AR 201-13044

Kevin Baer <pybaer@alpha.ulm.edu> on 05/15/2001 02:36:02 PM

To: ChemRTK HPV/DC/USEPA/US@EPA
cc: nlester@deltechcorp.com

Subject: Robust Summary for para-methylstyrene

The Robust summary for para-methylstyrene (CAS #622979) is attached. The Sponsor is Deltech Corporation (ID # The test plan was submitted earlier and is currently available on the ACC-maintained site (hpvchallenge.com). Please contact (pybaer@ulm.edu) if there are any questions or additional information is needed.
Thank you.

Dr. Kevin Baer

TOVivo_051020011445 ECDaphni_05102001144 ECFishTx_05102001144
ENBiodeg_05102001144 ENPhotoD_05102001144 ENStabil_051020011445
ENTransp_05102001144 Master_051020011445 PCBoil_051020011445
PCMelt_051020011445 PCParti_051020011445 PCVapor_05102001144
PCWatrSol_05102001144 TOAcute_051020011445 TODevel_051020011445
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Admin Record Number: **ROBUST SUMMARY** AR 201-13044B

Styrene, p-methyl-

CAS# 622979 **Study Number: 1**

ECOTOXICITY ELEMENTS:

TOXICITY TO AQUATIC PLANTS

Test Substance

Remarks

p-methylstyrene
Test Article ID: Paramethyl Styrene
Purity: 99.66% Active Ingredient
Additives: None reported
Carrier solvent: None
Contaminants: None reported
Chemical formula: C9H10

Chemical Category

Method

Method/Guideline Followed OECD Method 201

Test Type static

GLP **Yes**

Year Study Performed 2001

Species Selenastrum capricornutum

End Point Average specific growth rate; hemocytometer

Analytical Monitoring Gas chromatograph/FID; LOD, 0.0002 mg/L

Exposure Period 72

Statistical Method Weighted least squares non-linear regression

Remarks for Method

Algae used for the test (Selenastrum capricornutum, UTEX 1648) were from a culture originally obtained from the Culture Collection of Algae at the University of Texas at Austin and delivered to T.R. Wilbury Laboratories on March 1, 2001. The culture was transferred to sterile enriched media identical to media used for this test and maintained at test conditions for at least 14 days before the definitive test. During the acclimation period, the culture was actively growing in at least 2 subcultures prior to the start of the toxicity test. The subsample of algae used to inoculate media at the start of the definitive test came from a seven day old culture. Identification of the culture organisms, which are also referred to as Raphidocelis subcapitata, was verified using an appropriate taxonomic key.

Paramethyl styrene was shipped to T.R. Wilbury Laboratory at ambient temperature. Prior to use the test substance was stored at room temperature in the dark. Water used for acclimation of test organisms and for all toxicity testing was sterile freshwater AAP medium at a pH of 7.5 plus or minus 0.1.

A range-finding test was conducted under static conditions with a control and five concentrations of paramethyl styrene. The definitive study was performed at 24 degrees C (plus or minus 2 degrees C) with five concentrations and a control. A series of solutions was prepared by bringing 0.0010, 0.0020, 0.0040, 0.0081, and

ECOTOXICITY ELEMENTS:

TOXICITY TO AQUATIC PLANTS

0.0160 g of test substance to 1,000 mL with dilution water (measured using a Class A graduated cylinder) in sealed glass bottles. The solutions were mixed on magnetic stirrers for approximately four hours and each vortex was adjusted to approximately 5% of the distance from the surface to the bottom of the solution. The solutions were allowed to settle for approximately one hour and a portion of each solution was transferred into a 250 mL beaker through a spout at the bottom of the mixing vessels. A 250 mL portion of dilution water was also transferred to a glass flask to serve as a control. The pH was immediately adjusted to 7.5 with 0.1 N sodium hydroxide. Water quality measurements were made and each solution was inoculated with 10,000 algal cells/mL.

Solutions were subdivided into 11 clear glass 40 mL vials for each treatment (the control was subdivided into 20 replicates) and the vials, which were filled to capacity to eliminate any head space, were sealed with Teflon-lined caps. Nominal concentrations of paramethyl styrene were 0 mg/L (control), 1 .0, 2.0, 4.0, 8.0, and 16 mg/L. Test vessels were randomly arranged on a rotary shaker adjusted to approximately 100 rpm in an incubator during the test (a random numbers table was used to select the location for each vessel). A 24-hour light and O-hour dary photoperiod was automatically maintained with cool-white fluorescent lights that provided a light intensity of approximately 370 to 420 footcandles (approximately 50 to 53 uEin/m-squared sec).

The number of algal cells/ml in each test vessel and the occurrence of relative size differences, unusual cell shapes, colors, flocculations, adherence of cells to test containers, or aggregation of cells was determined visually by means of direct microscopic examination with a hemocytometer. At 24, 48, and 72 hours, three treatment vessels and six control vessels were randomly selected and sacrificed (opened to the atmosphere) to allow daily determination of the number of algal cells/ml. The remaining two vessels at each concentration were used for the determination of paramethyl styrene concentration at the end of the test.

Temperature of the incubator was measured and recorded daily (thermometer number 2968) and the temperature in a representative vessel of water incubated with the test vessels was continuously recorded. The pH of test solutions was measured and recorded in the single solution of each concentration prior to its distribution to test vessels at the beginning of the test, and in all test vessels used for the determination of the number of algal cells/ml at the end of the test.

A 0.5 mL aliquot of test media from each test vessel where growth was maximally inhibited (16 mg/L nominal paramethyl styrene concentration) was combined in a 250-mL flask with 100 mL of fresh media to determine whether toxic effects were algicidal or algistatic. This culture was incubated under test conditions for 192 hours.

Analytical determination of test concentration (active ingredient) was performed with 40 mL samples collected from each test solution prior to its distribution to test vessels at the beginning of the test. These samples were immediately sealed into 40 mL glass vials with no head space and analyzed immediately. Analytical samples were collected from two randomly selected replicates at each concentration at the end of the test. Samples collected from the four highest concentrations were removed with a syringe through the Teflon septa in the caps and analyzed. The control sample and

ECOTOXICITY ELEMENTS:

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samples collected at the lowest concentration were sampled from opened vials (the volume required for these analysis was too great to collect through the septa) and analyzed immediately. Each set of samples was accompanied by two laboratory control samples prepared at 4.0 mg/L paramethyl styrene in dilution water.

A 10 mL aliquot of each sample was transferred to a purge vessel with a 50 mL gas-tight syringe. Samples outside the calibration range were diluted with deionized water. Samples were concentrated using a 4460A O-I-Analytical Sample Concentrator fitted with a MPM-16 O-I-Analytical Multiple Purging Module. The typical purge, desorption, and bake times were 11, 4, and 15 minutes, respectively. The bake temperature was approximately 210 degrees C. Samples were analyzed using a Hewlett Packard model 5890 gas chromatograph with an FID detector. The column was a HP-5 (crosslinked 5%) PH ME Siloxane (1.5 micron thickness). The analytical method was validated in duplicate at 0.50, 5.0, and 20 mg/L in dilution water. Measured concentrations for samples with a nominal concentration of 0.5 mg/L were 0.19 and 0.28 mg/L, measured concentrations for samples with a nominal concentration of 5.0 mg/L were 3.5 and 2.8 mg/L, and measured concentrations for samples with a nominal concentration of 20 mg/L were 16 and 14 mg/L. The estimated water solubility limit of paramethyl styrene in a representative dilution water was 25 to 40 mg/L. The limit of quantitation (LDQ) during the definitive test was 0.0007 mg/L and the limit of detection (LOD) was 0.0002 mg/L.

The average specific growth rate was calculated as the natural log of the number of cells/ml at time tl minus the natural log of the number of cells/ml at time 0 divided by the exposure period. The area under the growth curve was calculated using a standard formula. The 72-hour EC50s were calculated using the weighted least squares non-linear regression estimation procedure. The no observed effect concentration (NOEC) was determined using a one-way analysis of variance (ANOVA) and Bonferroni's test (TOXSTAT 3.3). The effective concentrations and NOECs were determined using the mean measured concentration of paramethyl styrene and the number of cells/ml, average specific growth rate, and area under the growth curve.

Results

Nominal Concentration	1.0, 2.0, 4.0, 8.0, and 16 mg/L
Measured Concentration	0.41, 0.53, 1.6, 3.7, and 8.4 mg/L
Precision	=
Endpoint Type	EC50-CD
Endpoint Value	2
Unit used	mg/L
Endpoint Time	72
Concentration Type	Measured
NOEC Precision	=

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ROBUST SUMMARY

Styrene, p-methyl-

CAS# 622979 **Study Number: 1**

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NOEC	1
Unit Used	mg/L
NOEC Concentration Type	Measured
NOEC Effect(s) Assessed	Area under the growth curve
LOEC Precision	=
LOEC	2
Unit Used	mg/L
LOEC Concentration Type	Measured
LOEC Effect(s) Assessed	Area under the growth curve
Response of Control Group (was it satisfactory?)	**Yes**
Statistical Results	The 72-hour NOEC was 1.6 mg/L using the number of cells/mL or the average specific growth rate, and 0.53 mg/L when determined using the area under the growth curve ($p<0.05$).
Results Remarks	Insoluble material was not observed during the test. Nominal concentrations of paramethyl styrene were 0 mg/L (control), 1 .0, 2.0, 4.0, 8.0, and 16 mg/L. Mean measured concentrations of paramethyl styrene were: ND (none detected at or above the LOQ; control), 0.41, 0.53, 1.6, 3.7, and 8.4 mg/L. Mean measured concentrations ranged from 27. to 53% of nominal concentrations. Loss of the volatile test substance during preparation of test solutions was assumed to have occurred to the atmosphere. Final measured concentrations were 78 to 89% of the initial concentrations, indicating that once the aqueous solutions of paramethyl styrene were sealed into the test vessels with the algae, concentrations remained relatively constant. The algal population grew at an acceptable rate in the sealed vessels with no head space, resulting in an average of 229,000 cells/mL in the control after 72 hours. No effects (relative size differences, unusual cell shapes, colors, flocculations, adherence of cells to test containers, or aggregation of cells) were noted during the test. Water quality throughout the test was within acceptable limits. The incubator temperature ranged from 24.3 to 24.9 degrees C. The pH was decreased slightly by the test substance. The mean number of cells/mL at 24, 48, and 72 hours, respectively, was as follows: control, 23000, 52000, and 229000; 0.41 mg/L, 22000, 59000, and 217000; 0.53 mg/L, 22000, 50000, and 213000; 1.6 mg/L, 18000, 31000, and 184000; 3,7 mg/L, 13000, 19000, and 61000; 8.4 mg/L, <10000, <10000, and 16000. The average specific growth rate at 24, 48, and 72 hours, respectively, was as follows: control, 0.035, 0.034, and 0.043; 0.41 mg/L, 0.033, 0.037, and 0.043; 0.53 mg/L, 0.033, 0.034, and 0.042; 1.6 mg/L, 0.024, 0.024, and 0.040; 3.7 mg/L, 0.011, 0.013, and 0.025; 8.4 mg/L, 0.000, 0.000, and 0.007.

