AR 201-14160

Mariam_Azimi-Osman@americanchemistry.com on 12/20/2002 03:21:01 PM

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Subject: HPV Chemical Challenge- ACC HQPD Panel - HQMME Task Force - Consortium # - Test Plan

Please find attached the following documents in submission of the "Test Plan" for the chemical 4-hydroxyanisole, which is sponsored by the HQMME Task Force of the ACC Hydroquinone Precursors and Derivatives (HQPD) Panel :

Cover Letter to Governor Whitman
4-Hydroxyanisole Background Information
The Test Plan for the Hydroquinone Monomethyl Ether Category
Robust Summaries

(See attached file: Cover Letter.pdf)(See attached file: HQMME B'ground.pdf)(See attached file: HQMME Test Plan.pdf)(See attached file: HQMME Robust Summaries.pdf)

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201-14160

COURTNEY M. PRICE
VICE PRESIDENT
CHEMSTAR

American Chemistry Council
Good Chemistry Makes It Possible

December 20, 2002

2002 DEC 27 AM 11:22

Via US Mail and E-mail

Christine Todd Whitman
US Environmental Protection Agency
P.O. Box 1437
Merrifield, VA 22116

Re: Hydroquinone Precursors and Derivatives Panel
Consortium No
HPV Chemical Challenge Program Submission
Hydroquinone Monomethyl Ether Category Justification and Testing Rationale

Dear Governor Whitman:

The Hydroquinone Precursors and Derivatives Panel (HQPD) HQMME Task Force of the American Chemistry Council is pleased to submit the subject documents to EPA's HPV Chemical Challenge Program (Program) as our test plan for a category covering the chemical 4-hydroxyanisole (CAS Nos. 150-76-5). The HQMME Task Force includes the following member companies that are sponsoring the chemical under the Voluntary HPV Chemical Challenge Program: Rhodia, Inc., Eastman Chemical Company, Borregaard, and Specialtychem Products Corporation.

This submission includes the following documents:

- Background Information
- Test Plan
- Robust Summaries

This submission is also being sent electronically to the following e-mail addresses:

Oppt.ncic@epa.gov
Chem.rtk@epa.gov

COURTNEY M. PRICE
VICE PRESIDENT
CHEMSTAR

American Chemistry Council
Good Chemistry Makes It Possible

If you require additional information, please contact F.J. "Sonny" Maher, HQPD Panel Manager at (703) 741-5605 or sonny_maher@americanchemistry.com.

Sincerely yours,

Courtney M. Price
Vice President, CHEMSTAR

Attachments

cc: HQMME Task Force
Steven Russell, ACC
Jim Keith, ACC

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HIGH PRODUCTION VOLUME (HPV) CHALLENGE PROGRAM

HYDROQUINONE MONOMETHYL ETHER CATEGORY

TEST PLAN

4-HYDROXYANISOLE (CAS No. 150-76-5)

PREPARED BY:

MEMBER COMPANIES OF THE AMERICAN CHEMISTRY COUNCIL'S
HYDROQUINONE PRECURSORS AND DERIVATIVES PANEL
HYDROQUINONE MONOMETHYL ETHER TASK FORCE

EASTMAN CHEMICAL COMPANY
RHODIA INCORPORATED
SPECIALTYCHEM PRODUCTS CORPORATION
BORREGAARD

December 20, 2002

4-HYDROXYANISOLE
BACKGROUND INFORMATION

4-Hydroxyanisole CAS No.: 150-76-5
Background Information, Test Plan, and Robust Summaries

Introduction

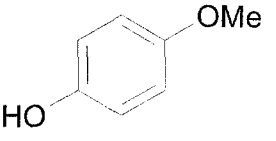
This test plan is provided by the American Chemistry Council HQMME Task Force of the HQPD Panel, in fulfillment of its commitment under the US HPV Chemical Challenge Program. Members of the HQMME Task Force are Rhodia, Inc., Eastman Chemical Company, Borregaard, and Specialtychem Products Corporation.

Summary

4-Hydroxyanisole has been manufactured in the US and abroad for many years. It is used primarily in industrial settings as an oxygen scavenger for polymerization inhibition in the manufacture of a broad array of products. Non-industrial applications for this material are very minor. A small quantity of 4-Hydroxyanisole is manufactured by organizations – not part of the HQMME Task Force – for pharmaceutical use. As shown in the Test Plan, the Physical/chemical Properties, Environmental Fate and Effects, and Mammalian Toxicity associated with 4-hydroxyanisole are well characterized. No further testing has been proposed for this material. Robust summaries are provided as Appendix A.

Chemical Identity:

4-Hydroxyanisole
CAS No.: 150-76-5



Background Information: Manufacturing and Commercial Applications

Manufacturing

4-Hydroxyanisole is made in batch processes using hydroquinone, sodium hydroxide and methyl chloride as the primary starting materials. This reaction produces crude 4-hydroxyanisole and a minor co-product 1,4-dimethoxybenzene. The crude 4-hydroxyanisole is distilled to remove trace impurities.

Commercial Applications

4-Hydroxyanisole has been manufactured in the United States for nearly 40 years, and is widely used throughout the chemical industry due to its excellent stability and functionality as

an oxygen scavenger. The majority of all usage is in industrial applications as a polymerization inhibitor for acrylic acid and esters, acrylonitrile and methacrylic acid and esters. It is also utilized as the starting material for the production of butylated hydroxyanisole. A very minor application that has only just recently been developed is as an active ingredient in a dermatological preparation used to lighten skin. The safety of the product for this consumer use was reviewed by the FDA and included data were not completely accessible to the Panel.

Shipping/Distribution

4-Hydroxyanisole is shipped extensively throughout the world from manufacturing plants located in the United States, Western Europe, Japan and India.

Worker Exposure

The synthetic fine organic batch manufacturing industry has a long safety record and only sophisticated industrial users handle this material. Exposure of workers to 4-hydroxyanisole is likely highest in the area of material solidification and packaging rather than from chemical manufacturing. This material is made as a molten material that is usually flaked or cast and then re-melted or dissolved at the user's facility. As there is little dust generation with the use of this material, the main exposure is from vapor generated in the hot liquid operations as it is solidified and packaged. This is easily controlled by any number of air control technology applications and good industrial hygiene practices by workers. There is a TWA exposure level of 5 mg/m^3 set by the ACGIH and by NIOSH.

Consumer exposure

Consumer exposure is minimal except through its use as an active agent in a dermatological product recently approved by the US FDA. This product is listed by the FDA as being available for use by prescription only. The product is applied directly to the skin in a cream/ointment form.

HQMME Test Plan

As shown in the Test Plan below, the Physical/chemical properties, Environmental Fate and Effects, and Mammalian Toxicity associated with 4-hydroxyanisole are well characterized. No further testing has been proposed for this material.

