymatic activity was observed in styrene-exposed workers, which was associated with hypersecretion of PRL. To further investigate the putative effects of styrene on PRL release, male rats were exposed to styrene vapors (645, 2150 and 6450 mg/m(3)) for 6 h/day on 5 consecutive days. Animals were killed either directly following the last exposure (immediate group) or after a recovery period of 24 h (recovery group). Serum PRL and DA levels were measured by radioimmunoassay. Concentrations of catecholamines and their metabolites in the striatum and mediobasal hypothalamus (MBH) were determined by high performance liquid chromatography with electrochemical detection. Neither in the immediate nor in the recovery group were any statistically significant changes of serum PRL levels observed. Likewise, concentrations of catecholamines and their metabolites in the striatum and MBH remained unaffected. We conclude from these data that styrene, even at very high concentrations, has no adverse effects on the neuroendocrine mechanisms regulating PRL release and DA levels in the brain. With the limitations inherent in any animal model, we suggest that our data indicate that styrene also has no adverse neuroendocrine effects in humans.

Attachment P – Styrene

Title: A re-assessment of styrene-induced clastogenicity in mice in a subacute inhalation study.
Author(s): Engelhardt G, Gamer A, Vodicka P, Barta I, Hoffmann HD, Veenstra G.
Source: Arch Toxicol. 2003 Jan;77(1):56-61.
Abstract: To date, a number of in vivo cytogenetic assays have studied the clastogenicity (chromosome aberrations, micronuclei formation) in bone marrow of rodents exposed to styrene by various routes. The majority of all these cytogenetic experiments yielded negative findings (Scott and Preston, Mutat Res 318:175-203, 1994). Recently published data from a micronucleus test in mice exposed via inhalation for up to 21 days showed some positive response, but was not fully conclusive (Vodicka et al., Chem Biol Interact 137:213-227, 2001). Since this exposure regimen has considerable relevance for workplace exposure, the present study was performed to further elucidate these findings. NMRI mice were exposed by whole body inhalation to styrene concentrations of 750 mg/m3 and 1,500 mg/m3 for 1, 3, 7, 14 and 21 consecutive days (6 h/day). Animals were killed directly after exposure and bone marrow was sampled for analysis of micronucleus induction. Under the experimental conditions used in the present investigation, there was no evidence of clastogenicity at any concentration or exposure interval.

Title: Styrene hepatotoxicity in rats treated by inhalation or intraperitoneally: a structural investigation.
Author(s): De Piceis Polver P, Fenoglio C, Nano R, Coccini T, Bertone V, Vaccarone R, Gerzeli G.
Source: Histol Histopathol. 2003 Jan;18(1):49-54.
Abstract: The purpose of this study was to investigate the toxicity of styrene in the liver of adult rats treated either by inhalation of styrene vapour (300 ppm, 6 h/d, 5 dlwk, for 2 wk) or intraperitoneally with different styrene doses (4, 40, 400 mg/Kg) for 3 consecutive days. Using a light microscope, some alterations of liver parenchyma and sinusoid dilation were noticed, more marked in the group treated with the intraperitoneal administration of the chemical. Using an electron microscope, some additional changes were observed (once again, more marked in the latter group of rats): a) an increase in the content of lipids inside hepatocytes, and b) the rise of intracytoplasmic, intercellular and perisinusoidal collagen fibres. Therefore, cell damage and functional disturbance of sinusoids due to perisinusoidal fibrosis are apparent in the liver of both groups of rats exposed to styrene treatment, but these changes are definitely more significant in those subjected to intraperitoneal administration.

Title: Dermal exposure to styrene in the fibreglass reinforced plastics industry.
Author(s): Eriksson K, Wiklund L.

Attachment P – Styrene

Source: Ann Occup Hyg. 2004 Apr;48(3):203-8.

Abstract: OBJECTIVES: The aim of this study was to assess the potential dermal exposure to styrene in the fibreglass reinforced plastics industry.
METHODS: Assessment was performed during spraying and rolling using a patch sampling technique. The patch was made of charcoal sandwiched between two layers of cotton fabric. Samplers were fastened at 12 different spots on a sampling overall, each spot representing a body area. One patch was fastened at the front of a cap. A patch fastened to a string worn around the neck assessed the exposure at chest level inside the clothing. Patches were fastened to cotton gloves at sites representing the dorsal side and the palm of the hand to evaluate exposure on these areas. Following sampling the patches were solvent desorbed and styrene was analysed by gas chromatography flame ionization detection.
RESULTS: The potential body exposure for the participating individuals was between 544 and 17 100 mg/h with a geometric mean (GM) of 3780 mg/h. The legs, arms and outer chest in general had the highest exposures. The left and right hands had mean (GM) exposures of 344 and 433 mg/h, respectively. Styrene was determined for the patch at the inside of the clothing, indicating contamination of the dermal layer.
CONCLUSIONS: The charcoal patch can be used to evaluate potential exposure to styrene. The results indicate that the dermal layer of the worker is exposed to styrene. Precautions should be performed to reduce dermal exposure.

Title: Effects of 5-day styrene inhalation on serum LH and testosterone levels and on hypothalamic and striatal amino acid neurotransmitter concentrations in male rats.

Author(s): Jerry H, Gamer A, Wuttke W.

Source: Inhal Toxicol. 2004 Apr;16(4):209-15.