Admin Record Number:

ROBUST SUMMARY

Styrene, p-methyl-

CAS# 622979

Study Number: 1

ECOTOXICITY ELEMENTS:

TOXICITY TO AQUATIC PLANTS

The mean area under the growth curve at 24, 48, and 72 hours, respectively, was as follows: control, 156000, 816000, and 3948000; 0.41 mg/L, 144000, 876000, and 3948000; 0.53 mg/L, 144000, 768000, and 3684000; 1.6 mg/L, 96000, 444000, and 2784000; 3.7 mg/L, 36000, 180000, and 900000; 8.4 mg/L, 0, 0, and 72000.

The 24-, 48-, and 72-hour EC50s (95% confidence limits) and NOEC, respectively, using the number of cells/mL was 5.5 (4.1 to 7.6), 2.3 (1.5 to 3.4), 2.6 (2.1 to 3.3), and 1.6 mg/L. The 24-, 48-, and 72-hour EC50s (95% confidence limits) and NOEC, respectively, using the average specific growth rate was 2.7 (2.2 to 3.2), 3.1 (2.6 to 3.7), 4.3 (3.9 to 4.7), and 1.6 mg/L. The 24-, 48-, and 72-hour EC50s (95% confidence limits) and NOEC, respectively, using the area under the growth curve was 2.2 (1.8 to 2.8), 1.9 (1.5 to 2.3), 2.3 (2.0 to 2.7), and 0.53 mg/L.

At the conclusion of the definitive toxicity test, a 0.5 mL aliquot of test media from each test vessel where growth was maximally inhibited (the 8.4 mg/L paramethyl styrene concentration) **was** combined with 100 mL of fresh media in a 250-mL flask. This culture was incubated under test conditions for 192 hours. During this period the number of algal cells increased from an initial calculated concentration of 250 cells/ml to 1,562,000 cells/ml, indicating that the toxic effect was algistatic rather than algicidal.

Conclusions

Exposure of Selenastrum capricornutum to paramethyl styrene for 72 hours resulted in an EC50 of 2.6 mg/L when calculated using the number of cells/mL, 4.3 mg/L when calculated using the area under the growth curve, and 2.3 mg/L when calculated using the average specific growth rate. The 72-hour NOEC is 1.6 mg/L when determined using the number of cells/ml or the average specific growth rate, and 0.53 mg/L when determined using the area under the growth curve.

Due to the high volatility of the test substance, the test was conducted in sealed containers with little or no head space. Mean, measured concentrations ranged from 27 to 53% of nominal concentrations. The loss of the test substance to the atmosphere occurred during preparation of the test solutions. Once the test solutions were prepared and the algae added, the final concentrations were 78 to 89% of the initial measured concentrations. Control algal growth was acceptable during the 72 hours.

Data Quality

Reliability Acceptable

Data Reliability Remarks All endpoints and experimental design were appropriate and adequately described in the study. Rigorous attempts were made to limit evaporation of this very volatile test substance, and all endpoints were based on mean, measured concentrations.

Reference

Remarks Growth and Reproductive Toxicity Test with Paramethyl Styrene and the Freshwater Alga, Selenastrum capricornutum. T.R. Wilbury Study Number 1922-UL.

General

Admin Record Number: 20113044B **ROBUST SUMMARY**

Styrene, p-methyl-

CAS# 622979 **Study Number: 1**

ECOTOXICITY ELEMENTS:

ACUTE TOXICITY TO AQUATIC INVERTEBRATE

Test Substance

Remarks

p-methylstryene
Test Article ID#: MCTR-197-79 (08217901)
Purity: 97% Active Ingredient
Additives: None reported
Carrier solvent: Dimethylformamide (DMF)
Contaminants: None reported
Chemical formula: C9H10

Chemical Category

Method

Method/Guideline Followed Am. Public Health Association, Standard Methods for the Examination of Water and Wastewater. 1975

Test Type static

GLP Unknown

Year Study Performed 1979

Species Daphnia magna

Analytical Monitoring None

Exposure Period 48

Statistical Method Spearman-Karber Estimator

Remarks for Method

Daphnia magna Straus used in this test came from a UCES laboratory stock culture, the original population having been obtained from the EPA Environmental Research Laboratory, Duluth, Minnesota. Stock cultures were maintained at 19-21 degrees C in 350 liter tanks. Neonates, less than 20 hours old, were used in the test. One hour before the test, they were fed, and no food was administered thereafter.

Dilution water for the test was obtained from a well on the Tarrytown, New York site. The water, stored in a 95 liter glass reservoir, was vigorously aerated before use and determined by analysis to have a pH of 7.95, total hardness of 220 mg/l as calcium carbonate, total alkalinity of 145 mg/l as calcium carbonate and a specific conductance of 600 microhos/cm.

A 100 mg/ml stock solution of the test material in reagent grade DMF was prepared by weight to a precision of 0.1 mg; additional stocks were prepared by serial dilution. A range-finding test was first conducted in disposable 250-ml polypropylene beakers. A definitive test was conducted, based on rangefinding results, which included five nominal test concentrations of 0.56, 1 .00, 1.80, 3.20, and 5.60 mg/l. Solvent and blank controls were also employed. There were four replicates per concentration (250 ml glass beakers containing 200 ml solution) with five organisms per replicate (total of 20 per concentration). Testing was begun by thoroughly mixing measured volumes of stock solution and dilution water in one-liter volumetric flasks. The test beakers were held for the duration of the test in a refrigerator incubator at a

ECOTOXICITY ELEMENTS:

ACUTE TOXICITY TO AQUATIC INVERTEBRATE

constant temperature of 20.8 degrees C. Dissolved oxygen and pH were determined inititally and at 48 hours for all test concentrations and controls. Mortalities were recorded at 24 and 48 hours.

The concentration of test material lethal to 50% of the test population and 95% confidence limits were determined for the 24 and 48 hour exposure periods by the Spearman-Karber Estimator. LC50 calculations were based on nominal concentrations of the test material in hard well water. The NOEC was determined, by observation, at 48 hours.

Results

Nominal Concentration	0.56, 1.00, 1.80, 3.20, and 5.60 mg/L
Measured Concentration	Not measured
Precision	=
Endpoint Type	LC50
Endpoint Value	3
Unit Used	mg/L
Concentration Type	Nominal
Endpoint Time	48
Statistical Results	The 48 hour LC50 with 95% confidence limits was 3.34 (2.69-4.13) mg/L
Results Remarks	Percent mortalities at 48-hours were 0, 0, 0, 20, 20, 25, and 75 mg/L for control, solvent control, 0.56, 1.00, 1.80, 3.20, and 5.60 mg/L, respectively. The 24-hour and 48-hour LC50s (95% confidence limits) were 5.60 (4.92-6.40) and 3.34 (2.69-4.13) mg/L, respectively. The 48-hour NOEC, based on mortality, was 0.56 mg/L. All dissolved oxygen and pH values were within acceptable limits.

Conclusions

The 48-hour LC50 and 95% confidence limits for MCTR-197-79 in Daphnia magna were 3.34 (2.69-4.13) mg/L. The 48-hour NOEC was observed to be 0.56 mg/L.

Data Quality

Reliability	Unacceptable
Data Reliability Remarks	The test substance was used in a static exposure system and not analytically measured. All values are based on nominal concentrations. The test beakers were apparently open to the air and, based on the high volatility of the test substance, substantial amounts of the the compound probably was lost due to evaporation.

Reference

Remarks	The Acute Toxicity of 08217901 (97% Active Ingredient) to the Water Flea Daphnia magna Straus, Union Carbide Corporation Environmental Services, Project No.: 11506-05-14, January 3, 1980.

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ROBUST SUMMARY

Styrene, p-methyl-

CAS# 622979

Study Number: 1

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General

Admin Record Number: 20113044B **ROBUST SUMMARY**

Styrene, p-methyl-

CAS# 622979 **Study Number: 2**

ECOTOXICITY ELEMENTS:

ACUTE TOXICITY TO AQUATIC INVERTEBRATE

Test Substance

Remarks

p-methylstyrene
Test Article ID: Paramethyl Styrene
Purity: 99.66% Active ingredient
Additives: None reported
Carrier solvent: None
Contaminants: None reported
Chemical formula:C9H10

Chemical Category

Method

Method/Guideline Followed OECD Method 202

Test Type static

GLP Yes

Year Study Performed 2001

Species Daphnia magna

Analytical Monitoring Gas chromatograph/FID; LOD, 0.0002 mg/L

Exposure Period 48 hours

Statistical Method Moving average method

Remarks for Method

Juvenile daphnids, Daphnia magna, less than 24 hours old, were produced from an in-house culture by adult daphnids that were maintained under test conditions at T.R. Wilbury Laboratories for 29 days. The original culture was obtained from Aquatic Biosystems Inc., Fort Collins, Colorado, on March 7, 2000. Prior to testing, the daphnid culture was maintained in 100% dilution water under static, renewal conditions, and the test organisms were not treated for disease. During the 48 hours prior to the beginning of the test, there was no mortality, and at the beginning of the test organisms were apparently free of disease, injuries, and abnormalities. The culture produced young before day 12 and a subsample of adults from this culture produced, on average, more than 3 young per day during the seven days before the start of the definitive toxicity test. During the 7 days prior to the beginning of the test, the temperature of the culture ranged from 20.0 to 20.6 degrees C, the pH ranged from 7.3 to 7.6, and the dissolved oxygen concentration was always at least 8.8 mg/L. Test organisms were fed the freshwater algae, Selenastrum capricornutum, and a mixture of yeast and trout chow once each day before the test. Daphnids were not fed during the test.