Physical-Chemical				
Melting Point	**Boiling Point**	**Vapor Pressure**	**Partition Coefficient**	**Water Solubility**
A	A	NA	A	A

Environmental Fate			
Photodegradation	**Stability in Water**	**Transport/ Distribution**	**Biodegradation**
Calc	NA	Calc	A

Ecotoxicity		
Acute Toxicity to Fish	**Acute Toxicity to Aquatic Plants (e.g., Algae)**	**Acute Toxicity to Aquatic Invertebrates (e.g., Daphnia)**
A	A	A

Mammalian Toxicity					
Acute Toxicity	**Bacterial Genetic Toxicity *In Vitro***	**Mammalian Genetic Toxicity *In Vitro***	**Repeat Dose Toxicity**	**Reproductive Toxicity**	**Developmental Toxicity**
A	A	A	A	A	A

Legend	
Symbol	**Description**
Calc	Endpoint requirement fulfilled based on calculated data
A	Endpoint requirement fulfilled with adequate existing data
NR	Not required per the OECD SIDS guidance
NA	Not applicable due to physical/chemical properties

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APPENDIX A
ROBUST SUMMARIES

1. General Substance Information

1.1 General Substance Information

Substance type: organic
Physical status: solid
Purity: 99.5 - % w/w

1.2 Synonyms and Trade names

4-hydroxyanisole
4-methoxyphenol (4-MP)
HQMME
hydroquinone monomethyl ether
p-hydroxyanisole
p-methoxyphenol
P-METHOXYPHENOL
Benzene, 1-methoxy-4-hydroxy-
1-Hydroxy-4-methoxybenzene
Hydroquinone methyl ether
Leucobasal
Leucodine B
Mechinolum
MEHQ
Novo-Dermoquinona
p-Guaiacol
p-Hydroxyanisol
p-Hydroxymethoxybenzene
PMF (antioxidant)

1.3 Impurities

CAS-No: 150-78-7
EC-No: 205-771-9
EINECS-Name: 1,4-dimethoxybenzene
Contents: ca. .1 - % w/w

1.4 Additives

None

2. Physico-chemical Data

Melting Point

Value: = 57 degree C
Method: other: Handbook value
Year: 1999
Reliability: (2) valid with restrictions; Reliability of 2 assigned because this is peer-reviewed published data.

(1)

Boiling Point

Value: 243 degree C
Method: other: Handbook value
Year: 1999
Reliability: (2) valid with restrictions; Reliability of 2 assigned because this is peer-reviewed published data.

(1)

Vapor Pressure

Result: not applicable, material is a solid

Partition Coefficient

log Pow: 1.58
Method: other (calculated)
Year: 1995
Test substance: purity not stated
Reliability: (2) valid with restrictions; Reliability of 2 assigned because this is modeling data.

(2)

log Pow: 1.34 at 25 degree C
Method: OECD Guideline 107 "Partition Coefficient (n-octanol/water), Flask-shaking Method"
Year: 1988
GLP: no data
Test substance: purity not stated
Remark: The Log Pow value of 1.34 represents the measured octanol-water log P value as cited in C. Hansch, A. Leo, S.H. Under, K.H. Kim, D. Nickaitani and E.J. Lien, J. Med. Chem., 1973, Vol 16, 1207. This determination of partition coefficient used a surface area approach. Two calculated log P values are derived: one from the surface area group contribution approach (4-MP = 1.59) and another from the -C log P program (4-MP = 1.57)
Reliability: (1) valid without restriction

(3)

Water Solubility

Value: 40 g/l at 25 degree C
Method: other
Year: 1992
Test substance: purity not stated
Reliability: (2) valid with restrictions; Reliability of 2 assigned because this is peer-reviewed published data.

(4)

Descr.: soluble (1000-10000 mg/L)
Method: other: Handbook
Year: 1999
Reliability: (2) valid with restrictions; Reliability of 2 assigned because this is peer-reviewed published data.

(1)

Photodegradation

Type: water
Light source: Xenon lamp
Light spect.: = 300 - 800 nm
Rel. intensity: ca. 1 based on Intensity of Sunlight
Conc. of subst.: 25.2 mg/l
INDIRECT PHOTOLYSIS
Sensitizer: water with additives
Conc. of sens.: 2.9 mg/l
Degradation: 100 % after 4 hour(s)
Method: other (measured)
Year: 1992
GLP: no data
Test substance: as prescribed by 1.1 - 1.4 : purity not stated
Method: A 50mM phosphate buffer solution containing 4-MP (100mM) and lumichrome (10mM) was irradiated for 4 hours with a 300-W xenon lamp through a 1.5mm plate glass to get reactivity similar to the environment. The disappearance of substrate was monitored by HPLC with a spectrometer at 254nm.
Result: First-order rate constants of lumichrome-sensitized photolysis of phenols:

pKa	pH	k (10e+3/min)
10.21	7.0	30.0
	8.0	30.0
	9.0	61.0

Test condition: Lumichrome was used a as sensitizer; water was taken from center of Kasumigaura Lake and a small pond, Hyotan Pond.
Reliability: (2) valid with restrictions; Reliability of 2 assigned because the study did not follow standardized guidelines and test material purity was not characterized.

(5)

Type: air
INDIRECT PHOTOLYSIS
Sensitizer: OH
Conc. of sens.: 1560000 molecule/cm^3
Rate constant: .000000000029798 cm^3/(molecule * sec)
Degradation: 50 % after 4.3 hour(s)
Method: other (calculated): AOPWin (vers. 1.90)
Test substance: other TS: molecular structure
Reliability: (2) valid with restrictions; Reliability of 2 assigned because this is modeling data.

(6)

Stability in Water

Remark: This remark has been prepared to document the known chemistry relevant to the stability of 4-Methoxyphenol, known commonly as Hydroquinone Monomethyl Ether (HQMME) in aqueous solution. Of particular concern in the evaluation of the stability of organic compounds in aqueous solution is the potential for hydrolysis. Hydrolysis is the reaction between water and an organic substrate resulting in the cleavage of existing chemical bonds and subsequent or simultaneous formation of new chemical bonds to form a different chemical compound. Typically, hydrolysis reactions involve incorporation of a water molecule into the structure of the reaction products. For organic substances that participate in hydrolysis reactions, various kinetic methods can be used to monitor the changes in concentration of reactants and determine the rate of transformation of the original substrate into reaction products. OECD Guideline 111 describes one such procedure for measuring the hydrolysis rate of water-soluble substrates as a function of pH. Substrates that exhibit high rates of hydrolysis are considered unstable in an aqueous environment.

Alkyl aryl ethers as a class and HQMME specifically, do not participate in hydrolysis reactions. The solubility of HQMME in water is 40 grams/liter at 25°C. However, HQMME does not possess a sufficiently labile leaving group that can be displaced by the nucleophilic attack of a water molecule, as is required in the mechanism of many hydrolysis reactions. Thus, it would not be meaningful to attempt to measure a hydrolysis rate using a protocol such as OECD Guideline 111.

Relatively severe conditions are required to affect the cleavage of alkyl aryl ethers in general, and methyl aryl ethers specifically (March, J., ed. "Advanced Organic Chemistry", 4th edition, pp. 373, 433-434, John Wiley & Sons, New York, 1992). Literature reviews of ether cleavage in general, and alkyl aryl ethers specifically, have been published (Bhatt; Kulkarni *Synthesis*, 1983, 249-282.
Tiecco, *Synthesis*, 1988, 749-759). Exemplary of the severity of the conditions of available methods, alkyl aryl ethers can be cleaved by heating with concentrated hydriodic or hydrobromic acids. The requisite severity of the conditions for alkyl aryl ether cleavage clearly establishes the resistance of the ether portion of the HQMME molecule to simple hydrolysis under conditions of moderate temperature and pH. The phenol substituent on the aromatic ring, and the aromatic ring itself, are well known to be stable to hydrolysis. The phenolic functionality may be converted to the corresponding phenoxide under conditions of high alkalinity, but the phenoxide remains hydrolytically stable.

Conclusion: Based on the properties of HQMME described above, it must be concluded that HQMME is not subject to hydrolysis, but may form the phenoxide under conditions of high alkalinity. Therefore, it is concluded that HQMME should be considered stable in aqueous solution at temperatures and pH levels relevant to environmental and human exposure.