Abstract: The volatile chemical styrene may impair male fertility. Testicular testosterone (T) production is controlled by the hypothalamic/pituitary/gonadal axis. From the mediobasal hypothalamus (MBH), gonadotropin-releasing hormone (GnRH) is released, which stimulates luteinizing hormone (LH) secretion from the pituitary, which in turn enhances T production. GnRH release is controlled by glutamate (GLU) and gamma-aminobutyric acid (GABA). GLU and GABA neurons are regulated by T. Thus, reduced fertility of styrene-exposed male workers may result from altered GLU/GABA neurotransmission, causing insufficient GnRH, LH, and T secretion. Therefore, we compared LH and T levels of male rats that have inhaled styrene (0, 150, 500, 1500 ppm for 6 h on 5 consecutive days) to GLU and GABA concentrations in the MBH and striatum. Animals were killed directly following the last exposure (immediate group) or after 24 h (recovery group). No suppression of LH or T levels was observed after styrene inhalation. LH levels of the immediate groups with 500 or 1500 ppm exposure were slightly

Attachment P – Styrene

but significantly elevated. Hypothalamic GLU and GABA concentrations remained unchanged. Increased striatal GABA concentrations were determined in recovery groups with 500 or 1500 ppm exposure. Striatal GLU concentrations remained unaffected. Thus, we demonstrate slightly increased LH and T levels in styrene-exposed male rats after inhalation of the two higher doses. This effect did not correlate with hypothalamic GLU and GABA concentrations. With the limitations inherent to any animal model, these data obtained from a 5-day exposure study with rats suggest, but do not unequivocally prove, that styrene may have also no reproductive toxicity effects in men chronically exposed to this chemical.

Title: Neurobehavioral effects of experimental exposures to low levels of styrene.
Author(s): Seeber A, Blaszkewicz M Golka K, Hallier E, Kiesswetter E, Schaper M Van Thriel C.
Source: Toxicol Lett. 2004 Jun 15;151(1):183-92.
Abstract: Two experimental studies were conducted with the intention to simulate exposure characteristics of work places with styrene exposure and to investigate the risk for neurobehavioral impairments. In experiment I 16 volunteers (8 in the morning, 8 in the afternoon) were exposed to 0.5 and 20 ppm styrene on a constant level for 3h. In experiment II 24 volunteers (12 in the morning, 12 in the afternoon) were exposed for 4h to 0.5 and 20 ppm styrene on a constant level as well as to a changing exposure between 0.5 and 40 ppm with a TWA of 14 ppm. Simple reaction, choice reaction, attention, acute symptoms, and ratings for well-being were measured. Exposure related performance effects could not be detected. However, 6h time change resulted in delayed choice reactions in the morning hours. Analysing acute symptoms and the state of well-being the impact of styrene did not reach adverse extents of impaired well-being.

Title: Response of human cord blood cells to styrene exposure: evaluation of its effects on apoptosis and gene expression by genomic technology
Author(s): Cristina Diodovich, Marco Giorgio Bianchi, Gerard Bowe, Francesco Acquati, Roberto Taramelli, Dominique Parent-Massie and Laura Gribaldo
Source: Toxicology, Volume 200. Issues 2-3, 5 August 2004, Pages 145-157
Abstract: Styrene is one of the most important monomers produced worldwide, and it finds major use in the production of polystyrene, acrylonitrile-butadiene—styrene resins and unsaturated polystyrene resins. Epidemiological studies on styrene showed that the malignancies observed most frequently in humans after exposure are related to the lymphatic and haemopoietic system. IARC classified styrene a possible carcinogenic to humans (Group 2B). In this study, we evaluated the effect of styrene on gene expression profiles of human cord blood cells, as well as its activity on the apoptosis and bcl-2 related protein expression.

Attachment P – Styrene

Data demonstrated that, after 24 and 48 h of exposure, styrene (800 pM) induced an increase in the necrosis of mononuclear cor d blood cells, whereas it did not cause any increase in the apoptotic process. Western blot analysis revealed a modified expression of Bax, BCl-2, c-Jun, c-Fos and Raf-1 proteins in the human cord blood cells after direct exposure to styrene, whereas p53 expression did not change. Furthermore, Macroarray analysis showed that styrene changed cord blood gene expression, inducing up-regulation of monocyte chemotacti c protein 1 (MCP-1), and down-regulation of CC chemokine receptor type 1 (CCR-1) and SLP-76 tyrosine—phosphoprotein.

Title: Styrene monomer does not induce unscheduled DNA synthesis in the mouse liver following inhalation exposure.
Author(s): Clay P.
Source: Mutagenesis. 2004 Nov;19(6):489-92.
Abstract: Styrene monomer is a commercially important chemical used extensively in the production of plastics. It has been shown to induce lung tumours in the mouse via the inhalation route. Styrene monomer has shown a low reactivity with DNA and also a lack of genotoxic response in the mouse lung in vivo. Together with the fact that the mouse lung tumours were late occurring and mostly benign, which suggest a promotional effect rather than initiation, these factors have led to a suggestion that the tumours may not be of genotoxic origin. The studies examining the genotoxicity of styrene monomer in vivo have to date been predominantly cytogenetic assessments, although low levels of DNA adducts have been reported in the mouse liver and lung. In order to extend this evaluation, a mouse liver unscheduled DNA synthesis study has been performed to assess the ability of styrene monomer to induce DNA damage/repair. The negative response observed in this assay is consistent with the theory that tumours observed in mouse oncogenicity studies are non-genotoxic in origin.

Title: Perspectives on the genotoxic risk of styrene.
Author(s): Nestmann ER, Lynch BS, Ratoan F.
Source: J Toxicol Environ Health B Crit Rev. 2005 Mar-Apr;8(2):95-107.
Abstract: Styrene is a highly reactive monomer widely used in the plastics industry. The potential for styrene to produce genotoxic effects has been studied extensively in experimental systems. Styrene can induce sister chromatid exchanges (SCE) and chromosome aberrations (CA) in vitro under test conditions that enhance metabolism of styrene to styrene 7,8-oxide (SO)or reduce detoxification of 50 by epoxide hydrolase. The in vivo animal data indicate that styrene is not clastogenic at concentrations (doses) likely encountered by humans under ambient or occupational exposure conditions. DNA binding studies with styrene in rats and mice demonstrated no increased adducts in mice compared to rats or

Attachment P – Styrene

in mouse lung compared to liver. As a result, DNA adducts in the lungs are unlikely to be the sole explanation of the development of lung tumors in mice exposed to styrene for 2 yr. Some epidemiological studies reported that DNA and/or protein adducts and DNA strand breaks result from occupational exposure to styrene and/or 50. Results of some of these studies, how-ever, are difficult to interpret, given that the statistical significance of reported effects (SCE, CA, and micronucleus formation) was often near or at p values of .05; dose and/or temporal response relationships often were missing; confounding variables could not be excluded; and, concomitant exposures to other industrial chemicals that are potentially genotoxic may also have occurred. These studies suggest that styrene, through metabolism to SO, could be clastogenic in humans at workplace levels in excess of 125 mg/m3. However, results from controlled animal studies involving in vivo exposure to styrene alone do not show clastogenic effects at exposures of up to 1500 mg/m3/d. In any event, these studies show that there is an apparent threshold for styrene-mediated effects.