Water for the acclimation of test organisms and for all toxicity testing was carbon filtered, deionized water collected at T.R. Wilbury Laboratories in Marblehead, Massachusetts. Water was adjusted to a hardness of 160 to 180 mg/L and stored in polyethylene tanks where it was aerated and recirculated through particle filters, activated carbon, and an ultraviolet sterilizer. Prior to use, the pH of the dilution

ECOTOXICITY ELEMENTS:

ACUTE TOXICITY TO AQUATIC INVERTEBRATE

water was adjusted to <8.0. A sample of dilution water collected at the start of the test had a hardness of 172 mg/L as calcium carbonate and an alkalinity of 100 mg/L as calcium carbonate. The dilution water sample contained less than 10 mg/L particulate matter, less than 1 .0 mg/L total organic carbon, and less than 0.01 mg/L total residual chlorine.

A rangefinding test was conducted under static conditions to determine the concentrations for the definitive test. The definitive test was conducted under static conditions at 20 degrees C (plus or minus 1 degree C) with five concentrations and a control. A series of solutions was prepared by bringing 3.8, 6.1, 9.6, 15.3, and 23.9 mg of test substance to 4,000 mL with dilution water (measured using a Class A gradualted cylinder) in sealed glass bottles. The solutions were mixed on magnetic stirrers for approximately four hours and each vortex was adjusted to approximately 5% of the distance from the surface to the bottom of the solution. The solutions were allowed to settle for approximately one hour and a portion of each solution was transferred into a 250 mL beaker through a spout at the bottom of the mixing vessels. A 250 mL portion of dilution water was also transferred to a glass beaker to serve as a control. Water quality measurements were made and each solution was subdivided into four clear glass 40 mL vials. Five daphnids were indiscrimantely added to each vial and the vials, which were filled to capacity to eliminate any head space, were sealed with Teflon-lined caps. Nominal concentrations were 0 mg/L (control), 0.95, 1.5, 2.4, 3.8, and 6.0 mg/L. Test vessels were randomly arranged in a water bath during the 48-hour test (a random numbers table was used to select the location of each vessel). A 16-hour light and 8-hour dark photoperiod was automatically maintained with cool-white fluorescent lights that provided a light intensity of approximately 33 footcandles. A 15 minute transition period was provided between dark and light. The numbers of surviving organisms, the occurrence of immobility and sublethal effects, and the presence of insoluble material were determined visually and recorded after 0, 24, and 48 hours. Vials remained sealed throughout the 48 hour exposure period to prevent the loss of paramethyl styrene to the atmosphere, so dead test organisms were not removed at 24 hours.

Dissolved oxygen concentration and conductivity were measured and recorded in a single solution of each concentration prior to its distribution to test vessels at the beginning of the test, and in all test vessels after analytical samples have been removed at the end of the test. The temperature in a vessel of water incubated among the test vessels was recorded continuously during the test.

Analytical determination of test substance concentration (active ingredient) was performed in 40 mL samples collected from each test solution prior to its distribution to test vessels at the beginning of the test. These samples were immediately sealed into 40 mL glass vials with no head space and analyzed immediately. Analytical samples were collected from two randomly selected replicates at each concentration at the end of the test. Samples collected from the four highest concentrations were removed with a syringe through the Teflon septa in the caps and analyzed immediately. The control sample and samples collected at the lowest concentration were sampled in open vials (the volume required for these analysis was too great to collect through the septa) and analyzed immediately. Each set of samples was accompanied by two laboratory control samples prepared at 2.4 mg/L paramethyl styrene in dilution water. A 10 mL aliquot of each sample was transferred to a purge vessel with a 50 mL gas-tight syringe. Samples outside the calibration range were diluted with deionized water. Sample were concentrated using a 4460A O-I-

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Styrene, p-methyl-

CAS# 622979 **Study Number: 2**

ECOTOXICITY ELEMENTS:

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Analytical Sample Concentrator fitted with a MPM-16 O-I-Analytical Multiple Purging Module. The typical purge, desorption, and bake times were 11, 45, and 15 minutes, respectively. The bake temperature was approximately 210 degrees C. Samples were analyzed using a Hewlett Packard model 5890 gas chromatograph and an FID detector. The column was a HP-5 (crosslinked 5%) PH ME Siloxane (1.5 micron thickness). The analytical method was validated in duplicate at 0.50, 3.0, and 10 mg/L in dilution water. Measured concentrations for samples with a nominal concentration of 0.50 mg/L were 0.30 and 0.35 mg/L, measured concentrations for samples with a nominal concentration of 3.0 mg/L were 2.3 and 2.4 mg/L, respectively, and measured concentrations for samples with a nominal concentration of 10 mg/L were 8.0 and 9.1 mg/L. A solubility study was conducted in the dilution water and the estimated water solubility limit was between 25 and 40 mg/L. The limit of quantitation (LOQ) was 0.0007 mg/L and the limit of detection (LOD) was 0.0002 mg/L.

The 24-hour LC50 was calculated using the binomial method, and the 48-hour LC50 and the 24- and 48-hour EC50s were calculated using the moving average method. The slopes of the concentration-response curves were calculated using the probit method. The no observed effect concentration (NOEC) was calculated using TOXSTAT 3.3. A Chi-square test was used to determine that the data were not normally distributed and Bartlett's test was used to determine that the variances were heteroscedastic. Survival and sublethal effect data in the treatments were compared to the control using a Williams' test.

Results

Nominal Concentration	0.95, 1.5, 2.4, 3.8, and 6.0 mg/L
Measured Concentration	0.51, 0.81, 1.5, 2.3, and 3.8 mg/L
Precision	=
Endpoint Type	EC50
Endpoint Value	1
Unit Used	mg/L
Concentration Type	Measured
Endpoint Time	48
Statistical Results	The 24- and 48-hour EC50s were 1.4 and 1.3 mg/L, respectively. The 48-hour NOEC was 0.81 mg/L ($p<0.05$).
Results Remarks	Exposure of daphnids to paramethyl styrene resulted in a 24-hour EC50 (immobility) of 1.4 mg/L (95% confidence interval = 1 .1 to 1.7 mg/L) and a 48-hour EC50 (immobility) of 1.3 mg/L (95% confidence interval = 1.0 to 1.6 mg/L). The 24-hour LC50 was 1.9 mg/L (95% confidence interval = 1.5 to 2.3 mg/L) and a 48-hour LC50 was 1.7 mg/L (95% confidence interval = 1.4 to 2.1 mg/L). The slopes of the dose response curves based on effects (immobility) and mortality were 5.3 and 6.6, respectively. The 48-hour NOEC was 0.81 mg/L. All values were based on mean, measured concentrations.

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The 24-hour survival data (number dead/total) was as follows: control, 0/20; 0.51 mg/L, 1/20; 0.81 mg/L, 0/20; 1.5 mg/L, 0120; 2.3 mg/L, 20/20; 3.8 mg/L, 20/20. The 48-hour survival data (number dead/total) was as follows: control, 0/20; 0.51mg/L, 1/20; 0.81 mg/L, 0120; 1.5 mg/L, 1/20; 2.3 mg/L, 20/20; 3.8 mg/L, 20/20. The 24-hour immoblization data (number immobilized/total alive) was as follows: control, 0120; 0.51 mg/L, 1/19; 0.81 mg/L, 0/20, 1.5 mg/L, 9/20; 2.3 mg/L, all dead; 3.8 mg/L, all dead. The 48-hour immobilization data (number immobilized/total alive) was as follows: control, 0/20; 0.51 mg/L, 1/19; 0.81 mg/L, 0/20; 1.5 mg/L, 8/19; 2.3 mg/L, all dead: 3.8 mg/L, all dead.

Insoluble material was not observed during the test. Mean, measured concentrations ranged from 54 to 63% of nominal concentrations. Final measured concentrations were 85 to 92% of the initial measured concentrations, indicating that once the aqueous solutions of paramethyl styrene were sealed into the test vessels with the daphnids, concentrations remained constant. Loss of the volatile test substance during the preparation of test solutions was assumed to have occurred to the atmosphere.

One hundred percent survival occurred in the control and no sublethal effects were noted during the exposure period. Water quality was within the normal range throughout the toxicity test. During the definitive toxicity test the conductivity was 560 umhos/cm, the pH ranged from 7.2 to 7.7, the temperature ranged from 20.1 to 20.9 degrees C (mean = 20.7 degrees C), and the dissolved oxygen concentration during the test was 79% saturation (saturation equals 9.1 mg/L at 20 degrees C).

Conclusions

Exposure of daphnids to paramethyl styrene resulted in a 24 hour EC50 (immobility) of 1.4 mg/L (95% confidence interval = 1 .1 to 1.7 mg/L) and a 48-hour EC50 (immobility) of 1.3 mg/L (95% confidence interval = 1.0 to 1.6 mg/L). The study was conducted in a closed system with little or no headspace in order to limit volatility of this compound. Mean, measured concentrations ranged from 54 to 63% of nominal concentrations.

Data Quality

Reliability Acceptable

Data Reliability Remarks All endpoints and experimental design were appropriate and adequately described in the study. Rigorous attempts were made to limit evaporation of this very volatile test substance, and all endpoints were based on mean, measured concentrations.

Reference

Remarks Acute Toxicity of Paramethyl Styrene to the Daphnid, Daphnia magna. T.R. Wilbury Study Number 1921-UL.

General

Admin Record Number: **ROBUST SUMMARY**

Styrene, p-methyl-

CAS# 622979 **Study Number: 1**

ECOTOXICITY ELEMENTS:

ACUTE TOXICITY TO FISH

Test Substance

Remarks

p-methylstyrene
Test Article ID#: 08217901
Purity: 97% Active Ingredient
Additives: None reported
Carrier Solvent: Dimethylformamide (DMF)
Contaminants: None reported
Chemical formula: C9H10

Chemical Category

Method

Method/Guideline Followed Am. Public Health Association, Standard Methods for the Examination of Water and W

Test Type static

GLP Unknown

Year Study Performed 1979

Species Lepomis macrochirus

Analytical Monitoring Not measured

Exposure Period 96 hours

Statistical Method None reported

Remarks for Method

Bluegill sunfish, Lepomis macrochirus Rafinesque, used in the study were obtained from a commerical hatchery in Nebraska and maintained in the UCES laboratory at 22 degrees C. Mortalities in the stock culture over a one month period were less than 2%. Forty-eight hours before starting the test, the fish were taken off feed, and no food was administered thereafter.