Transport between Environmental Compartments (Fugacity)

Media: air - biota - sediment(s) - soil - water
Method: EPIWIN; Calculation according to Mackay, Level III
Year: 1999
Result: Level III Fugacity Model:

	Distribution (percent)	Half-Life (hr)	Emissions (kg/hr)	Fugacity (atm)
Air	1.26	8.62	1000	2.12 E-11
Water	46.8	360	1000	8.63E-12
Soil	51.8	360	1000	1.57E-10
Sediment	0.103	1.44e+003	0	6.91E-12

Persistence Time: 285 hr
Reaction Time: 343 hr
Advection Time: 1.68e+003 hr
Percent Reacted: 83.1
Percent Advected: 16.9

Reliability: (2) valid with restrictions; Reliability of 2 assigned because this is modeling data.

(8)

Biodegradation

Type: anaerobic
Inoculum: activated sludge, domestic
Concentration: 50 mg/l related to test substance
Contact time: 2 months
Degradation: > 90 % after 7 day(s)
Result: inherently biodegradable
Method: other
Year: 1983
GLP: no data
Test substance: as prescribed by 1.1 - 1.4
Method: A 10% sewage sludge inoculum was continuously purged with 10% CO_2 - 90% N_2 while being mixed and dispensed (100ml) into 160ml serum bottles. 4-MP was injected directly into the bottle to give a final concentration of 50 mg/l. The bottles were sealed with rubber stoppers and aluminum crimpseals. All tests were conducted in triplicate. Autoclaved bottles were used as sterile controls. All bottles were incubated at 35 deg C for 8 weeks. Methane production was determined weekly by analyzing 0.02 ml headspace gas in a Carle 8500 gas chromatograph. Net methane production was compared to theoretical CH_4 production based on the stoichiometry of complete mineralization.
Remark: 4-MP was transformed to the corresponding dihydroxybenzene compound, which was subsequently mineralized.
Reliability: (2) valid with restrictions; Reliability of 2 assigned because test exceeds standardized test duration of 28 days.

(9)

Type: aerobic
Inoculum: activated sludge
Concentration: 100 mg/l related to test substance

Contact time: 28 day(s)
Degradation: 79 - 95 % after 28 day(s)
Result: inherently biodegradable
Method: OECD Guideline 302 C "Inherent Biodegradability: Modified MITI Test (II)"
Year: 1981
Test substance: as prescribed by 1.1 - 1.4
Result: Measurement of three replicate tests:

	% biodegradation			average (%)
BOD:	79	95	84	86
TOC:	100	98	98	99
analysis by HPLC:	100	100	100	100

Test condition: Concentration of activated sludge = 30 mg/l
Volume of test solution = 300 ml
Cultivation temperature = 25 degree C
Cultivation duration = 28 days
Remark: Source of activated sludge inoculum not reported.
Reliability: (2) valid with restriction; Reliability of 2 assigned because some study details are not available.

(4)

Type: aerobic
Inoculum: other: mixed microbial cultures
Concentration: .4 mmol/l related to COD (Chemical Oxygen Demand)
3.2 mmol/l related to COD (Chemical Oxygen Demand)
Contact time: 5 day(s)
Degradation: ca. 57 % after 5 day(s)
Result: other: more work necessary to establish biodegradability
Method: other: Closed Bottle Test (5 day)
Year: 1987
GLP: no data
Test substance: as prescribed by 1.1 - 1.4
Method: Mixed microbial cultures were acclimated by seeding 10(e5) - 10(e6) cells/ml and 100mg/l chemical for 24 hours. Biodegradation of the test material was measured by the BOD technique. The test material and 1 ml of the seed were added to 20 ml of dilution water contained in a 300-ml BOD bottle. Bottles were filled to capacity with the same water, and sealed and incubated for 20 days at 21 +/- 3 deg C. Each test was run in duplicate. A seed control and 2 or more chemical concentrations ranging from 0.4 to 3.2 mg/l were employed. Glucose-glutamic acid controls for assessing the dilution water quality was included. Initial and 5, 10 or 11, 14 or 15, and 20-day dissolved oxygen (DO) concentrations were determined using a YSI 54 oxygen meter equipped with a self-stirring probe. The DO concentrations in randomly selected bottles were measured by the azide modification of the iodometric method. The test BOD values showing DO depletions of at least 2 mg/l and residual DO of at least 1 mg/l were acceptable. These values were adjusted for the seed control and used for calculating the 5-day mmol BOD/mmol chemical at various chemical concentrations.
Reliability: (2) valid with restrictions; Reliability of 2 assigned because test does not meet standardized test duration of 28 days. (10)

4. Ecotoxicity (Aquatic Organisms)

Acute/Prolonged Toxicity to Fish

Type:	flow through
Species:	Pimephales promelas (Fish, fresh water)
Exposure period:	96 hour(s)
Unit:	mg/l Analytical monitoring: yes
LC50:	110
Method:	Comparable to OECD Guideline 203 "Fish, Acute Toxicity Test"
Year:	1983
GLP:	no data
Test substance:	as prescribed by 1.1 - 1.4 : purity = 98%; purchased from Aldrich Chemical Co.
Method:	pH was adjusted to approximate that of Lake Superior water (pH 7.8) with NaOH or HCL. Compound analyses were done by Gas-Liquid Chromatography: all exposure chambers at 0, 24 48, 72 and 96 hr. Fathead minnows (age 32 days; mean length of 22.0 mm; mean weight 0.177g; loading 3.54 g/L) used in this experiment were cultured at US EPA Environmental Research Laboratory, Duluth, MN and University of Wisconsin - Superior campus. 20 fish/concentration and control. Behavior and toxic signs were noted at 4,24,48,72 and 96 hours and used to calculate EC50.
Result:	96 hr EC50 = 94.9mg/l; Affected fish lost schooling behavior, swam near the tank bottom in a corkscrew/spiral pattern, were hyperactive and then became under reactive to external stimuli, had convulsive movements, and lost equilibrium prior to death.
Test condition:	temperature =24.9 deg C (+/-0.3); dissolved oxygen = 5.7 mg/l; pH=7.41 (+/- 0.04); hardness = 45.3 (+/- 1.94) mg/l CaCO3; tank volume = 1 liter; concentrations 69.6, 107, 164, 253, 389 mg/l.
Reliability:	(1) valid without restriction

(11)

Type:	flow through
Species:	Salmo gairdneri (Fish, estuary, fresh water)
Exposure period:	96 hour(s)
Unit:	µmol/l Analytical monitoring: yes
LC50:	230
Method:	Comparable to OECD Guideline 203 "Fish, Acute Toxicity Test" Traditional continuous-flow, aqueous-exposure tests were used to measure the LC50 values of water-soluble compounds. Preliminary tests of five fish each at 1.0, 10, 100 and 1,000 mg/L gave the concentration range around the LC50. Bioassays within this range were then conducted at 0 (control), 10, 18, 32, 56 and 100% of the maximum test concentration. Ten fish were exposed at each concentration and the bioassay was repeated three times (n = 10 fish x 6 concentrations x 3 replicates = 180). Chemical was added to water by Hamilton syringe pump (PLD II) to create the 100% concentration and the dilutions were achieved by a Mount-Brungs diluter. Each bioassay tank contained 14 liters of water and the flow per tank varied between tests from 21 to 111 mL/min.