Title: Developmental neurotoxicity study of styrene by inhalation in Crl-CD rats.

Author(s): Cruzan G, Faber WD Johnson KA, Roberts LS Hellwiq J, Maurissen J, Beck MJ Radovsky A, Stump DG.

Source: Birth Defects Res B Dev Reprod Toxicol. 2005 Jun;74(3):221-32.

Abstract: This study was conducted to assess potential adverse functional and/or morphological effects of styrene on the neurological system in the F2 offspring following FO and Fl generation whole-body inhalation exposures. Four groups of male and female Crl:CD (SD)IGS BR rats (25/sex/group) were exposed to 0, 50, 150, and 500 ppm styrene for 6 hr daily for at least 70 consecutive days prior to mating for the FO and Fl generations. Inhalation exposure continued for the FO and Fl females throughout mating and through gestation day 20. On lactation days 1 through 4, the FO and Fl females received styrene in virgin olive oil via oral gavage at dose levels of 66, 117, and 300 mg/kg/day (divided into three equal doses, approximately 2 hr apart). Inhalation exposure of the FO and Fl females was re-initiated on lactation day 5 and continued through weaning of the Fl or F2 pups on postnatal day (PND) 21. Developmental landmarks were assessed in Fl and F2 offspring. The neurological development of randomly selected pups from the F2 generation was assessed by functional observational battery, locomotor activity, acoustic startle response, learning and memory evaluations, brain weights and dimension measurements, and brain morphometric and histologic evaluation. Styrene exposure did not affect survival or the clinical condition of the animals. As expected from previous studies, slight body weight and histopathologic effects on the nasal olfactory epithelium were found in FO and Fl rats exposed to 500 ppm and, to a lesser extent, 150 ppm. There were no indications of adverse effects on reproductive performance in

Attachment P – Styrene

either the FO or Fl generation. There were exposure-related reductions in mean body weights of the Fl and F2 offspring from the mid and high-exposure groups and an overall pattern of slightly delayed development evident in the F2 offspring only from the 500-ppm group. This developmental delay included reduced body weight (which continued through day 70) and slightly delayed acquisition of some physical landmarks of development. Styrene exposure of the FO and Fl animals had no effect on survival, the clinical condition or necropsy findings of the F2 animals. Functional observational battery evaluations conducted for all Fl dams during the gestation and lactation periods and for the F2 offspring were unaffected by styrene exposure. Swimming ability as determined by straight channel escape times measured on PND 24 were increased, and reduced grip strength values were evident for both sexes on PND 45 and 60 in the 500-ppm group compared to controls. There were no other parental exposure-related findings in the F2 pre-weaning and post-weaning functional observational battery assessments, the PND 20 and PND 60 auditory startle habituation parameters, in endpoints of learning and memory performance (escape times and errors) in the Biel water maze task at either testing age, or in activity levels measured on PND 61 in the 500-ppm group. Taken together, the exposure-related developmental and neuromotor changes identified in F2 pups from dams exposed to 500 ppm occurred in endpoints known to be both age- and weight-sensitive parameters, and were observed in the absence of any other remarkable indicators of neurobehavioral toxicity. Based on the results of this study, an exposure level of 50 ppm was considered to be the NOAEL for growth of F2 offspring; an exposure level of 500 ppm was considered to be the NOAEL for F2 developmental neurotoxicity.

Title: Two generation reproduction study of styrene by inhalation in Crl-CD rats.

Author(s): Cruzan G, Faber WD, Johnson KA, Roberts LS Hellwig J, Carney E, Yarrington JT, Stump DG.

Source: Birth Defects Res B Dev Reprod Toxicol. 2005 Jun;74(3):211-20.

Abstract: This study was conducted to evaluate the potential adverse effects of styrene on reproductive capability from whole-body inhalation exposure of FO and Fl parental animals. Assessments included gonadal function, estrous cyclicity, mating behavior, conception rate, gestation, parturition, lactation, and weaning in the FO and Fl generations, and Fl generation offspring growth and development. Four groups of male and female Crl:CD(SD)IGS BR rats (25/sex/group) were exposed to 0, 50, 150, and 500 ppm styrene for 6 hr daily for at least 70 consecutive days prior to mating for the FO and Fl generations. Inhalation exposure for the FO and Fl females continued throughout mating and gestation through gestation day 20. Inhalation exposure of the FO and Fl females was suspended from gestation day 21 through lactation day 4. On lactation days 1

Attachment P – Styrene

through 4, the FO and F1 females received styrene in virgin olive oil via oral gavage at dose levels of 66, 117, and 300 mg/kg/day (divided into three equal doses, approximately 2 hr apart). These oral dosages were calculated to provide similar maternal blood peak concentrations as provided by the inhalation exposures. Inhalation exposure of the FO and Fl females was re-initiated on lactation day 5. Styrene exposure did not affect survival or clinical observations. Rats in the 150- and 500-ppm groups in both parental generations gained weight more slowly than the controls. There were no indications of adverse effects on reproductive performance in either the FO or Fl generation. Male and female mating and fertility indices, pre-coital intervals, spermatogenic endpoints, reproductive organ weights, lengths of estrous cycle and gestation, live litter size and postnatal survival were similar in all exposure groups. Additionally, ovarian follicle counts and corpora lutea counts for the Fl females in the high-exposure group were similar to the control values. No adverse exposure- related macroscopic pathology was noted at any exposure level in the FO and Fl generations. A previously characterized pattern of degeneration of the olfactory epithelium that lines the dorsal septum and dorsal and medial aspects of the nasal turbinates occurred in the FO and Fl generation animals from the 500-ppm group. In the 500-ppm group, F2 birthweights were reduced compared to the control and F2 offspring from both the 150- and 500-ppm exposure groups gained weight more slowly than the controls. Based on the results of this study, an exposure level of 50 ppm was considered to be the NOAEL for FO and Fl parental systemic toxicity; the NOAEL for FO and Fl reproductive toxicity was 500 ppm or greater.