Dilution water for the test was obtained from a well on the Tarrytown, New York site, treated with a Continental Model 3020 Reverse Osmosis Water System, and deionized. The water was then reconstituted to a pH of 7.82, a total hardness and alkalinity (as calcium carbonate) of 44 and 33 mg/L, respectively, and a specific conductance of 155 microhos/cm. A fresh working stock solution of the test material in reagent grade DMF was prepared by weight to a precision of 0.1 mg. A range-finding test was first conducted in two liters of soft reconstituted water.

The definitive test was conducted in 19.6 liter glass jars, each containing 15 liters of soft reconstituted dilution water and immersed in a constant temperature recirculating water bath. Five nominal test concentrations of 18, 32, 56, 100, and 180 mg/L, and a control and solvent control were used. Testing was begun by adding measured volumes of stock solution to the water in five test vessels, and mixing thoroughly, to obtain the nominal test concentrations. There was one replicate per test concentration with ten fish per replicate (approximately 3 months old; 33 mm length; weight, 0.43 grams; loading 0.28 g/l). At the beginning of the test, and every 24 hours thereafter,

ECOTOXICITY ELEMENTS:

ACUTE TOXICITY TO FISH

dissolved oxygen and pH of all groups were determined. Mortalities among test fish, and any observable abnormal behavioral responses, were noted and recorded every 24 hours. The NOEC was determined, by observation, at 96 hours.

Results

Nominal Concentration	18, 32, 56, 100, and 180 mg/L
Measured Concentration	Not measured
Precision	>
Endpoint Type	LC50
Endpoint Value	180
Unit Used	mg/L
Concentration Type	Nominal
Endpoint Time	96
Statistical Results	None reported
Results Remarks	No mortalites were observed in any test concentration. All dissolved oxygen and pH values were within acceptable limits. Animals at 56 mg/L exhibited irritation at 24-72 hours. All animals at 100 and 180 mg/L exhibited hyperexcitablity. Based on these results, the NOEC was observed to be 32 mg/L.

Conclusions

The 96-hour LC50 of p-methylstyrene to bluegill sunfish was greater than 180 mg/L, nominal concentration. The 96-hour NOEC, based on behavioral observations, was 32 mg/L.

Data Quality

Reliability	Unacceptable
Data Reliability Remarks	The test substance was used in a static exposure system and was not analytically measured. The test vessels were apparently open to the air and, based on the high volatility of the test substance, a substantial amount was probably lost due to evaporation.

Reference

Remarks	The Acute Toxicity of 08217901 to the Bluegill Sunfish Lepomis macrochirus Rafinesque, Union Carbide Corporation Environmental Services, Project No.: 11506-05-12, November 19, 1979.

General

Admin Record Number:

ROBUST SUMMARY

Styrene, p-methyl-

CAS# 622979

Study Number: 2

ECOTOXICITY ELEMENTS:

ACUTE TOXICITY TO FISH

Test Substance

Remarks	p-methylstyrene Test Article ID#: 08217901 Purity: Not reported Additives: None reported Carrier Solvent: Dimethylformamide (DMF) Contaminants: None reported Chemical formula: C9H10
Chemical Category	

Method

Method/Guideline Followed	Am. Public Health Association, Standard Methods for the Examination of Water and W
Test Type	static
GLP	Unknown
Year Study Performed	1979
Species	Salmo gairdneri
Analytical Monitoring	Not measured
Exposure Period	96 hours
Statistical Method	Spearman-Karber Estimator
Remarks for Method	Rainbow trout (Salmo gairdneri Richardson) used in this test were cultured in the UCES laboratory from eggs obtained from a commerical hatchery in Washington and maintained at 13 degrees C. Mortalities in the stock culture over a one month period was less than 2%. Two days before starting the test the fish were selected at random from the stock culture, taken off feed, and isolated in a jar of dilution water for 48 hours before testing. Dilution water for the test was obtained from a well on the Tarrytown, New York site, treated with a Reverse Osmosis Water System, deionized, and reconstituted to a pH of 7.46, total hardness and alkalinity (based on calcium carbonate) of 40 and 30 mg/L, respectively, and a specific conductance of 155 microhos/cm. A fresh working solution of the test material in reagent grade DMF was prepared by weight to a precision of 0.1 mg. A rangefinding test was first conducted in two liters of soft reconstituted water. The definitive test was conducted in 19.7 liter glass jars, each containing 15 liters of soft reconstituted dilution water and immersed in a constant temperature water bath (11.5 to 12.0 degrees C). Five concentrations were tested, with a control and solvent control. Testing was begun by adding measured volumes of stock solution to the water in five test vessels, and mixing thoroughly, to yield nominal test material concentrations of 3.2, 5.6, 10, 18, and 32 mg/L. Ten fish were introduced at random into each of the vessels. The fish were approximately five months old, with a mean

ECOTOXICITY ELEMENTS:

ACUTE TOXICITY TO FISH

length of 58 mm and mean weight of 1.64 grams. The loading was 1 .10 g/L, which exceeded the protocol maximum of 0.80 g/L.

At the beginning of the test, and every 24 hours thereafter, dissolved oxygen and pH were measured in all test vessels. Mortalities among the test fish, and any observable abnormal behavioral responses, were recorded every 24 hours. The NOEC was determined by observation at 96 hours. The LC50s were determined by the Spearman-Karber Estimator.

Results

Nominal Concentration	3.2, 5.6, 10, 18, and 32 mg/L
Measured Concentration	Not measured
Precision	=
Endpoint Type	LC50
Endpoint Value	8
Unit Used	mg/L
Concentration Type	Nominal
Endpoint Time	9 6
Statistical Results	The 96-hour LC50 was 7.6 mg/L
Results Remarks	Total mortality was observed in 10, 18, and 32 mg/L at 24 hours. No mortality was observed in 5.6 and 3.2 mg/L. Sublethal effects (surfacing, irritated) were observed in all surviving animals at 3.2 and 5.6 mg/L. All dissolved oxygen and pH values were within normal limits.

Conclusions

The 96-hour LC50 for 08217901 to rainbow trout was 7.6 mg/L (nominal concentration). No confidence limits could be obtained due to the lack of partial **mortality.** The 96-hour NOEC was observed to be less than 3.2 mg/L.

Data Quality

Reliability	Unacceptable
Data Reliability Remarks	The test substance was used in a static exposure system and was not analytically measured. The test vessels were apparently open to air and, based on the high volatility of the test substance, a substantial amount was probably lost due to evaporation.

Reference

Remarks	The Acute Toxicity of 08217901 to the Rainbow Trout Salmo gairderi Richardson, Union Carbide Corporation Environmental Services, Project No.: 11506-05-I 3, December 27, 1979.

Admin Record Number:

ROBUST SUMMARY

Styrene, p-methyl-

CAS# 622979

Study Number: 2

ECOTOXICITY ELEMENTS:

ACUTE TOXICITY TO FISH

General

Admin Record Number: **ROBUST SUMMARY**

Styrene, p-methyl-

CAS# 622979 **Study Number: 3**

ECOTOXICITY ELEMENTS:

ACUTE TOXICITY TO FISH

Test Substance

Remarks

p-methylstyrene
Test Article ID#: PMS
Purity: 100% assumed for dosage calculations
Additives: None reported
Solvent Carrier: Nanograde acetone
Contaminants: None reported
Chemical formula: C9H10

Chemical Category

Method

Method/Guideline Followed EPA-66013-75-009, April, 1975

Test Type flow-through

GLP **Yes**

Year Study Performed 1981

Species Lepomis macrochirus

Analytical Monitoring HPLC, limit of detection, 0.05 micrograms/ml

Exposure Period 168

Statistical Method LC50 determined by Stephen, ANOVA and Fisher's LSD

Remarks for Method Bluegill sunfish (Lepomis macrochirus; mean length 40 mm, mean weight 1.9 g) used in the test were obtained from Fattig Fish Hatchery in Brady, Nebraska. All test fish were held in culture tanks on a 16-hr light photoperiod and observed for at least 14 days prior to testing. A Mount and Brungs proportional dilutor system was used for the intermittent introduction of PMS and diluent water into the test aquaria. Aerated well water was delivered to the glass aquaria at a rate of 250 ml/minute/aquarium, an amount which was sufficient to replace the 30 liter test volume at least 10 times in a 24 hour period. The test aquaria were immersed in a circulating water bath held at 22 degrees C by submerged heating elements. The fish (20/concentration, 1 replicate/concentration) were exposed to the following nominal concentrations: 0.76, 1.5, 3.0, 6.1, and 12 mg/L. An acetone control (18.14 mg/ml) was also employed. The fish were observed for mortality and abnormal behavior initially and once every 24 hours for the 7-day test period. Water quality parameters of temperature, dissolved oxygen, pH and ammonia were measured throughout the test. Analysis of aquarium water for PMS was accomplished using an HPLC unit consisting of a Waters Model 6000A solvent delivery system (mobile phase, 70% methanol in 30% deionized water, 1 ml/minute), a Cl8 reverse phase column and a Schoeffel Model Sprectroflow UV detector set at 254 nm. The detection limit was 0.05 microgram/ml. Analysis of the test water was performed on day 0, 1, 4, and 7. The results were statistically analyzed for daily LC50 values and their corresponding 95% confidence limits. Weight and length measurements were subjected to ANOVA followed by Fisher's protected Least Significant Difference (LSD) if the overall F-test was significant

ECOTOXICITY ELEMENTS:

ACUTE TOXICITY TO FISH

(p<0.05).