The fish weighed between 1 and 4 g each. Although the 95% molecular replacement time ranged from 6 to 33 h, the size of test fish was chosen such that the flow rate was always greater than 2 liters per gram of fish per day. The tanks were not aerated, to reduce volatilization. The levels in water of the compounds were measured daily. The assay method was the measurement of the absorbance of ultraviolet light by the test solutions in a quartz cell with a 1-cm path length. Concentrations were calculated by reference to standard curves of the chemical dissolved in control tank water. The reported LC50 value is based on the means of measured concentrations.

Fish: The rainbow trout were purchased from Goosen's Trout farm, R.R. #1, Otterville, ON, Canada. In the laboratory, they were held at 15°C on a 16 h light: 8 h dark photoperiod for at least 1 week prior to use. The trout were fed daily, except on weekends with Ewos trout pellets.

Water: The water for stock and bioassay tanks was a municipal supply drawn from Lake Ontario. It was dechlorinated to less than 10 micrograms Cl/L by charcoal filtration and the subsequent addition of 1 mg/L sodium sulfite. Alkalinity and conductivity averaged 86 mg/L as $CaCO_3$ and 340 µmhos/cm^2, respectively. Average pH, temperature and dissolved oxygen levels in the bioassay tanks were recorded.

Statistics: All LC50 values were calculated from records of percent mortality using computerized probit analysis validated by analyzing data sets from Finney. When the number of partial mortalities was too low for probit analysis, the slope of the probit lines were quite high. Hence, a graphical method for estimating the LC50 values was an accurate alternative.

Year: 1983
GLP: no data
Test substance: as prescribed by 1.1 - 1.4
The test substance was purchased from Aldrich Chemical Company at the highest degree of purity and refined by a toluene-hexane technique.
Result: The 96-Hr LC50 in rainbow trout was 230 umol/l
Reliability: (1) valid without restrictions.

(12)

Type: static
Species: Pimephales promelas (Fish, fresh water)
Exposure period: 96 hour(s)
Unit: mg/l Analytical monitoring: no data
NOEC: >= 10
LC0: >= 10
LC50: ca. 55
Method: other: Acute toxicity test
Year: 1978
GLP: no data
Test substance: as prescribed by 1.1 - 1.4
purity not stated
Method: Static test; dose levels of 10 and 100 mg/l where the solid chemical was dissolved in 0.5ml acetone; n=10 fish /dose

level; temperature= 19-20C; Dissolved Oxygen= 7.5-8.4 at test start and 4.8-6.0 at test end; pH= 7.6-7.8 at test start and pH= 7.4-7.6 at test end.

Value: = 57 degree C

Reliability: (2) valid with restrictions; Reliability of 2 assigned because some study details not available.

(13)

Type: static
Species: Leuciscus idus (Fish, fresh water)
Exposure period: 48 hour(s)
Unit: mg/l Analytical monitoring:
LC0: 10
LC50: 33
LC100: 100
Method: other: Fish test acc. to Deutsche Einheitsverfahren zur Wasser-,Abwasser- und Schlammuntersuchung L15
Year: 1976
Reliability: (4) not assignable, original report not available

(14)

Acute Toxicity to Aquatic Invertebrates

Species: Daphnia magna (Crustacea)
Exposure period: 24 hour(s)
Unit: mg/l Analytical monitoring: yes
EC0: 1.6 - 3.5
EC50: 7.2 - 19.3
EC100: 100 - 200
Method: Other: A largely standardized procedure for testing the potential toxic action of water pollutants measured by the immobilization of Daphnia magna Straus is presented. The standardized test strain IRCHA served as a test organism. The stock cultures were fed standardized dry algae. Testing of the toxic action of the water pollutants was performed in a chemically and physically defined standardized culture medium ("artificial fresh water"). When evaluating the test results, the EC50 values of the substances were determined according to a mathematically standardized method.)
Year: 1982
GLP: no data
Test substance: as prescribed by 1.1 - 1.4
purity not stated
Reliability: (2) valid with restrictions; Reliability of 2 assigned because some study details not available.

(15)

Species: Daphnia magna (Crustacea)
Exposure period: 48 hour(s)
Unit: mg/l Analytical monitoring: no data
EC50: ca. 2.2
Method: other: Acute Immobilisation Test
Year: 1978
GLP: no data
Test substance: as prescribed by 1.1 - 1.4 Purity not stated

Method: Static test; dose levels of 1, 10, 100mg/l where the solid chemical was dissolved in 0.5ml acetone; n=10 Daphnia/dose level; temperature= 19-20 degree C; Dissolved Oxygen= 7.7-8.6 at test start and 5.5-6.1 at test end; pH= 7.5-7.8 at test start and pH= 7.4-7.5 at test end.

Reliability: (2) valid with restrictions; Reliability of 2 assigned because some study details not available.

(13)

Species: Daphnia magna (Crustacea)
Exposure period: 48 hour(s)
Unit: mg/l Analytical monitoring: no data
EC0: = .04
EC50: ca. .09
EC100: = .31
Method: other: Acute immobilization test according to Bringmann and Kuehn: The 24-hr LC50 of 173 substances hazardous in water was determined by means of a standardized procedure using 24-hr-old animals from a clone of Daphnia magna. At the same time, the LC_0 and LC_{100} for each of these pollutants were determined. The test medium was tap water free from chlorine, saturated with oxygen, hardness 16° (German), pH 7.6-7.7, and temperature 20-22°C.)
Year: 1977
GLP: no data
Test substance: as prescribed by 1.1 - 1.4; purity not reported
Reliability: (2) valid with restrictions; Reliability of 2 assigned because some study details not available.

(16)

Toxicity to Aquatic Plants e.g. Algae

Species: Microcystis aeruginosa (Algae, blue, cyanobacteria)
Endpoint: growth rate
Exposure period: 24 hour(s)
Unit: mg/l Analytical monitoring: no data
NOEC: 20
Method: other
Year: 1952
GLP: no
Test substance: as prescribed by 1.1 - 1.4
Method: Pure cultures of Microcystis aureginosa were grown in 125 ml Erlenmeyer flasks with 75 ml of nutrient enriched solution (modified Chu No. 10 solution) under continuous fluorescent illumination and temperature approximately 22C. The chemical was added 5 days after algal inoculation (1.0-2.0 x10e+6 cells/ml), as the cells were entering the logarithmic growth phase. Microscopic examination after 24 hours and failure of treated cultures to grow in subculture were used to determine lethal dosages.
Remark: "However, not all compounds with quinone structure and/or marked redox properties are highly toxic to blue-green algae. The following compounds were non-toxic in concentrations up to 20 mg per L, or 21 per cent saturation in the cases of less soluble compounds: tetrachlorobenzoguinone (chloronil); tetrahydroxyquinone (disodium salt); 2,5-diphenyl-p-quinone; p-

dimethyl-aminoazobenzene; 1,2-acenaphthenequinone; 1,4-anthraquinone (quinizarin) and 9 substituted anthraquinones; o-aminophenol-p-sulfonic acid; aminophenol methyl ether (p-anisidine); hydroquinone monomethyl ether; aminohydroquinone dimethyl ether; 2-aminoresorcinol·HCl; m-aminophenol; and m-phenylenediamine·di-HCl."

Reliability: (2) valid with restrictions; Reliability of 2 assigned because some study details not available.

(17)

Species: Scenedesmus quadricauda (Algae)
Endpoint: growth rate
Exposure period: 10 day(s)
Unit: mg/l Analytical monitoring:
TGK : 4.4
Method: other: analogous to the cell multiplication inhibition test
Year: 1977
GLP: no data
Test substance: as prescribed by 1.1 - 1.4; purity not reported
Result: TGK (Toxische Grenzkoncentration = Toxicity Threshold Concentration)
Reliability: (2) valid with restrictions; Reliability of 2 assigned because some study details not available.