Title: 7-Alkylguanine adduct levels in urine, lungs and liver of mice exposed to styrene by inhalation

Author(s): Pavel Erik Vodicka, Igor Linhart, Jan Novak, Mikko Koskinen, Ludmila Vodickova and Kari Hemminki

Source: Toxicology and Applied Pharmacology, Volume 210, Issues 1-2, January 2006, Pages 1-8

Abstract: This study describes urinary excretion of two nucleobase adducts derived from styrene 7,8- oxide (SO), i.e., 7-(2-hydroxy-1-pheny 'ethyl) guanine (N7aG) and 7-(2-hydroxy-2- phenylethyl)guanine (N7 pG), as well as a formation of N7-SO-guanine adducts in lungs and liver of two month old male NMRI mice exposed to styrene by inhalation in a 3-week subacute study. Strikingly higher excretion of both isomeric nucleobas e adducts in the first day of exposure was recorded, while the daily excretion of nucleoba se adducts in following time intervals reached the steady-state level at 4.32 + 1.14 and 6.91 + 1.17 pmol/animal for lower and higher styrene exposure, respectively. 6-S0-guanine DNA adducts in lungs increased with exposure in a linear way (F = 13.7 for linearity and 0.17 for

Attachment P – Styrene

non-linearity, respectively), reaching at the 21st day the level of 23.0 adducts/10^8 normal nucleotides, i.e., 0.74 fmol/pg DNA of 7-alkylguanine DNA adducts for the concentration of 1500 mg/m^3, while no 7-SO-guanine DNA adducts were detected in the liver after 21 days of inhalation exposure to both of styrene concentrations. A comparison of 7 -alkylguanines ex creted in urine with 7-SO-guanines in lungs (after correction for depurination and for missing a-isomers) revealed that persisting 7-SO-guanine DNA adducts in lungs account for about 0.5% of the total alkylation at N7 of guanine. The total styrene-specific 7-guanine alkylation accounts for about 1.0 x 10^{-5}% of the total styrene uptake, while N1-adenine alkylation contributes to this percentage only negligibly.

Title: Inhalation exposure to 1,3-butadiene and styrene in styrene—butadiene copolymer production

Author(s): Tiina Anttinen-Klemetti, Raija Vaaranrinta, Pertti Mutanen and Kimmo Peltonen

Source: International Journal of Hygiene and Environmental Health, Volume 209, Issue 2, 17 March 2006, Pages 151-158

Abstract: This study assessed personal ex posure to 1,3- butadiene (BD) and styrene in three plants manufacturing sty rene—butadiene (SB) copolymers. Air samples were collected from the breathing zone of 28 workers over 4 months in three SB plants using di ffusive samplers. The total number of samples was 885 with the number of samples per participant varying from 19 to 39. Samples were collected by use of 3M 3500 passive monitors and analyzed with a gas chromatograph (GC). Sampling proved to be simple and inexpensive and laboratory analysis of BD could detect 0.01 and 0.007 part per millions (ppm) of sty rene in the 8 h samples. In the case of BD, 624 samples were below t he limit of quantification (LOQ), 240 samples were between the LOQ and 1 ppm, and 21 samples exceeded the threshold limit value (TLV). In the case of styrene 336 samples were below the LOQ, 548 samples were between the LOQ and 20 ppm. The TLV was exceeded once. The data gives a comprehensive picture of personal exposure of workers in modern SB latex manufacturing plants. The study illustrates also how the new TLV of BD is being implemented.

Title: Comparison of genotoxic potency of styrene 7,8-oxide with gamma radiation and human cancer risk estimation of styrene using the rad-equivalence approach.

Author(s): Godderis L, Aka P, Kirsch-Volders M, Veulemans H.

Source: Mutagenesis. 2007 May;22(3):209-15.

Abstract: Styrene is suspected to cause lympho-hematopoietic malignancies through the formation of styrene 7,8-oxide. However, we are still unable to calculate the cancer risk for workers exposed to styrene using epidemiological data. The aims of this study were to determine the blood dose after styrene exposure and to compare the genotoxic potency of styrene 7,8-oxide and gamma radiation in

Attachment P – Styrene

order to calculate the cancer risk by means of the rad-equivalence approach. Leucocytes of 20 individuals were exposed to 0, 0.1, 0.2 or 0.3 mM styrene 7,8-oxide (1 h) or 1, 2 or 3 gray (=100, 200, 300 rad) gamma radiation. Genotoxicity was evaluated with the cytokinesis-block micronucleus assay. Comparison of the two slopes of the regression lines between micronuclei and dose revealed a genotoxic potency for styrene 7,8-oxide of 37 rad/mMh, corresponding with a median value derived from mutagenicity studies (1, 37, 208 rad/mMh). At exposure levels of 1 ppm styrene, a blood styrene 7,8-oxide concentration between 0.03 x 10(-)(6) and 0.42 x 10(-)(6) mM is to be expected using data of toxicokinetic models and human exposure studies. With the cancer risk per unit dose of gamma radiation as benchmark, we calculated a lifetime risk of acquiring a fatal lympho-hematopoietic cancer of 0.17 in 10(3) workers (between 0.037 x 10(-)(3) and 5.0 x 10(-)(3)) exposed to 20 ppm styrene during 40 years.