Results

Nominal Concentration	0.76, 1.5, 3.0, 6.1, and 12 mg/L
Measured Concentration	0.41, 0.66, 1.2, 2.3, and 5.4 mg/L
Precision	=
Endpoint Type	LC50
Endpoint Value	3
Unit Used	mg/L
Concentration Type	Measured
Endpoint Time	168
Statistical Results	The 7-day LC50 with 95% confidence limits was 2.6 (1.2-5.4) mg/L. The NOEC, based on sublethal effects, was 0.66 mg/L.
Results Remarks	No mortality was observed in 0.76 to 3.0 mg/L. Cummulative percent mortality in 6.1 mg/L on days l-7 was 0, 0, 15, 30, 35, 40, and 40, respectively. Cummulative percent mortality in 12 mg/L on days 1-7 was 95, 95, 100, 100, 100, 100, and 100, respectively. The mean, measured concentrations of PMS were 0.41, 0.66, 1.2, 2.3, and 5.4 mg/L. These values ranged from 38 to 54% of nominal concentrations of 0.76, 1.5, 3.0, 6.1, and 12 mg/L, respectively. Because of this, only the measured values were used in the statistical calculations of the LC50 levels. The mean, measured LC50 values with 95% confidence limits were as follows: Days 1 and 2, 3.7 (2.3-5.4) mg/L; day 3, 3.1 (2.3-5.4) mg/L; day 4, 2.8 (1.2-5.4) mg/L; day 5, 2.7 (1.2-5.4) mg/L; and days 6 and 7, 2.6 (1.2-5.4) mg/L. The NOEC was 0.66 mg/L. Adverse behavior observations noted during the study were loss of equilibrium and surfacing at 1.2 and 2.3 mg/L. Weight measurements of surviving fish at the end of the study yielded the following weight percentages of the control group mean weight: 0.41 mg/L, 100%; 0.66 mg/L, 89%; 1.2 mg/L, 100%; and 2.3 mg/L, 89%. The weight analysis indicated no significant effects on growth of the test fish at any test concentration. Water quality parameters of temperature, dissolved oxygen, pH and ammonia were all within normal limits.

Conclusions

A dynamic 7-day toxicity study **was** conducted to determine the lethal threshold (incipient LC50) of PMS to bluegill sunfish (Lepomis macrochirus). A flow-through proportional diluter system was used to maintain **constant** test concentrations. Exposure concentrations were measured by HPLC methods. The mean measured levels were 0.41, 0.66, 1.2, 2.3, and 5.4 mg/L. The nominal concentrations were 0.76, 1.5, 3.0, 6.1, and 12 mg/L. Nominal concentrations were approximately 45% of the measured values. The volatility of the compound was thought to account for this discrepancy. For this reason, only measured values were used in statistical calculations of the LC50 values.

Admin Record Number:

ROBUST SUMMARY

Styrene, p-methyl-

CAS# 622979

Study Number: 3

ECOTOXICITY ELEMENTS:

ACUTE TOXICITY TO FISH

The LC50s for PMS during the study ranged from 3.7 to 2.6 mg/L for days l-7, respectively. An apparent lethal threshold for PMS to bluegill sunfish was reached within the 7 days of the study and was estimated to be 2.6 mg/L.

Based on behavior responses, the NOEC after 7 days of exposure was 0.66 mg/L.

Data Quality

Reliability Acceptable

Data Reliability Remarks The test substance was measured during the study and a flow-through design was used. Although recoveries ranged from 38 to 54%, the LC50 values were calculated using mean measured concentrations.

Reference

Remarks

Dynamic Acute Toxicity of para-methylstyrene (PMS) to Bluegill Sunfish (Lepomis macrochirus). Analytical Biochemistry Laboratories, Inc., Project No.:281 67, October 30, 1981.
Uptake, Depuration and Bioconcentration of 14C-para-methylstyrene by Bluegill Sunfish (Lepomis macrochirus), Analytical BioChemistry Laboratories, Inc. Project No.: 28170, February 26, 1982.
Uptake, Depuration and Bioconcentration of 14C-para-methylstyrene by Channel Catfish (Ictalurus punctatus), Analytical Biochemistry Laboratories, Inc. Project No.:281 71, March 10, 1982.

General

A dynamic bioconcentration study was conducted exposing bluegill sunfish to a 14C-PMS concentrations of 0.25 mg/l in constant flow-through water. During a 30-day uptake period, tissue contents of 14C-PMS ranged from 24-64 micrograms/g for whole fish, 6-23 micrograms/g for fillet, and 25-79 micrograms/g for viscera. 14C-PMS accumulation in the fish reached a steady-state after about day 3 of uptake. A bioconcentration factor (BCF) was calculated to be 110 based on a two-compartment kinetic model. Radioanalysis throughout the 14-day depuration period inidcated 94-95% clearance rates of 14C-PMS from whole fish, fillet, and viscera to respective levels of 1.2, 0.7, and 2.6 micrograms/g. The BCF of 110 indicated that PMS is not substantially accumulated in fish under the conditions of this study.

A static bioconcentration study was conducted exposing channel catfish to 14C-PMS at a level of 0.34 mglkg in sandy loam soil. It consisted of four continuous periods: 30-day aging, 8-day equilibration, 30-day uptake, and 14-day depuration. The application rate of 14C-PMS was intended to be 2.0 mglkg. Due to the volatility of PMS, 82% of the material was lost during application and mixing and 14C-radioactivity level in the treated soil was reduced to 0.14 mg/kg on day 30 of uptake. The mean water concentrations ranged from 0.34 micrograms/l on day 1 of equilibration to 1.3 micrograms/l on day 30 of uptake. There was no significant increase in water concentration after day 22 of uptake. Based on semi-quantitative values, maximum tissue concentrations of 14C-PMS were estimated to be 0.0073 mg/kg for whole fish, 0.0067 mg/kg for fillet and 0.0060 mglkg for viscera. Dividing these concentrations by the respective water concentrations gave the corresponding BCFs of 4.9, 9.2, and

Admin Record Number:

ROBUST SUMMARY

Styrene, p-methyl-

CAS# 622979

Study Number: 3

ECOTOXICITY ELEMENTS:

ACUTE TOXICITY TO FISH

4.0. Because all tissue values in both uptake and depuration phases were below minimum quantifiable limits, it was not possible to evaluate the elimination of 14C-PMS. The results indicate that PMS is not substantially accumulated in fish under the conditions of this study.

Admin Record Number:

ROBUST SUMMARY

Styrene, p-methyl-

CAS# 622979

Study Number: 4

ECOTOXICITY ELEMENTS:

ACUTE TOXICITY TO FISH

Test Substance

Remarks

p-methylstyrene
Test Article ID: Paramethyl Styrene
Purity: 99.66% Active Ingredient
Additives: None reported
Carrier solvent: None
Contaminants: None reported
Chemical formula: C9H10

Chemical Category

Method

Method/Guideline Followed OECD Method 203

Test Type semi-static

GLP **Yes**

Year Study Performed 2001

Species Pimephales promelas

Analytical Monitoring Gas chromatograph/FID; LOD, 0.0002 mg/L

Exposure Period 96 hours

Statistical Method Probit method

Remarks for Method

Juvenile fathead minnows, Pimephales promelas, employed as test organisms were from a single source and were identified using an appropriate taxonomic key. Fish were obtained from a commercial supplier (Aquatic Biosystems, Fort Collins, Colorado) and maintained in a 20 L glass aquarium. Because the test was performed in sealed containers with a minimum of head space (to minimize the loss of the volatile test substance), small fish were used to reduce loading and allow the maintenance of acceptable water quality. At the conclusion of the test, the control fish had an average total length of 9.3 mm and an average wet weight of 3.6 mg (loading rate was approximately 0.018 g/L). During acclimation, fish were not treated for disease and they were free of apparent disease, injuries, and abnormalities at the beginning of the test. During the 14-day period prior to the start of the test, the acclimation temperature range was 22.3 to 22.9 degrees C and the dissolved oxygen concentration was at least 8.6 mg/L. Fish were fed newly hatched Artemia salina nauplii once per day except during the 48 hours preceding the test and they were not fed during the toxicity test. Mortality during the entire acclimation period was 0%.

Paramethyl styrene was shipped to T.R. Wilbury Laboratory at ambient temperature. Prior to use the test substance was stored at room temperature in the dark. Water used for the acclimation of test organisms and for all toxicity testing was carbon filtered, deionized water collected at T.R. Wilbury Laboratories in Marblehead, Massachusetts. Water was adjusted to a hardness of 40 to 48 mg/L and stored in polyethylene tanks where it was aerated and recirculated through particle filters,

ECOTOXICITY ELEMENTS:

ACUTE TOXICITY TO FISH

activated carbon, and an ultraviolet sterilizer. A sample of dilution water collected at the start of the definitive test had a hardness of 48 mg/L as calcium carbonate, and an alkalinity of 36 mg/L as calcium carbonate. The dilution water sample contained less than 10 mg/L particulate matter and less than 1 .O mg/L total organic carbon.

A range-finding test was conducted under static conditions to determine the concentrations to be used in the definitive test. In the definitive test, a series of solutions was prepared at the beginning of the test and at the time of media renewal at 48 hours by bringing 6.6, 10.3, 16.4, 26.2, and 41 .1 mg of test substance to 4,100 mL with dilution water (measured using a Class A graduated cylinder) in sealed glass bottles. The solutions were mixed on magnetic stirrers for approximately four hours and each vortex was adjusted to approximately 5% of the distance from the surface to the bottom of the solution. The solutions were allowed to settle for approximately one hour and a 2.0 L portion of each solution was transferred into a 1/2 gallon glass jar through a spout at the bottom of the mixing vessels. A 2.0 L portion of dilution water was also transferred to a glass jar to serve as a control. Water quality measurements were made and 10 fathead minnows were indiscriminately added to each jar at the start of the test. At 48 hours, most of the solution in each test vessel was removed using a siphon and replaced with freshly prepared solutions. The jars, which were filled nearly to capacity to minimize head space, were sealed with Teflon-lined caps. Nominal concentrations of paramethyl styrene were 0 mg/L (control), 1.6, 2.5, 4.0, 6.4, and 10 mg/L.