(18)

5. Toxicity

Acute Toxicity

Type: LD50
Species: rat
No. of Animals: 26
Value: ca. 1630 mg/kg bw
Method: other
Year: 1949
GLP: no
Test substance: as prescribed by 1.1 - 1.4: hydroquinone mono methyl ether; purity not stated
Method: Varying numbers of rats (1 to 10 per group) were dosed orally (via stomach tube) with 150-350mg/rat of HQMME. The rats ranged in weight from 129-160gm. A dose of 200 mg killed 4 of 10 rats; a dose of 300 mg killed 5 of 6 rats. No clinical signs were reported.
Reliability: (2) valid with restrictions; Reliability of 2 assigned because some study details not available.

(19)(26)

Repeated Dose Toxicity

Species: rat Sex: male/female
Route of administration: oral feed
Exposure period: 7 weeks
Frequency of treatment: daily
Doses: 0.02, 0.1, 0.5, 2.0, 5.0% of diet

Control Group: yes, concurrent vehicle
NOAEL: = .1 %
LOAEL: = .5 %
Method: other
Year: 1949
GLP: no
Test substance: other TS: hydroquinone mono methyl ether; purity not stated
Method: Eight groups of 10 male and female rats were maintained on diets with HQMME or feed only (controls). The rats were weighed weekly. Urinalyses for glucose and protein were done on pooled urine samples collected near the end of the feeding period. Qualitative observations were made for sugar and protein. Hematological studies (hemoglobin, red blood cell count, white blood cell count, and differential counts) were done a few days before the start of the experiment, mid-way in the high dose groups, and at termination for all levels. After 5 to 7 weeks on the diets, the animals were sacrificed. At sacrifice, the organs were weighed (heart, lungs, spleen, liver, kidneys, brain, and testes) and tissue samples were taken from the organs as well as from parts of the gastro-intestinal tract and prepared for histological examination.
Result: No mortality was seen in any group. The growth curves exhibit a gradation from zero to a consistent effect. Adding 0.02% to the diet produced little effect on the growth of either male or female rats. The female rats fed 0.1% grew as well as the controls; whereas the male rats showed a small growth depression. When 0.5% was mixed in the diets, small and probably insignificant variations in growth were observed in female rats. In the case of the male rats, however, each test with 0.5% showed a fairly consistent depression which amounted to 12 and 14 gm, respectively, at the end of the feeding period. When the diets contained 2.0%, the female rats showed a depression in growth of about 10 gm. The male rats suffered a growth depression which amounted to about 20 gm. The inclusion of 5% produced a marked depression in growth: at the end of the feeding period the female rats weighed some 30 gm less than the controls, and the male rats 63 gm less than their corresponding control group. However, it was suggested that the odor and flavor of the test item at these two dietary concentrations may have reduced both the palatability and, thus, the intake of the diet. Dose resulting in no growth depression is between 0.1 and 0.5%. This represents a daily dosage of the order of at least 100 mg/kg/day. Male rats showed increased urine glucose with increased levels of HQMME with 0.5% showing highest values. The protein values were all low and within the range of those frequently found in the colony. All hematology results (red blood cell counts, differential counts, and hemoglobin concentrations) were normal. A few high leukocyte counts were observed, but these were likely the result of mild respiratory infection. Decrease in organ weights were attributed to general body weight depression. In rats on the low dietary percentages, there was some questionable tendency to decreases in the spleen and liver weights; and in the male rats, of the kidney and testes weights. None of these were large-order changes; and in general all of the values showed a high degree of consistency, and only minimal responses to the addition of HQMME in the diets. In the rats fed the higher dietary percentages, the organ weights showed a tendency to

decrease as the percentage of HQMME increased. Calculating the weights on the basis of body weights at the time of sacrifice, this weight tendency was reversed, which may be interpreted as a sparing effect; the carcass growth was sacrificed to maintain the weights of the vital organs. Histological examination of heart, lungs, spleen, liver, kidneys, brain, testes, and gastro-intestinal tract showed no toxic changes that could be ascribed to the administration of HQMME.

Reliability: (2) valid with restriction

(19)(26)

Species: rabbit Sex:
Route of administration: oral feed
Exposure period: 5-9 weeks
Frequency of treatment: continuous in diet
Doses: 1.0, 5.0, 10.0% of diet
Control Group: yes, concurrent vehicle
NOAEL: 5 %
LOAEL: 10 %
Method: other
Year: 1949
GLP: no
Test substance: other TS: hydroquinone mono methyl ether; purity not stated
Method: Four groups of 6 rabbits each were maintained on diets with HQMME or feed only (controls). The rabbits were weighed weekly. Urinalyses for glucose and protein were done for each rabbit at the start and near the end of the feeding period. Hematological studies (hemoglobin, red blood cell count, white blood ell count, and differential count) were done on 3 selected rabbits 5 days before the start of the experiment, and again at 24 and 59 days of the experiment. At
sacrifice, the organs were weighed (heart, lungs, spleen, liver, kidneys, brain, stomach and testes) and tissues prepared for histological examination.

Result: No mortality was seen in any group. Rabbits fed 1 and 5% p-Hydroxyanisole gained weight at the same rate as control animals. Rabbits fed 10% had transient weight loss. The "appetite and general condition" of the animals fed 1% p-Hydroxyanisole for 5 weeks was described as "excellent". The dose resulting in no growth depression is greater than 5%. Occasional urine samples showed decreased glucose and protein levels but with no evidence of kidney damage. Blood samples taken mid-way through the study at the end of the study generally had normal hemoglobin concentrations and normal white and red cell counts. Rabbits fed 10% p-Hydroxyanisole had low red cell counts. The high dose group may have
developed low-grade anemia. Specifically, the hemoglobin values ranged from 9.8 to 13.6 gm; the red blood cell counts from 3.3 to 6.6 millions; the white blood cell counts ranged from 6 to 15 thousand. The differential counts showed considerable variation. Most of the values appeared to be normal, although the red blood cell counts on the 3 rabbits fed 5% and later 10% HQMME were low, specifically, 3.5 to 4.0 millions at the completion of the experiment. Average heart, lungs, and testes were smaller and liver and brain larger in the 10% HQMME group. Histological examination of heart, lungs, spleen, liver, kidneys, brain,

testes, and gastro-intestinal tract showed no pathological changes that could be
attributed to the administration of HQMME.

Reliability: (2) valid with restriction

(19)(26)

Species: rabbit Sex: male
Route of administration: dermal
Exposure period: 30 days
Frequency of treatment: 5 days/week
Doses: 1.0 and 10.0% in a suntan lotion base
Control Group: yes, concurrent vehicle
NOAEL: 1 %
LOAEL: 10 %
Method: other
Year: 1949
GLP: no
Test substance: other TS: hydroquinone mono methyl ether; purity not stated
Method: Three groups of 6 rabbits had dermal applications of suntan lotion or suntan lotion with 1.0 and 10.0% HQMME. The sun tan lotions were prepared on two basic formulae. One contained the specified amount of ether, 5 gm of castor oil, 6 gm of water, and alcohol to make 100 gm. The other formula contained the specified amount of ether, 15 gm of butyl stearate, and alcohol to make 100 gm. The skin was prepared by clipping the fur and applying a depilatory. Body weights were recorded weekly. Urinalyses for glucose and protein were done prior to, mid-way, and at the end of the experiment. Hematological (hemoglobin, red blood cell count, white blood ell count, and differential count) studies were done prior to, and after 2 and 4 weeks of the experiment. At sacrifice, the organs were weighed (heart, lungs, spleen, liver, kidneys, brain, stomach, and tested) and tissues prepared for histological examination.