Title: Excretion of urinary N7 guanine and N3 adenine DNA adducts in mice after inhalation of styrene.
Author(s): Mikes P, Korinek M Linhart I, Krouzelka J, Frantik E, Vodicková L, Neufussova L.
Source: Toxicol Lett. 2009 Jan 10;184(1):33-7.
Abstract: New urinary adenine adducts, 3-(2-hydroxy-1-phenylethy)adenine (N3alphaA), 3-(2-hydroxy-2-phenylethyl)adenine (N3betaA), were found in the urine of mice exposed to styrene vapour. These styrene 7,8-oxide derived adenine adducts as well as previously identified guanine adducts, 7-(2-hydroxy-1-phenylethyl)guanine (N7alphaG) and 7-(2-hydroxy-2- phenylethyl)guanine (N7betaG) were quantified by HPLC-ESI-MS(2) and the excretion profile during and after a repeated exposure to 600mg/m(3) or 1200mg/m(3) of styrene for 10 consecutive days (6h/day) was determined. The excretion was dose dependent. Total N3 adenine adducts (N3alphaA+N3betaA) excreted amounted to nearly 0.8x10(-5)% of the absorbed dose while urinary N7 guanine adducts (N7alphaG+N7betaG) amounted to nearly 1.4x10(-5)% of the dose. No accumulation of the adducts was observed. Due to rapid depurination from the DNA, the excretion of both N3 adenine and N7 guanine adducts ceased shortly after finishing the exposure. Both N3 adenine and N7 guanine adducts may be used as non-invasive biomarkers of effective dose reflecting only a short time exposure to styrene.

Title: Effect of physical exertion on the biological monitoring of exposure to various solvents following exposure by inhalation in human volunteers: III. Styrene.
Author(s): Truchon G, Brochu M, Tardif R.
Source: J Occup Environ HVQ. 2009 Aug;6(8):460-7.
Abstract: This study evaluated the impact of different work load intensities on biological indicators of styrene exposure. Four adult Caucasian men, aged 20 to 44 years,

Attachment P – Styrene

were recruited. Groups of 2-4 volunteers were exposed to 20 ppm of styrene in an exposure chamber according to scenarios involving either aerobic, muscular, or both types of physical exercise for 3 or 7 hr. The target intensities for each 30-min exercise period-interspaced with 15 min at rest-were the following: REST, 38 watts AERO (time-weighted average intensity), 34 watts AERO/MUSC, 49 watts AERO/MUSC, and 54 watts AERO for 7 hr and 22 watts MUSC for 3 hr. End-exhaled air samples were collected at 15 time points during and after 7-hr exposures for the determination of styrene concentrations. Urine samples were collected before the start of exposure, after the first 3 hr of exposure, and at the end of exposure for the determination of mandelic acid (MA) and phenylglyoxilic acid (PGA) concentrations. Compared with exposure at rest, styrene in alveolar air increased by a factor up to 1.7, while the sum of urinary MA and PGA increased by a factor ranging from 1.2 to 3.5, depending on the exposure scenario. Concentrations of biological indicators of styrene fluctuated with physical exertion and were correlated with the magnitude of the physical activity and pulmonary ventilation. Despite the physical exertion effect, urinary concentrations of styrene metabolites after a single-day exposure remain below the current biological exposure index value recommended by ACGIH; therefore, no additional health risk is expected. However, results shows that work load intensities must be considered in the interpretation of biological monitoring data and in the evaluation of the health risk associated with styrene exposure.

Attachment P – Styrene

Welfare Effects Studies (toxicity in aquatic organisms, toxicity in terrestrial species, toxicity to plants/bacteria, effects on visibility/weather/climate, effects on materials, economic effects, effects on personal comfort and well-being)

Title: Ecotoxicity hazard assessment of styrene.
Author(s): Cushman JR, Rausina GA Cruzan G Gilbert J, Williams E, Harrass MC Sousa JV, Putt AE, Garvey NA, St Laurent JP, Hoberci JR, Machado MW.
Source: Ecotoxicol Environ Saf. 1997 Jul;37(2):173-80.
Abstract: The ecotoxicity of styrene was evaluated in acute toxicity studies of fathead minnows (Pimephales promelas), daphnids (Daphnia magna), amphipods (Hyalella azteca), and freshwater green algae (Selenastrum capricornutum), and a subacute toxicity study of earthworms (Eisenia fostida). Stable exposure levels were maintained in the studies with fathead minnows, daphnids, and amphipods using sealed, flowthrough, serial dilution systems and test vessels. The algae were evaluated in a sealed, static system. The earthworms were exposed in artificial soil which was renewed after 7 days. Styrene concentrations in water and soil were analyzed by gas chromatography with flame ionization detection following extraction into hexane. Test results are based on measured concentrations. Styrene was moderately toxic to fathead minnows, daphnids, and amphipods: fathead minnow: LC50 (96 hr), 10 mg/liter, and NOEC, 4.0 mg/liter; daphnids: EC50 (48 hr), 4.7 mg/liter, and NOEC, 1.9 mg/liter; amphipods: LC50 (96 hr), 9.5 mg/liter, and NOEC, 4.1 mg/liter. Styrene was highly toxic to green algae: EC50 (96 hr), 0.72 mg/liter, and NOEC, 0.063 mg/liter; these effects were found to be algistatic rather than algicidal. Styrene was slightly toxic to earthworms: LC50 (14 days), 120 mg/kg, and NOEC, 44 mg/kg. There was no indication of a concern for chronic toxicity based on these studies. Styrene's potential impact on aquatic and soil environments is significantly mitigated by its volatility and biodegradability.