Test vessels were randomly arranged in a water bath (22 plus or minus 1 degree C) during the 96 hour test (a random numbers table was used to select the location of each vessel). A 16-hour light and 8-hour dark photoperiod was automatically maintained with cool-white fluorescent lights that provided a light intensity of approximately 83 footcandles (11 uEin/m-squared sec). A 15 minute transition period was provided between dark and light. The numbers of surviving organisms, the occurrence of immobility and sublethal effects, and the presence of insoluble material were determined visually and recorded after 0, 24, 48, 72, and 96 hours. Jars remained sealed throughout the exposure period except during the 48 hour media renewal in order to prevent the loss of paramethyl styrene to the atmosphere, so dead test organisms were not removed at 24 or 72 hours.

Dissolved oxygen concentration, temperature, pH, and conductivity were measured and recorded in each test vessel prior to media renewal at 48 hours and end of the test. Measurements were also made in each test solution just prior to its distribution to replicate test vessels at the start of the test and at the time of media renewal at 48 hours. Measurements were not made at 24 or 72 hours to allow the test vessels to remain sealed and prevent loss of paramethyl styrene from solutions. The temperature in a vessel of water incubated among the test vessels was recorded continuously during the test.

Analytical determination of test substance concentration (active ingredient) was performed with samples collected from each test vessel at the beginning and end of the test, and before and after media renewal at 48 hours. Samples from replicate vessels at each concentration were pooled and immediately sealed into 40 mL glass vials with no head space and analyzed immediately. Samples were removed with a syringe through the Teflon septa in the caps and analyzed immediately. Each set of samples was accompanied by two laboratory control samples prepared at 4.0 mg/L

ECOTOXICITY ELEMENTS:

ACUTE TOXICITY TO FISH

paramethyl styrene in dilution water.

A IO mL aliquot of each sample was transferred to a purge vessel with a 50 mL gas-tight syringe. Samples outside the calibration range were diluted with deionized water. Samples were concentrated using a 4460A O-I-Analytical Sample Concentrator fitted with a MPM-1 6 O-I-Analytical Multiple Purging Module. The typical purge, desorption, and bake times were II, 4, and 15 minutes, respectively. The bake temperature was approximately 210 degrees C. Samples were analyzed using a Hewlett Packard model 5890 gas chromatograph and an FID detector. The column was a HP-5 (crosslinked 5%) PH ME Siloxane (1.5 micron thickness). The analytical method was validated in duplicate at 0.50, 3.0, and 10 mg/L in dilution water. Measured concentrations for samples with a nominal concentration of 0.5 mg/L were 0.30 and 0.35 mg/L, measured concentrations for samples with a nominal concentration of 3.0 mg/L were 2.3 and 2.4 mg/L, and measured cocentrations for samples with a nominal cocentration of 10 mg/L were 8.0 and 9.1 mg/L. The estimated water solubility limit of paramethyl stryene in dilution water was 25 to 40 mg/L. The limit of quantitation (LOQ) and limit of detection (LOD) was 0.0007 and 0.0002 mg/L, respectively.

The 24- and 48-hour LC50s could not be calculated because greater than 50% survival occurred at each test concentration. The 72- and 96-hour LC50s were calculated using the probit method. The slope of the concentration-response curve was also calculated using the probit method. The no observed effect concentration (NOEC) was determined as the highest tested concentration of paramethyl styrene that allowed at least 95% survival and did not allow any sublethal effects.

Results

Nominal Concentration	1.6, 2.5, 4.0, 6.4, and 10 mg/L
Measured Concentration	0.82, 1.3, 2.6, 4.2, and 6.8 mg/L
Precision	=
Endpoint Type	LC50
Endpoint Value	5
Unit Used	mg/L
Concentration Type	Measured
Endpoint Time	96
Statistical Results	The 96-hour NOEC (mortality) was 2.6 mg/L; $p<0.05$.
Results Remarks	Insoluble material was not observed during the test. Mean measured concentrations ranged from 51 to 68% of nominal concentrations. Final measured concentrations were 87 to 125% of the initial concentrations, indicating that once the aqueous solutions of paramethyl styrene were sealed into the test vessels with the fathead minnows, concentrations remained constant. Loss of the volatile test substance during preparation of test solutions was assumed to have occurred to the atmosphere.

Admin Record Number:

ROBUST SUMMARY

Styrene, p-methyl-

CAS# 622979 **Study Number: 4**

ECOTOXICITY ELEMENTS:

ACUTE TOXICITY TO FISH

Water quality was within the normal range throughout the toxicity test. During the definitive toxicity test the conductivity ranged from 150 to 210 umhos/cm, the pH ranged from 6.9 to 7.5, the temperature ranged from 21 .1 to 22.6 degrees C, and the dissolved oxygen ranged from 6.8 to 9.0 mg/L. The minimum dissolved oxygen concentration during the test was 78% saturation (saturation equals 8.7 mg/L at 22 degrees C). One hundred percent survival occurred in the control and no sublethal effects were noted in the control fish during the exposure period.

No mortality or sublethal effects were noted in fish exposed to 0 mg/L (control), 0.82, 1.3 or 2.6 mg/L. The number of fish dead/total in 4.2 mg/L at 24, 48, 72, and 96 hours was 0/20, 2/20, 2/20, and 4/20 (all surviving fish at 96 hours exhibited a loss of equilibrium or were intermittently or completely immobilized). The number of fish dead/total in 6.8 mg/L at 24, 48, 72 and 96 hours was 1/20, 1/20, 1 1/20, and 17/20 (all surviving fish at 96 hours exhibited a loss of equilibrium or were intermittently or completely immobilized).

Exposure of fathead minnows to paramethyl styrene resulted in 24- and 48-hour LC50s greater than 6.8 mg/L (the highest tested concentration), a 72-hour LC50 of 6.5 mg/L (95% confidence interval = 5.6 to 8.5 mg/L) and a 96-hour LC50 of 5.2 mg/L (95% confidence interval = 4.6 to 5.9 mg/L). The slope of the 96-hour concentration-response curve based on mortality was 9.2.

Conclusions

The 96-hour LC50 was 5.2 mg/L (95% confidence interval = 4.6 to 5.9 mg/L). All surviving fish exposed to 4.2 and 6.8 mg/L paramethyl styrene were affected at 96 hours. No mortality or sublethal effects were observed at 0.82, 1.3, or 2.6 mg/L. The 96-hour NOEC was 2.6 mg/L.

Due to the high volatility of the test substance, the test was conducted in sealed containers with little or no head space. Mean, measured concentrations ranged from 51 to 68% of nominal concentrations. The loss of the test substance to the atmosphere occurred during preparation of the test solutions. Once the test solutions were prepared and the fish added, the final measured concentrations were 87 to 125% of initial measured concentrations. These recoveries exceeded the recoveries in a bluegill sunfish 7-day dynamic toxicity study (measured concentrations were approximately 45% of nominal concentrations).

Data Quality

Reliability Acceptable

Data Reliability Remarks All endpoints and experimental design were appropriate and adequately described in the study. Rigorous attempts were made to limit evaporation of this very volatile test substance, and all endpoints were based on mean, measured concentrations.

Reference

Remarks Acute Toxicity of Paramethyl Styrene to the Fathead Minnow, Pimephales promelas. T.R. Wilbury Study Number 1920-UL.

Dynamic Acute Toxicity of para-methylstyrene (PMS) to Bluegill Sunfish (Lepomis

ECOTOXICITY ELEMENTS:

ACUTE TOXICITY TO FISH

macrochirus). Analytical BioChemistry Laboratories, Inc., Project No.:28167, October 30, 1981.

General

Admin Record Number: 201130446

ROBUST SUMMARY

Styrene, p-methyl-

CAS# 622979 Study Number: 1

ENVIRONMENTAL FATE AND PATHWAY ELEMENTS:

BIODEGRADATION

Test Substance

Remarks

p-methylstyrene
14C/13C-PMS
Purity: 100% assumed for solution calculations
Additives: None reported
Carrier solvent: Nanograde acetone
Contaminants: None reported
Chemical formula: C9H 10

Chemical Category

Method

Method/Guideline Followed EPA, 1979, Federal Register, March 16, 1979. 16275-l 6277.

Test Type aerobic

GLP **Yes**

Year Study Performed 1982

Contact Time 33

Inoculum activated sludge, domestic, adapted

Remarks for Method

The specific objectives of the study were to assess the effects of the test material on the wastewater treatment process, to assess the efficiency of removal of the test agent by the sludge microflora, to assess the effect of the test agent on the sludge microflora, to monitor the test material concentration in the mixed liquor over a nineteen day biodgradation period, and to provide mixed liquor samples for the identification of such primary degradation products of the test agent which could be found in the mixed liquor samples taken over the nineteen day biodegradation period.

The activated sludge used in this study was obtained from sewage treatment plant #1 City of Columbia, Columbia, Missouri within four hours of the initiation of the study. Prior to initiation of the study, the sludge was kept refrigerated. Also, the solids concentration of the sludge was determined and subsequently adjusted to approximately 2500 mg/L by dilution with tap water. The initial microbial population of the sludge was found to have an average of 3.8E+7 organisms per milligram of solids.

A fourteen day acclimation period was employed using nonradiolabeled test material. Concentration #1 consisted of increasing concentrations of 1 O-50 mg/L and concentration #2 consisted of increasing concentrations of 20-I 00 mg/L. An acetone solvent (1 ml daily) and positive control (diethylene glycol) were also employed. The aeration vessels were standard tall form 3000 ml resin-pots. The specific similated influents were prepared in the following manner: 1) Acetone control influents were prepared by adding 15 ml of synthetic sewage and 1 .O ml of acetone to tap water to a volume of two liters, 2) Positive control influents were prepared by adding 15 ml of synthetic sewage and the corresponding amount of diethylene glycol to tap water to a volume of two liters, 3) test concentrations #1 and #2 influents were prepared by adding 15 ml of synthetic sewage, 1 .0 ml of the appropriate spiking solution of PMS in acetone to tap water to a volume of two liters. Aliquots of the sludge from each of the two control and two test chambers were taken for microbial analysis on days 0, 3, 6, 9, 1 1, and 15 of acclimation and at the end of the study. On day 15 of the acclimation 14C/13C -PMS was introduced with the daily influent at 50 and 100 ppm (as organic carbon) for test concentration #1 and test concentrations #2, respectively. Daily "draw and fill" was discontinued at that time. Trapping solutions for collection of 14C-C02 and 14C-radioactivity from volatile organics (ethylene glycol) in effluent air and representative aliquots of the mixtures in each test vessel, 50 ml, were taken at 0.5, 3, 6, 24 hours, days 2, 3, 4, 6, 8, 10, 13 16 and 19. Total residues of 14C/13C-PMS in the test mixtures were determined by triplicate sample combustion to 14C-CO2 followed by liquid scintillation counting.