In a follow-up experiment, 12 dermal applications over 2 weeks were made on three additional groups of rabbits in which sun tan lotion without and with 10.0% HQMME was applied and thoroughly washed after a 3 hour exposure. Body weights were recorded and the skin examined microscopically.

Result: Depression in body weight was insignificant. Urinalyses were negative. Hematological findings were within normal limits. Autopsy findings were essentially negative except for marked irritation of the skin.
The follow-up experiment showed mild to moderate erythema and escharificaton of the skin.

Reliability: (2) valid with restriction

(19)(26)

Species: dog Sex: 2 male;1 female
Route of administration: oral unspecified
Exposure period: 1 to 3 months
Frequency of treatment: daily
Doses: 1 -12 gm daily
Control Group: no data specified
Method: other

Year: 1949
GLP: no
Test substance: other TS: hydroquinone mono methyl ether; purity not stated
Method: Three dogs were fed various doses of HQMME as follows: dog #1 (male; 12.5 kg)- 1 gm daily for 2 months; dog #2 (female; 6.8 kg)- 2 gm daily for 1 month, then 4gm daily for 6 weeks; dog #3 (male; 14.4 kg)- 3 gm daily for 6 weeks, then 6 gm daily for 1 month, then 12 gm daily for 2 weeks. The dogs were weighed biweekly. Urinalyses for glucose and protein were done twice during the experiment. Hematological studies (hemoglobin, red blood cell count, white blood ell count, and differential count) were done prior to the experiment, and at day 16,38, and 87 of the experiment. At sacrifice, the organs were weighed (heart, lungs, spleen, liver, kidneys, brain, and uterus, tubes, and ovaries) and tissues prepared for histological examination.
Result: NOAEL= greater than 6 gm /day. No mortality was seen in any group. The body weight of dog #1 varied but in general was maintained throughout the study. Dog #2 had very little change in body weight. Dog #3 lost weight during the first month, regained weight in the next two weeks, maintained weight during the period when the dosage was 6 g daily, then lost weight again when the dosage was increased to 12 g daily. This weight loss associated with the 12 g per day dosage was not considered excessive. Dose resulting in no growth depression is greater than 6 gm daily. Occasional urine samples (examined twice during the study) were normal for protein (0.02 to 0.07%) and sugar (0.2 to 0.4%). Hematological results were normal, except for the high dose animal which showed a pronounced decrease in hemoglobin (from 13-14 down to 9.5 gm). The red blood cell count diminished in the same period from 6.8 to 4 million. The white blood cell counts and differential counts were normal. The total leukocyte and differential leukocyte counts were normal. Organ weights were within normal limits except for an enlarged spleen in the high-dose animal and increased weight of the brain of dog #2 (with no evidence of toxic effect). Histological examination of heart, lungs, spleen, liver, kidneys, brain, testes, and gastro-intestinal tract showed no pathological changes that could be attributed to the administration of HQMME.
Reliability: (2) valid with restriction

(19) (26)

Genetic Toxicity - Mutation

Type: Ames test
System of testing: Salmonella typhimurium TA 98, 100, 1535, 1537.
Concentration: 3umol/plate
Metabolic activation: with and without
Result: negative with and without
Method: other: analogous to OECD Guideline 471
The substances were tested in spot tests using histidine-requiring mutants of Salmonella typhimurium (TA98, TA100, TA1535 and TA1537) with and without S-9 from Aroclor 1254-induced rats. Each substance was tested at a single concentration of 3 µmol/plate. The vehicle was ethanol.
Year: 1980
GLP: no data

Test substance: other TS; The test substance was a commercially available compound; the source was not indicated; purity was reported as > 97

Reliability: (2) valid with restrictions; Reliability of 2 assigned because only one concentration was tested.

(20) (26)

Type: Ames test
System of testing: Salmonella typhimurium strains TA100,TA1530
Concentration: up to 4 umoles/plate
Metabolic activation: with and without
Result: negative with and with out activation
Test substance: 4-Methoxyphenol (obtained from Merck-Schuchardt, Darmstadt (F.R.G.)

Method: Directive 84/449/EEC, B.14
4-Methoxyphenol was tested using the plate incorporation method at concentrations up to 4 µmoles/plate in strains TA100 and TA1530 with phenobarbitone sodium-induced OF-1 mouse liver S9 mix. *Salmonella typhimurium* strains TA100 and TA1530 were provided by Professor B.N. Ames, Berkeley, CA (U.S.A.). The mutability of the TA strain was confirmed by using methylmethane sulphonate and the TA1530 strain was confirmed by using N-nitroso-N'-nitro-N-methylguanidine. 4-Methoxyphenol was obtained from Merck-Schuchardt, Darmstadt (F.R.G.). Adult male OF-1 mice (30-40 g) were bred either in the IARC laboratory or were obtained from Iffa-Credo, St. Germain-sur-l'Arbresle (France), and were fed on a Charles River CFR diet. Groups of 2-6 animals received phenobarbitone sodium (PB), 1 mg/ml in the drinking-water for 7 days before the experiment. A 9000 x g post-mitochondrial supernatant fraction (S9) was prepared at 0-4° from the pooled livers of the animals by centrifugation of a homogenate (3 ml of 0.15 M KCl/5 mM Sørensen buffer, pH 7.4, per g of wet liver). The resulting fractions were kept at 0-4° for less than 2 h and then used or stored at ~70° for up to 3 weeks before use. All procedures were carried out with sterile glassware and solutions.

Plate-incorporation assay: The test compound, dissolved in 100 µl DMSO, 0.5 ml of a mixture containing various amounts of tissue S9 (normally up to 150 µl), cofactors (2 µmol $NADP^+$ and 2.5 µmol G6P), 4 µmol magnesium chloride, 50 µmol Sørenson phosphate buffer, pH 7.4, and 0.1 ml of a suspension containing 1-2 x 10^8 bacteria were combined with 2 ml histidine-poor soft agar (0.55%w/v agar, 0.55% w/v sodium chloride, 45.5 µM each of biotin and histidine, in 5 mM Sørensen buffer, pH 7.4). The agar mixture (final volume, 2.7 ml) was agitated vigorously and immediately poured onto plates of minimal agar; these were then incubated at 37°. The number of *his*$^+$ revertant colonies was counted, usually after incubation for 48 h. The presence of a background lawn of bacteria on the histidine-poor soft agar plate was used as an indication that gross toxic effects were absent. Mutagenicity assays were carried out at least in triplicate.

Year: 1980

Remark: The data presented were obtained in collaborative studies carried out at the IARC laboratories essentially between 1974 and 1978. The citation summarizing the results of testing with 4-

methoxyphenol is "Bartsch, H., C. Malaveille, R. Montesano, and L. Tomatis (1975b) Tissue-mediated mutagenicity of vinylidene chloride and 2-chlorobutadiene in *Salmonella typhimurium*, Nature (London), 255, 641-643."

Reliability: (2) valid with restrictions; Reliability of 2 assigned because only two strains were tested.

(21)(26)

Genetic Toxicity - Aberrations

Type: Micronucleus assay
Species: rat Sex: no data
Strain: Sprague-Dawley
Route of admin.: dermal
Exposure period: 6 months
Doses: no data
Result: negative
Method: other: no data
Year: 1997
GLP: no data
Test substance: other TS: 2% 4-hydroxyanisole (4-methoxyphenol, mequinol, hydroquinone methyl ether, BMS-181158), and 0.01% All-Trans Retinoic Acid (ATRA, tretinoin, BMS- 181159) solution in ethanol (77.8%)
Method: The test substance was applied topically for 6 months.
Remark: Original study submitted as part of NDA No. 20-922.
Reliability: (4) Not assignable; Data were summarized in FDA documents as part of an NDA and minimal details were available.