Title: Styrene toxicity: an ecotoxicological assessment.
Author(s): Gibbs BF, Mulligan CN.
Source: Ecotoxicol Environ Saf. 1997 Dec;38(3):181-94.
Abstract: Although other aromatic compounds (e.g., benzene, toluene, polycyclic aromatic hydrocarbons (PAH), etc.) have been thoroughly studied over the years, styrene has been given little attention probably due to its lower rate of industrial use. In addition, it is less toxic than benzene and PAH, proven carcinogens. However, it is classified as a mutagen and thus potentially carcinogenic. Its main use is in the production of the polymer polystyrene and in the production of plastics, rubber, resins, and insulators. Entry into the environment is mainly through industrial and municipal discharges. In this review, the toxicological effects of styrene on humans, animals, and plants are discussed. Its mode of entry and methods of

Attachment P – Styrene

monitoring its presence are examined. Although its effects on humans and aquatic life have been studied, the data on short- or long-term exposures to plants, birds, and land animals are insufficient to be conclusive. Since exposure to workers can result in memory loss, difficulties in concentration and learning, brain and liver damage, and cancer, development of accurate methods to monitor its exposure is essential. In addition, the review outlines the present state of styrene in the environment and suggests ways to deal with its presence. It might appear that the quantities are not sufficient to harm humans, but more data are necessary to evaluate its effect, especially on workers who are regularly exposed to it.

Title: Styrene dimers and trimers affect reproduction of daphnid (Ceriodaphnia dubia).
Author(s): Tatarazako N, Takao Y, Kishi K, Onikura N, Arizono K, Iquchi T.
Source: Chemosphere. 2002 Aug;48(6):597-601.
Abstract: The endocrine disruptor activity of styrene in humans and other vertebrates appears to be negligible. However, offspring numbers were reduced in Ceriodaphnia dubia bred in polystyrene cups. Styrene dimers and trimers were found to be eluted from the polystyrene cups by hexane and methanol with gas chromatography-mass spectrometry. Styrene dimers and trimers at concentrations of 0.04-1.7 microgll affected C. dubia fertility (25% reduction after seven days), suggesting that styrenes have the potential to impair crustacean populations in the aquatic environment.

Attachment Q – trans-1,2-Dimethylcyclohexane

Welfare Effects Studies (toxicity in aquatic organisms, toxicity in terrestrial species, toxicity to plants/bacteria, effects on visibility/weather/climate, effects on materials, economic effects, effects on personal comfort and well-being)

Title: Comparison of Volatile Compounds in Water- and Oil-Soluble Annatto *(Bixa orellana* L.) Extracts
Author(s): Veronica Galindo-Cuspinera, Meryl B. Lubran, and Scott A. Rankin
Source: *J. Agric. Food Chem.,* 2002, 50 (7), pp 2010-2015
Abstract: Annatto is a natural food colorant extracted from the seeds of the *Bixa orellana* L. plant. Annatto is used in Latin American cuisine to add a deep red color as well as distinctive flavor notes to fish, meat, and rice dishes. In the United States, annatto extracts are primarily used to impart orange/yellow hues to cheese and other dairy foods. The objective of this study was to identify and compare volatile compounds present in water- and oil-soluble annatto extracts. Volatile compounds were recovered using dynamic headspace-solvent desorption sampling and analyzed using GC-MS. Compounds were identified by comparison to a mass spectral database, Kovats indexes, and retention times of known standards. Of the 107 compounds detected, 56 compounds were tentatively identified and 51 were positively identified. Volatile profile differences exist between water- and oil- soluble extracts, and annatto extracts contain odorants with the potential to influence food aroma.

Title: Sequential Chemical Oxidation and Aerobic Biodegradation of Equivalent Carbon Number-Based Hydrocarbon Fractions in Jet Fuel
Author(s): Guibo Xie and Michael J. Barcelona
Source: Environ. Sci. Technol., 2003, 37 (20), pp 4751-4760
Abstract: Remediation of petroleum mixtures is complicated by the differing environmental degradabilities of hundreds of individual hydrocarbons in the mixtures. By grouping the individual hydrocarbons into a few fractions based on equivalent carbon number (EC), the present study examined the chemical and biological degradation of the fractions. With or without prechemical oxidation (25 days) by three oxidants ($KMn0_4$, H_20_2, $Mg0_2$), sterile and live microcosms were constituted with aquifer samples for aerobic biodegradation (134 days) of JP-4 jet fuel. Eighty-seven hydrocarbons were recovered and grouped into nine EC fractions. The apparent removal and actual transformation rate constants were estimated for both chemical and biological degradations. The data show that prechemical oxidations facilitated removal of total petroleum hydrocarbons (TPH) (up to 80%) within shorter times (<50 days) than biological alone. $KMn0_4$ and H_20_2 were better oxidants in terms of mass reduction in shorter times yet to some extent inhibited the subsequent microbial activity. $Mg0_2$ was a moderate oxidant with

Attachment Q – trans-1,2-Dimethylcyclohexane

less inhibition of microbial activity. Selective degradation of the EC fractions was observed for both chemical and biological processes. The biological processes were much less effective than the prechemical oxidations in transforming aromatic fractions, the more toxic fractions. The favorable substrates (i.e., aliphatic EC 10) for microbial growth were also those most subject to chemical oxidation. The results suggest that for remediation of petroleum contaminants, sequential chemical and biological technologies may surpass biological alone and more moderate oxidants such as $Mg0_2$ may be better candidates. More work is needed on the optimal dose and residence time for applied oxidants and on the application to engineering design and formulation of cleanup standards.