Results

Precision	>
Degradation Value	95
Upper Value	0
Time Frame	19
Time Units	Days
Breakdown Products	Yes

Results Remarks

The effects of PMS on the wastewater treatment process were found to be negligible when dosed at or below a 100 ppm rate as organic carbon equivalents. Dissolved organic carbon removal in all test concentrations was above 90% efficiency with a mean efficiency of 95.6% (relative standard deviation of 1.9%). Assessment of the effect of PMS on the sludge microbial populations showed no discernable effect. A statistical analysis of the test concentration population levels indicated that no significant differences existed within a 95% confidence interval. No effect, real or apparent, was reflected in the dissolved organic carbon removal efficiencies for the test concentrations.

The test compound was found to be eliminated from the sludge principally as volatile organic and/or carbon dioxide. After only one-half hour, test material concentration in the sludge was

measured to be less than 40% of the dose rate. Less than 5% of the dosed test material remained after nineteen days. The elimination of test material as volatile components reached a maximum by the second day and decreased over the remainder of the test period.

It was postulated the high rate of loss from the reaction vessel was due to the volatility of PMS. A subsequent experiment introducing PMS into a completely closed system resulted in 58.8% accountability at zero time with the PMS concentrations decreasing to 2.7% of the amount added to the reactor vessel after 3 hours of aeration. Since only 4.3% of the PMS was recovered by trapping, the material must have been absorbed by the experimental system.

Conclusions

The test compound was found to be eliminated from the sludge primarily as volatile organic and/or carbon dioxide. Less than 5% of the dosed test material remained after nineteen days. It was postulated that the high rate of loss from the reaction vessel was due to the volatility of PMS.

Data Quality

Reliability Acceptable

Data Reliability Remarks All key parameters (i.e., concentrations used, sludge, and experimental design) were appropriate and described in the study.

Reference

Remarks Activated Sludge Biodegradation of Paramethylstyrene, Analytical Biochemistry Laboratories, Inc., Final Report #28172, March 5, 1982.
Biochemical and Chemical Oxygen Demand of 08217901, Union Carbide Environmental Services Project No.: 11506-05.

General

Chemical oxygen demand (COD) was determined according to standard procedures (Standard Methods for the Examination of Water and Wastewater 14th Ed. APHA, pp 543-554, 1976) using 2 and 4 microliter samples of the test material. A carbon standard yielded 100.9% of theoretical COD. Biological Oxygen Demand (BOD) was determined according to standard procedures with the modification described below. The 5 day test period was extended to 20 days with periodic reaeration to maintain acceptable DO concentrations (> 2 mg/l). This extended testing allows consideration of acclimation of test organisms to the compound under study. Test material was tested at two concentrations (2 and 4 microliters/300 ml test system volume) with four replicates being prepared for each concentration. Test material was introduced into the BOD-bottle using a syringe injection modification of the standard procedure because of its low water solubility. However, the material was completely dissolved prior to the 5 day DO determination. Test vessels were seeded with domestic sewage and seed controls were maintained throughout the 20 day test period to correct for oxygen uptake of the seed. A glucose control and glucose/glutamic acid control were prepared and were analyzed

after 5 days of incubation to check the activity of the seed and also to check for contamination of the BOD dilution water. The glucose/glutamic acid sample yielded 99.1% of theoretical oxygen demand. DO determinations were made using a Yellow Springs oxygen electrode.

A low COD value (2.24 mg oxygen/mg compound, measured; 3.1 calculated) was obtained for PMS. However, the hot chromic acid solution used to determine COD does not oxidize aromatic structures efficiently and therefore the low COD is not uncommon. BOD for day 5, 10, 15 and 20 (percent ratio of the measured BOD to the theoretical oxygen demand) was <3, 6, 13, and 32, respectively. The data indicate that the compound is not readily biodegradable. However, the substantial increase in bio-oxidation between day 15 and 20 suggests that the organisms had acclimated to the test material. More significant bio-oxidation would probably be observed if acclimated organisms were to be used as seed.

Admin Record Number:

ROBUST SUMMARY

Styrene, p-methyl-

CAS# 622979

Study Number: 1

ENVIRONMENTAL FATE AND PATHWAY ELEMENTS:

PHOTODEGRADATION

Test Substance

Remarks	p-methylstyrene Test Article ID: Paramethyl Styrene Purity: Assume 100% Additives: Unknown Carrier solvent: Unknown Contaminants: Unknown Chemical formula: C9H10
Chemical Category	

Method

Method/Guideline Followed	
Light Source	
Light Source Spectrum in nm	0
Relative Intensity	
Absorption Spectrum of Substance	1) 210; 2)0.62; 3) 0.41
GLP	Unknown
Year Study Performed	1965
Remarks for Method	Absorption Spectrum methods were not reviewed. A photolysis study was not conducted on this substance. The rate constants for the vapor-phase reaction of para methylstyrene with photochemically produced hydroxyl radicals and ozone were estimated using structure-activity relationship models (Atkinson, R.; Intern J. Chem. Kin. 19: 799-828, 1987)(Atkinson R., Carter, W.P.L.; Chem. Rev. 84: 437-70, 1984).

Results

Concentration Value	
Unit	
Temperature	

Direct Photolysis Precision	
Direct Photolysis	0

Admin Record Number:

ROBUST SUMMARY

Styrene, p-methyl-

CAS# 622979

Study Number: 1

ENVIRONMENTAL FATE AND PATHWAY ELEMENTS:

PHOTODEGRADATION

Direct Photolysis Upper Value	0
Direct Photolysis Unit	

Indirect Photolysis Precision	
Indirect Photolysis	0
Indirect Photolysis Upper Value	0
Indirect Photolysis Unit	

Sensitizer	Ozone
Sensitizer Concentration	7E+11
Sensitizer Unit	mg/cubic centimeter
Rate Constant	2.1E-17
Breakdown Products	Unknown
Results Remarks	Para-methylstyrene in methanol weakly absorbs UV light in the environmentally significant range (>290 nm) (Sadtler Res. Lab; Sadtler Standard UV Spectra No. 78), and has the potential to undergo direct photolysis in sunlit waters or in ambient air. However, reactions with photochemically produced hydroxyl radicals and ozone in the atmosphere are likely to be important fate processes. The rate constant for the vapor-phase reaction of para-methylstyrene with photochemically produced hydroxyl radicals has been estimated to be 3.15E-11 cu cm/molecule -sec at 25 deg C, which corresponds to an atmospheric half-life of about 12.2 hr at an atmospheric concentration of 5E+5 hydroxyl radicals per cu cm. The rate constant for the vapor-phase reaction of para-methylstyrene with ozone has been estimated to be 2.1 E-l 7 cu cm/molecule -sec at 25 deg C, which corresponds to an atmospheric half-life of 13 hr at an atmospheric concentration of 7E+11 ozone molecules per cu cm.

Conclusions

No direct photolysis studies **have been** conducted. But the estimated rate constants for the vapor-phase reaction with hydroxyl radicals and ozone have been calculated.

Data Quality

Reliability	Acceptable
Data Reliability Remarks	The UV spectrum and rate constants have been determined either experimentally or estimated by structure-activity relationships.

Admin Record Number:

ROBUST SUMMARY

Styrene, p-methyl-

CAS# 622979

Study Number: 1

ENVIRONMENTAL FATE AND PATHWAY ELEMENTS:

PHOTODEGRADATION

Reference

Remarks

General

Index of refraction = 1.5420 at 20 deg C (Lide, D.R. (ed.). CRC Handbook of Chemistry and Physics. 75th ed. Boca Raton, FL: CRC Press Inc., 1994-1995., p. 3-47.

Admin Record Number: **ROBUST SUMMARY**

Styrene, p-methyl-

CAS# 622979 **Study Number: 1**

ENVIRONMENTAL FATE AND PATHWAY ELEMENTS:

STABILITY IN WATER

Test Substance

Remarks	p-methylstyrene Test Article ID: Paramethyl Styrene Purity: Assume 100% Additives: Unknown Carrier solvent: Unknown Contaminants: Unknown Chemical formula: C9H10
Chemical Category	

Method

Method/Guideline Followed	Other
Test Type	
GLP	Unknown
Year Study Performed	
Remarks for Method	No direct stability or hydrolysis studies involving para methylstyrene have been conducted.

Results

Nominal Concentration	
Measured Concentration	
Precision	
Hydrolysis Result	0
Upper Value	0
Unit	
pHVal	0
Temperature	
Breakdown Products	
Results Remarks	No direct stability or hydrolysis studies involving para methylstyrene have been conducted. However, para methylstyrene is not expected to undergo hydrolysis in natural waters because of the lack of hydrolyzable functional groups (Lyman WJ, et al.; Handbook of Chemical Property Estimation Methods, NY: McGraw-Hill, pp. 15-l 5 to 15-29, 1982).

Admin Record Number:

ROBUST SUMMARY

Styrene, p-methyl-

CAS# 622979

Study Number: 1

ENVIRONMENTAL FATE AND PATHWAY ELEMENTS:

STABILITY IN WATER

Based on a Henry's Law constant of 3.01 E-3 atm-cu m/mole, which has been calculated using a group contribution method (Meylan, W.M., Howard, P.H. Environ. Toxicol. Chem. 10:1283-l 293, 1991), volatilization of p-methylstyrene from natural waters should be an important fate process. The volatilization half-life from a model river (1 meter deep flowing 1 m/sec with a wind speed of 3 m/sec) has been estimated to be 3.5 hr (Lyman, WJ, et al.; Handbook of Chemical Property Estimation Methods, NY:McGraw-Hill, pp. 15-l 5 to 15-29, 1982). The volatilization half-life from a model pond, which considers the effect of adsorption, has been estimated to be 10.6 days (USEPA; EXAMS II Computer Simulation, 1987).