(27)

Carcinogenicity

Species: mouse Sex: female
Strain: Swiss
Route of administration: dermal
Exposure period: 100 weeks
Frequency of treatment: twice a week
Doses: 5%, 10% in acetone or methanol
Result: negative
Control Group: yes, concurrent no treatment

Method: other:
Year: 1977
GLP: no
Test substance: other TS: 4-hydroxyanisole purchased from Aldrich Chemical Co; purity not stated
Method: Mice (50 per concentration) received dermal exposure of 5% or 10 % hydroxyanisole (volume of 0.02 ml) twice a week on a one - inch square area of the dorsal skin between the shoulder blades which was shaved regularly. Treatment was begun at 7 weeks. Animals were checked weekly and all lesions and tumors recorded. Complete autopsies were performed on all animals. Skin samples, grossly observed tumors and other lesions of the lungs, livers, kidneys, etc. were studied

histologically. Formalin-fixed, paraffin-embedded specimens were cut and stained with hematoxylin-eosin and other strains as needed. Positive controls treated with DMBA were included. The statistical significance of results was evaluated using Armitage (1971).

Result: No significant decrease in survival rates, body weights, or tumor incidence in both dose groups as compared to controls.

Reliability: (2) valid with restrictions; Reliability of 2 assigned because some study details not available.

(22)

Species: mouse Sex: female
Strain: other: Sutter mice, University of Wisconsin colony
Route of administration: dermal
Exposure period: 20 weeks
Frequency of treatment: twice weekly
Doses: 13.1% 4-MP (equimolar to 10% phenol)
Result: negative
Control Group: yes, concurrent vehicle
Method: other
Year: 1959
GLP: no
Test substance: other TS: 4-MP was obtained from Aldrich Chemical Company, Milwaukee, WI and Distillation Products Industries, Rochester, NY.;
purity not stated

Method:
Thirty female Sutter mice, aged 2-3 months were picked at random for the test. The test areas on the backs of the mice were shaved with electric clippers one week prior to first application. Due to the possibility of mechanical irritation, the mice were not shaved after the experiment started. The solution (13.1% 4-MP in benzene) was applied as a single drop (25ul) to the mid-dorsal section of each mouse twice weekly. The mice were inspected for tumors weekly. The gross identification of both benign and malignant tumors were confirmed by microscopic examination.

Result: 25/30 mice survived the 20 week experiment. 4% of the survivors had papillomas; average papilloma per survivor = 0.04; 0% of the survivors had epithelial carcinomas. The presence of a carbonyl, carboxyl, hydroxyl or methoxy group destroyed the tumor-promoting activity of phenol

Remark: Previous studies have reported that repeated applications of phenol and some substituted phenols were capable of promoting the appearance of skin tumors in mice following a single initiating dose of dimethylbenzanthracene. Tumors also developed in mice not exposed to DMBA but treated with phenol alone for long periods of time. In this report, more extensive data are presented on the role of phenol and its derivatives in promoting the formation of both papillomas and carcinomas. Particular attention was given to the effect of levels of phenol, the strain of mice, and the purity of phenol. The phenol used in this study was purified in the following manner: The phenol was dissolved in an excess of NaOH, and the alkaline solution was extracted 5 times with redistilled, reagent-grade diethyl ether. Excess

sulfuric acid was added, and the phenol was extracted into diethyl and dried over magnesium sulfate. Ether was removed by distillation, and the phenol was twice distilled from zinc dust at 179° -181°F.

Reliability: (4) not assignable; Reliability of 4 assigned because the study was experimental in nature and did not follow standard methods.

(23)

Species: rabbit Sex: male/female
Strain: New Zealand white
Route of administration: dermal
Exposure period: 80 weeks
Frequency of treatment: twice weekly
Doses: 5%, 10% in acetone or methanol
Result: negative
Control Group: yes, concurrent no treatment
Year: 1977
GLP: no
Test substance: other TS: 4-hydroxyanisole purchased from Aldrich Chemical Co; purity not stated

Method: Eight week old rabbits received dermal exposure of 5% or 10 % hydroxyanisole (volume of 0.02 ml) twice a week to the interior left ear. Animals were checked weekly and all lesions and tumors recorded. Complete autopsies were performed on all animals. Skin samples, grossly observed tumors and other lesions of the lungs, livers, kidneys, etc. were studied histologically. Formalin-fixed, paraffin-embedded specimens were cut and stained with hematoxylin-eosin and other strains as needed. The statistical significance of results was evaluated using Armitage (1971).

Result: No treatment-related decrease in survival rates or local dermal changes in both dose groups as compared to controls. No tumors were seen in the test animals.

Reliability: (2) valid with restrictions Reliability of 2 assigned because some study details not available.

(22)

Species: rat Sex: male
Strain: Fischer 344
Route of administration: oral feed
Exposure period: 104 weeks
Frequency of treatment: daily
Post exposure period: none
Doses: 0.4% in feed
Result: positive
Control Group: yes, concurrent vehicle
Method: other
Year: 1997
GLP: no data
Test substance: other TS: 4-methoxy phenol purchased from Tokyo Kasei Kogyo Co. Ltd; purity not stated

Method: A long-term (2 year) study was conducted with 30-31 rats/group. Control group was given the basal diet alone. All animals were weighed every 1-4 weeks during the experiment. All surviving animals were sacrificed at the

end of 104 weeks. Liver and kidneys were weighed and stomach tissues processed for hemotoxylin/eosin staining.

A medium-term (28 week) multiple organ study was also done with low and high doses (0.08 and 0.4% 4-MP in feed) either alone or in combination with the other chemicals. Groups of animals were pretreated with N-diethylnitrosamine, N-methylnitrosourea, 1,2-dimethylhydrazine, N-butyl-N-(4-hydroxybutyl)nitrosamine for 2-4 weeks prior to dosing with 4-MP in feed for the remainder of the 28 weeks. All surviving animals were terminated at the end of 4 weeks, the liver and kidney were weighed, and histopathological examinations were done on the major organs (lung, liver, esophagus, stomach, intestines, urinary bladder).

Result: In the 2 year carcinogenicity study, there was a significant increase of forestomach papillary or nodular hyperplasia incidence as compared to the basal diet control group (31%, p=<0.05). There was a non-significant increase in papilloma incidence (3/26 rats). No incidence of glandular stomach submucosal hyperplasia or adenoma was seen. Final average body weights were significantly lower than the basal diet controls (p <0.01), however relative liver and kidney weights were not significantly different.

In the medium-term multi-organ study, the only significant findings were increased incidence of forestomach papillary or nodular hyperplasia and papilloma in the high dose group. There were no additive or synergistic effects seen in the groups receiving a combination of chemicals.