Title: Anaerobic biodegradation of alicyclic constituents of gasoline and natural gas condensate by bacteria from an anoxic aquifer

Author(s): G. Todd Townsend, Roger C. Prince and Joseph M. Suflita

Source: FEMS Microbiology Ecology, Volume 49, Issue 1, July 2004, Pages 129-135

Abstract: The biodegradation of alicyclic compounds was studied under methanogenic and sulfate- reducing conditions in samples from a gas condensate-contaminated aquifer amended with whole gasoline. Aquifer microorganisms exhibited previously unrecognized anaerobic alicyclic hydrocarbon metabolism of a broad range of substrates at relatively rapid rates. Simple unsubstituted, methyl-substituted, and ethyl-substituted cyclopentenes, cyclopentanes and cyclohexanes were consumed without a substantial lag in the presence of sulfate, but rather less effectively under methanogenic conditions. Dimethyl-substituted cyclopentanes and cyclohexanes were biodegraded only in the presence of sulfate and a limited isomer- specific biodegradative pattern was seen. These results extend the range of hydrocarbons known to be susceptible to anaerobic decay and help indicate the patterns of alicyclic hydrocarbon biodegradation that can be expected.

Title: Metabolism of BTEX and Naphtha Compounds to Methane in Oil Sands Tailings

Author(s): Tariq Siddique, Phillip M. Fedorak, Michael D. MacKinnon, and Julia M. Foght

Source: Environ. Sci. Technol., 2007, 41 (7), pp 2350-2356

Abstract: Naphtha, comprising low molecular weight aliphatics and aromatics (C_3-C_{14}), is used as a diluent in processing of bitumen from oil sands. A small fraction (<1%) is lost to tailings waste and incorporated into mature fine tailings (MFT). BTEX (benzene, toluene, ethylbenzene, and xylenes) and whole naphtha were assessed for biodegradation under methanogenic conditions using MFT from an oil sands tailings settling basin. MFT spiked with 0.05-0.1% w/v of BTEX compounds produced up to 2.1 (±0.1) mmol of methane during 36 weeks of incubation. Metabolism of 0.5-1.0% w/v naphtha in MFT yielded up to 5.7 (±0.2)

Attachment Q – trans-1,2-Dimethylcyclohexane

mmol of methane during 46 weeks of incubation. Gas chromatographic analyses showed that BTEX degraded in the sequence: toluene > o-xylene > m- plus p-xylene > ethylbenzene > benzene. Only 15-23% of whole naphtha, mainly n-alkanes (in the sequence: nonane > octane > heptane) and some BTEX compounds (toluene > o-xylene > m-xylene), was metabolized. Other naphtha constituents, such as iso-paraffins and naphthenes, remained unchanged during this period. These results suggest that the microbial communities in the MET can readily utilize certain fractions of unrecovered naphtha in oil sands tailings and support methanogenesis in settling basins. Current study findings could influence extraction process, MFT management, and reclamation options.

Title: Anaerobic biodegradation of natural gas condensate can be stimulated by the addition of gasoline

Author(s): Roger C. Prince and Joseph M. Suflita

Source: Biodegradation, 2007, Volume 18, Number 4, 515-523

Abstract: Biodegradation of a broad range of linear and branched alkanes, parent and alkyl alicyclic hydrocarbons, and benzene and alkyl-substituted benzenes was observed when sediment and groundwater samples collected from a gas condensate-contaminated aquifer were incubated under methanogenic and especially under sulfate-reducing conditions, even though no exogenous nitrogen or phosphorus was added. This finding expands the range of hydrocarbon molecules known to undergo anaerobic decay and confirms that natural attenuation is an important process at this site. The addition of 1 pl of gasoline to the samples (-10 ppm) had minimal impact on the biodegradation of saturated compounds, but substantially increased the diversity and extent of aromatic compounds undergoing transformation. We attribute this to the promotion or induction of biodegradation pathways in the indigenous microflora following the addition of the gasoline components. The promoting compounds are not precisely known, but may have been present in the initial condensate and reduced in concentration by various mechanisms (dissolution, biodegradation, etc.) such that their concentration in the aquifer fell below necessary levels. A variety of aromatic hydrocarbons would appear to be likely candidates.

Attachment R – trans-1,4-Dimethylcyclohexane

Health Effects Studies (acute toxicity, subchronic toxicity, chronic toxicity – e.g., including reproductive/developmental toxicity, neurotoxicity, immunotoxicity, organ system effects, mutagenicity/genotoxicity, carcinogenicity)

Title: Non-Linear QSAR Treatment of Genotoxicity
Author(s): M. Karelson; S. Sild; U. Maran
Source: Molecular Simulation, Volume 24, Issue 4 & 6, 2000, Pages 229 - 242
Abstract: The nonlinear QSAR approach using the Chebyshev polynomial expansion and neural networks has been applied for the prediction of genotoxicity of compounds. The mutagenic toxicity of heteroaromatic and aromatic amines, measured by the Ames test, was correlated with the molecular descriptors calculated from the molecular structures using quantum-chemical methods. The quantitative models obtained were compared with the results of the linear QSAR treatment. The descriptors appearing in the models reveal the importance of mutagenic interactions of heteroaromatic amines via hydrogen bonding, of effects induced by the solvent, and of the size of compound. The dependence of molecular descriptors on environmental effects and on molecular conformations was analysed.

Title: A case study on identification of airborne organic compounds and time courses of their concentrations in the cabin of a new car for private use
Author(s): Toshiaki Yoshida and Ichiro Matsunaga
Source: Environment International, Volume 32, Issue 1, January 2006, Pages 58-79
Abstract: The cabin of an automobile can be considered to be a part of the living environment because many people spend long periods of time during business, shopping, recreation or travel activities. However, little is known about the interior air contamination due to organic compounds diffusing from the interior materials used in the interior of automobiles. In the present study, the compounds in the interior air of a new car were identified, and the time courses of their concentrations were examined for over 3 years after the delivery (July, 1999). A total of 162 organic compounds, involving many aliphatic hydrocarbons and aromatic hydrocarbons, were identified. High concentrations of n-nonane (458 $\mu g/m^3$ on the day following delivery), n-decane (1301 $\mu g/m^3$), n-undecane (1616 $\mu g/m^3$), n-dodecane (716 pg/m^3), n-tridecane (320 fig/m^3), 1-hexadecene (768 $\mu g/m^3$), ethylbenzene (361 $\mu g/m^3$), xylene (4003 $\mu g/m^3$) and 2,2'-azobis (isobutyronitrile) (429 tag/m^3) were detected, and the sum of the concentrations determined for all compounds ex cluding formaldehyde (TVOC) was approximately 14 mg/m^3 on the day after the delivery. The concentrations of most compounds decreased with time, but increased with a rise of the interior temperature. The TVOC concentration in the next summer (July, 2000) was