Reliability: (1) valid without restriction

(24)

Toxicity to Fertility

Type: Two generation study
Species: rat
Sex: male/female
Strain: Crl:CD®(SD)BR
Route of administration: dermal
Exposure Period: no data
Frequency of treatment: 7 days/week
Duration of test: no data
Doses: 0.6, 2.0, 6.0 ml/kg/day
Control Group: yes, concurrent vehicle (6 ml/kg/day)
NOAEL Maternal: 40/0.2 mg/kg/day (12 ul/cm2)
NOAEL Neonatal: 40/0.2 mg/kg/day (12 ul/cm2)
NOAEL Developmental: 40/0.2 mg/kg/day (12 ul/cm2)

Method: 25 pregnant female rats/group, approximately 12 weeks old at the time of breeding. Animals were collared and exposed to test article for 6 hours/day. Dams were allowed to deliver naturally, and the litters were monitored. At postnatal day 4, litters were culled to 8 pups each (4/sex when possible). At 8-13 days of age, 25 male and 25 female F1 pups per group were randomly selected for evaluation of physical and functional development and reproductive performance. Of those, 10/sex/group were selected for evaluation of sensory function and behavioral testing (motor

activity, learning and memory). F1 animals were mated; females underwent laparotomy on gestation day 20 and F2 fetuses were evaluated. F1 males were necropsied after F1 females.

Year: 1997

GLP: yes

Test substance: other TS: clinical formulation of 2% 4-hydroxyanisole / 0.01% Tretinoin

Result: "Deaths- No F0 animals died spontaneously during the study. During the first week of lactation, all dams and offspring in the high dose group were euthanized due to extreme irritation at the application site.

Clinical signs - In F0 animals, dose related irritation was noted in treated groups, consisting of very slight to severe erythema (first noted on study day 8), very slight to moderate edema, including fissuring (especially at the high dose), desquamation (first noted on study day 13), eschar, focal eschar and exfoliation (first noted on study day 14) at the treatment sites. Vocalization was observed on application of the test material in mid and high dose groups. High dose animals exhibited significant decrease in body weight on gestation day 20 and lactation day 1, in mean body weight gain during gestation, and in food consumption during gestation days 9-12. Increased food consumption in the first few days of lactation was observed in those animals before they were sacrificed for humane reasons. In F1 animals, drug-related changes were only observed at the maternally toxic high dose. In that group, there was increased pup mortality, decreased pup body weight, and an increased incidence of clinical signs; signs seen in high dose pups included small size, hypoactivity, cool to the touch, and pale in appearance. As adults F1 males from the treated groups exhibited body weights that appeared to lag behind those of controls in a dose-dependent manner, but that finding was not statistically significant. There was no apparent effect on male body weight gain at any dose. No significant effect was seen on body weight, body weight gain, gestation body weight or gestation body weight gain in females.

Reproductive parameters - Six dams in the mid- and high-dose groups failed to deliver by post-mating day 25, as compared to two each in the control and low dose groups; all but one of the controls were found to be nongravid. Four high dose females had total litter loss between lactation days 1 and 5. Reduced F1 pup survival and a higher rate of missing or cannibalized pups were seen in high dose litters after postnatal (PND) 1. High dose F1 pups had reduced body weights, and there was an increased incidence of F1 pup clinical and necropsy findings. Developmental parameters were included in F1 pup observations. Balanopreputial separation and vaginal patency were unaffected by treatment, although females in treated groups appeared to lag behind controls in timing of the latter measure, but not in a dose-related manner. Auditory startle testing on or about PND 21 and 60 revealed no treatment-related effects. Motor activity (total and ambulatory) measurements were made on or about PND 13, 17, 21 and 60. On PND 13 there was apparently less activity in treated groups, but the variability was so great in controls at this time point that there was no significant difference. It is

likely that this is too early an age for motor activity to be a sensitive measure. Variability was too high at PND 17 and 21 for meaningful interpretation as well. At PND 60, variability was less and there was no effect of treatment on total or ambulatory counts. Testing in the water maze was initiated between PND 20-23 and between PND 57-62 and evaluated swimming ability, learning and memory. No effect of treatment was seen. Estrous cycling in F1 females and reproductive performance in F1 animals were unaffected by treatment. Gravid uterine weights and F2 fetuses were also unaffected. There did appear to be an increase in early resorptions (and therefore post-implantation loss) in treated groups compared to control (not statistically significant).

Pathological examination - On gross necropsy, the only treatment-related finding in F0 dams was reddening, thickening and scabbing of skin at treated sites. In F1 pups found dead or euthanized, gross findings in the high dose group included absence of milk in the stomach, renal papilla not developed or not fully developed and/or distended ureters or urinary bladder. One external malformation (anury) was noted in one animal in one litter. At the low dose, one pup was found to have the renal papilla not fully developed. In F1 euthanized surplus pups, high dose animals were again noted with the absence of milk in the stomach, and one litter had pups in which the renal papilla was not developed or not fully developed and/or ureters or urinary bladder were distended. Also at the high dose there was one pup in one litter with a hemorrhagic ring around the iris. In F1 adults, no significant findings were seen that could be attributed to treatment. One low dose female had an enlarged spleen (also seen in a control female) and one mid-dose female had clear fluid in one uterine horn. In F2 pups, two low dose fetuses in two different litters were seen with external malformations (one with omphalocele and a second with craniorachischisis and a curly tail). These were considered to be within the range of historical controls. No external malformations were seen in the mid-dose group."

Remark: 0.6, 2.0, 6.0 ml/kg/day (12/0.06, 40/.2, 120/.6 mg/kg/day or 72/.36, 240/1.2, 720/3.6 mg/m2/day of 4-hydroxyanisole/tretinoin, respectively), or 2, 6.5 and 19 times the human dose on a mg/m2 basis). The topical application to 10% total body surface area would be 0.072/0.00036, 0.240/0.0012, and 0.720/0.0036 mg/cm2/day of 4-hydroxyanisole/tretinoin, respectively (3.6, 12 and 36 ul applied per cm2 of skin). These represent 1, 3.2 and 10 times the human application rate.

Conclusion: Based on this study the maternal, neonatal and developmental NOAELs were determined to be 40/0.2 mg/kg/day or 12 ul/cm2 (3.2 the human application rate)

Reliability: (2) Valid with restrictions; Data were summarized in FDA documents as part of an NDA. While original report was not available, the study was summarized in significant detail.

(27)

Developmental Toxicity/Teratogenicity

Species:	rat	Sex: female
Strain:	other: albino	
Route of administration:	dermal	
Exposure period:	throughout gestation (days 1-20)	
Frequency of treatment:	daily	
Duration of test:	20 days, observation of pups for 2 months post-partum	
Doses:	0.5ml of 5% and 25% HQMME in bleach creme base	
Control Group:	other: no treatment and concurrent vehicle groups	

NOAEL

Method:	other
Year:	1981
GLP:	no data
Test substance:	other TS: HQMME, purity not noted
Method:	Groups of 10-12 female white rats were administered 5% HQMME and 1% ascorbylpalmitate in bleach creme base or 25% HQMME or 5% ascorbylpalmitate in water-oil emulsions daily by dermal application from 1st through 20th day of pregnancy. There were two additional groups: a control group receiving bleach creme base and a non-treated control group. The animals were terminated on day 20 shortly before labor. The non-treated control group and the bleach creme (5% HQMME with 1% ascorbylpalmitate) group received the dermal application for the entire pregnancy. Time of delivery, number of live/stillborn offspring, and number of malformed fetuses were recorded. Offspring development for the bleach crème control group was observed for two months.
Result:	No significant differences were observed between treated and control groups with respect to skeletal anomalies, post implantation mortality, craniocaudal dimensions and weight of embryos, or placental weights. No teratogenic effects of bleaching creme, or its components (HQMME and ascorbylpalmitate), were detected. Both test items produced increased preimplantation mortality of embryos, whereas the bleach cream additionally produced subcutaneous hemorrhages in the embryos of treated rats.
Reliability:	(4); not Assignable, Data were only available in abstract form. (25)(26)

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