Attachment R – trans-1,4-Dimethylcyclohexane

approximately one-tenth of the initial concentration. During the 3-year study period, the TVOC concentrations in summer exceeded the indoor guideline value (300 μg/m^3) proposed by Seifert (1995) [Seifert B. Volatile organic compounds . In: Maroni M, Seifert B, Lindvall T, editors. Indoor air quality. A comprehensive reference book. Air quality monographs, vol. 3. Netherlands: Elsevier Science; 1995. p. 819-21]. The interior temperature and day s lapsed after delivery were the main factors affecting the interior concentrations of most compounds according to multiple linear regression analysis. The results of this study offer useful fundamental data for investigations on air pollution in automotive cabins due to the organic compounds diffusing from the interior materials.

Title: Interior Air Pollution in Automotive Cabins by Volatile Organic Compounds Diffusing from Interior Materials: I. Survey of 101 Types of Japanese Domestically Produced Cars for Private Use

Author(s): Toshiaki Yoshida, Ichiro Matsunaga, Kimiko Tomioka, Shinji Kumagai

Source: Indoor and Built Environment October 2006 vol. 15 no. 5 425-444

Abstract: The types and concentrations of organic compounds in the interior air of 101 different types of Japanese domestically produced private-use cars were examined. All the vehicles had been registered in the summer season as new cars and were less than 3 years old. The airborne compounds in the cabins were collected for 24h under static condition with the engine stopped and the windows, doors and vents closed. A total of 275 organic compounds, including many aliphatic hydrocarbons and aromatic hydrocarbons, were identified, and 242 of them could be quantitated for each cabin. The sum of the concentrations of 241 compounds excluding formaldehyde was approximately 600 gm's as a median, ranging from 136 to 3968 g-rn^{-3} for the tested cars. The findings demonstrated that the air in the cabin of these cars was contaminated by high concentrations of e large variety of organic compound diffusing from the interior materials.

Title: Interior Air Pollution in Automotive Cabins by Volatile Organic Compounds Diffusing from Interior Materials: II. Influence of Manufacturer, Specifications and Usage Status on Air Pollution, and Estimation of Air Pollution Levels in Initial Phases of Delivery as a New Car

Author(s): Toshiaki Yoshida, Ichiro Matsunaga, Kimiko Tomioka, Shinji Kumagai

Source: Indoor and Built Environment October 2006 vol. 15 no. 5 445-462

Abstract: Air pollution in the cabins of 101 Japanese cars due to organic compounds diffusing from the interior materials has been described in our previous report. In the present study, the influence of the manufacturer, specifications and usage status of these cars on the interior air pollution was evaluated by covariance analysis. Also, the levels of air pollution in the initial phases of delivery as a new

Attachment R – trans-1,4-Dimethylcyclohexane

car were estimated using previous data for the time-courses of interior concentrations of organic compounds measured in another new oar. The findings showed greater air pollution in the cabins of luxury cars, with leather seals or leather steering wheels, or high-end catalogue prices. Differences in the specifications contributed more markedly to interior air pollution than differences in manufacturers. Also, usage status, such as everyday ventilation affected the long-term interior air quality. The sum values of interior concentrations of 154 compounds, for which there were time-course data, were estimated to be approximately 1700 $pals^{-3}$ as a median (max. 11,000 $pg \cdot m^{-3}$) at 1 month from delivery (interior temperature; 32T interior humidity, 46%).

Attachment R – trans-1,4-Dimethylcyclohexane

Welfare Effects Studies (toxicity in aquatic organisms, toxicity in terrestrial species, toxicity to plants/bacteria, effects on visibility/weather/climate, effects on materials, economic effects, effects on personal comfort and well-being)

Title: Sequential Chemical Oxidation and Aerobic Biodegradation of Equivalent Carbon Number-Based Hydrocarbon Fractions in Jet Fuel

Author(s): Guibo Xie and Michael J. Barcelonai

Source: Environ, Sci, Technol. 2003, 37, 4751-4760

Abstract: Remediation of petroleum mixtures is complicated by the differing environmental degradabilities of hundreds of individual hydrocarbons in the mixtures. By grouping the individual hydrocarbons into a few fractions based on equivalent carbon number (EC), the present study examined the chemical and biological degradation of the fractions. With or without prechemical oxidation (25 days) by three oxidants ($KMn0_4$, H_20_2, $Mg0_2$), sterile and live microcosms were constituted with aquifer samples for aerobic biodegradation (134 days) of JP-4 jet fuel. Eighty-seven hydrocarbons were recovered and grouped into nine EC fractions. The apparent removal and actual transformation rate constants were estimated for both chemical and biological degradations. The data show that prechemical oxidations facilitated removal of total petroleum hydrocarbons (TPH) (up to 80%) within shorter times (<50 days) than biological alone. $KMn0_4$ and H_20_2 were better oxidants in terms of mass reduction in shorter times yet to some extent inhibited the subsequent microbial activity. $Mg0_2$ was a moderate oxidant with less inhibition of microbial activity. Selective degradation of the EC fractions was observed for both chemical and biological processes. The biological processes were much less effective than the prechemical oxidations in transforming aromatic fractions, the more toxic fractions. The favorable substrates (i.e., aliphatic EC —10) for microbial growth were also those most subject to chemical oxidation. The results suggest that for remediation of petroleum contaminants, sequential chemical and biological technologies may surpass biological alone and more moderate oxidants such as $Mg0_2$ may be better candidates. More work is needed on the optimal dose and residence time for applied oxidants and on the application to engineering design and formulation of cleanup standards.