Conclusions

Based on the computer simulation models (USEPA EXAMS II), the volatilization half-life from a model pond has been estimated to be 10.6 days. Since volatilization may be the most important fate process of para methylstyrene (hydrolysis is not predicted based on the lack of hydrolyzable functional groups), these results are acceptable for predicting water stability and environmental fate.

Data Quality

Reliability

Not determined.

Data Reliability Remarks

No water stability or hydrolysis studies have been performed. However, computer simulations using Henry's Law constant provide a reliable estimate of environmental fate in aquatic systems.

Reference

Remarks

General

Admin Record Number:

ROBUST SUMMARY

Styrene, p-methyl-

CAS# 622979

Study Number: 1

ENVIRONMENTAL FATE AND PATHWAY ELEMENTS:

TRANSPORT BETWEEN ENVIRONMENTAL COMPARTMENTS (FUGACITY)

Test Substance

Remarks

p-methylstyrene
Test Article ID: Paramethyl Styrene
Purity: Assume 100%
Additives: Unknown
Carrier solvent: Unknown
Contaminants: Unknown
Chemical formula: C9H10

Chemical Category

Method

Method/Guideline Followed Computer modeling

Test Type Level I fugacity model

Year Study Performed 2001

Remarks for Method EQC Model, Version 1 .01 , May, 1997

Input parameters: Temperature, 25 deg C; Molecular mass in g/mol, 118.2; water solubility (g/m+3), 0.040; vapor pressure (Pa), 239.4; log Kow, 3.35; melting point (deg C), -34; reaction half lives in air (h), 13; reaction half lives in water (h), 254.4; reaction half lives in soil and sediment, negligible.

Environmental conditions (default): transport velocities in m/h
Air side air-water MTC = 5.000; water side air-water MTC = 0.050; rain rate = 1 .00E-4; aerosol deposition = 6.00E-10; soil-air phase diffusion MTC = 0.020; soil-water phase diffusion MTC = 1 .OOE-5; soil-air boundary layer MTC = 5.000; sediment-water diffusion MTC = 1 .00E-4; sediment deposition = 5.00E-7; sediment resuspension = 2.00E-7; soil-water runoff rate = 5.00E-5; soil-solids runoff rate = 1 .00E-8.

Results

Media 100% air, 7.01 E-4% water, 1.39E-3 soil, 3.09E-5 sediment, 9.65E-7 suspended sediment, 7.84E-8 fish

Distribution Concentration

Amount in kg (total, 1 .00E+5)
Air, 99998
Water, 0.701
Soil, 1.389
Sediment, 0.031
Suspended sediment, 9.65E-4
Fish, 7.84E-5

Results Remarks Octanol-water partition coefficient = 2239

Admin Record Number:

ROBUST SUMMARY

Styrene, p-methyl-

CAS# 622979 **Study Number: 1**

ENVIRONMENTAL FATE AND PATHWAY ELEMENTS:

TRANSPORT BETWEEN ENVIRONMENTAL COMPARTMENTS (FUGACITY)

Organic C-water partition coefficient = 918
Fish-water partition coefficient = 112
Air-water partition coefficient = 285
Soil-water partition coefficient = 44.1
Sediment-water partition coefficient = 88.1
Suspended sediment-water partition coefficient = 275
Aerosol-air partition coefficient = 25063
Aerosol Z value = 10.1
Aerosol density in kg/m+3 = 2000

Conclusions

Only limited data was available for input in the Level I EQC Model. Additional data including the reaction half lives in soil and sediment was not available; the input of "negligible" was used. The water solubility used was the upper end estimated in the preliminary studies for algae, fish and daphnid ecotoxicity testing at T.R. Wilbury Laboratories, Inc. The reaction half-life in air **was** the estimated half life with ozone. The reaction half-life in water was the volatilization half-life from a model pond.

Data Quality

Reliability Not reliable

Data Reliability Remarks The data input was limited.

Reference

Remarks Mackay D., Guardo A., Paterson S. And Cowan, C. (1996). Evaluating the environmental fate of a variety of types of chemicals using the EQC model. Environ. Toxicol. Chem. 15:9, 1627-l 637.

General

The environmental fate as referenced in Toxnet was as follows:
Terrestrial fate: sufficient data are not available to predict the importance of biodegradation of p-methylstyrene in terrestrial environments. It is not expected to undergo hydrolysis in soils because of the lack of hydrolyzable functional groups. P-methylstyrene weakly absorbs UV light in the environmentally significant range (>290 nm) and has potential to undergo direct photolysis in sunlit surface soils. A calculated Koc range of 360 to 1585 indicates an uncertainty in soil mobility class for p-methylstyrene.

Aquatic fate: Sufficient data are not available to predict the importance of biodegradation of p-methylstyrene in aquatic systems. It is not expected to undergo hydrolysis in natural waters because of the lack of hydrolyzable functional groups. P-methylstyrene weakly absorbs UV light in the environmentally significant range (>290 nm) and has the potential to undergo direct photolysis in sunlit waters. It is not expected to bioconcentrate in aquatic organisms. An estimated Koc suggest p-methylstyrene may partition from the water column via adsorption to organic matter contained in sediments and suspended solids. An estimated Henry's Law constant of 3.01 E-3 atm-cu m/mole at 25 deg C indicated volatilization of p-methylstyrene from

ENVIRONMENTAL FATE AND PATHWAY ELEMENTS:

TRANSPORT BETWEEN ENVIRONMENTAL COMPARTMENTS (FUGACITY)

environmental waters may be an important fate process. Based on this Henry's Law constant, the volatilization half-life from a model river has been estimated to be 3.5 hr. The volatilization half-life from a mode pond, which considers the effect of adsorption, has been estimated to be about 10.6 days.

Atmospheric fate: Based on a vapor pressure of 1.81 mm Hg at 25 deg C, p-methylstyrene is expected to exist entirely in the vapor phase in ambient air. P-methylstyrene in methanol weakly absorbs UV light in the environmentally significant range (>290 nm) and therefore has the potential to undergo direct photolysis in ambient air. However, reactions with photochemically produced hydroxyl radicals and ozone in the atmosphere are likely to be important fate processes. The rate constant for the vapor-phase reaction of p-methylstyrene with photochemically produced hydroxyl radicals has been estimated to be 3.15E-11 cu cm/molecule-set at 25 deg C, which corresponds to an atmospheric half-life of about 12.2 hr at an atmospheric concentration of 5E+5 hydroxyl radicals per cu cm. The rate constant for the vapor-phase reaction of p-methylstyrene with ozone has been estimated to be 2.1 E-l 7 cu cm/molecule-set at 25 deg C, which corresponds to an atmospheric half-life of 13 hr at an atmospheric concentration of 7E+11 ozone molecules per cu cm.

Environmental Abiotic Degradation:
Benzenes and alkenes are generally resistent to hydrolysis, therefore p-methylstyrene is not expected to undergo hydrolysis in the environment.

Environmental bioconcentration: A log BCF of 1.50 for p-methylstyrene in goldfish indicates p-methylstyrene is not expected to bioconcentrate in aquatic organisms.

Admin Record Number: **ROBUST SUMMARY**

Styrene, p-methyl-

CAS# 622979 **Study Number: 1**

CHEMICAL AND PHYSICAL PROPERTIES:

BOILING POINT

Test Substance

Remarks
p-methylstyrene
Test Article ID: Paramethyl Styrene
Purity: Assume 100%
Additives: Unknown
Carrier solvent: Unknown
Contaminants: Unknown
Chemical formula: C9H10

Chemical Category

Method

Method/Guideline Followed Other

GLP Unknown

Year Study Performed

Remarks for Method Methods were not reviewed.

Results

Precision =

Boiling Point Value 173

Upper Value 0

Unit §C

Pressure 760

Pressure Unit mm Hg

Decomposition

Results Remarks
The boiling point is 172.8 deg C at 760 mm Hg.
Decomposition of substance during boiling point test is unknown.
Boiling points @:
100 mm = 104.7 deg C
30 mm = 76.5 deg C
10 mm = 54.7 deg C
1 mm = 18 deg C

Conclusions

Data Qualitv

Reliability Not determined

Admin Record Number:

ROBUST SUMMARY

Styrene, p-methyl-

CAS# 622979

Study Number: 1

CHEMICAL AND PHYSICAL PROPERTIES:

BOILING POINT

Data Reliability Remarks Methods were not reviewed.

Reference

Remarks Lide, D.R. (ed.). CRC Handbook of Chemistry and Physics. 75th ed. Boca Raton, Fl: CRC Press, Inc., 1994-1995., p. 3-47.

General

Critical temperature = 665 K
Critical pressure = 487.33 psia
Heat of polymerization (Kcal mole -1) = 15-l 7
Heat of combustion = -17,545.4 BTU/lbm
Heat of vaporization = 177.16 BTU/lbm at 77 deg F

Admin Record Number:

ROBUST SUMMARY

Styrene, p-methyl-

CAS# 622979

Study Number: 1

CHEMICAL AND PHYSICAL PROPERTIES:

MELTING POINT

Test Substance

Remarks	p-methylstyrene Test Article ID: Paramethyl Styrene Purity: Assume 100% Additives: Unknown Carrier solvent: Unknown Contaminants: Unknown Chemical formula: C9H10
Chemical Category	

Method

Method/Guideline Followed	Not available
GLP	Unknown
Year Study Performed	
Remarks for Method	Methods were not reviewed.

Results

Precision	=
Melting Point Value	-34
Upper Value	0
Unit	§C
Decomposition	Ambiguous
Sublimation	Ambiguous
Results Remarks	The test substance is a liquid so the value reported is a freezing point. The decomposition during boiling point test is unknown.

Conclusions

Data Quality

Reliability	Not determined
Data Reliability Remarks	Methods were not reviewed.

Reference

Remarks	Lide, D.R. (ed.). CRC Handbook of Chemistry and Physics. 75th ed. Boca Raton, FL: CRC Press Inc., 1994-1995., p. 3-47.

Admin Record Number: ROBUST SUMMARY

Styrene, p-methyl-

CAS# 622979 Study Number: 1

CHEMICAL AND PHYSICAL PROPERTIES:

MELTING POINT

General

Admin Record Number:

ROBUST SUMMARY

Styrene, p-methyl-

CAS# 622979

Study Number: 1

CHEMICAL AND PHYSICAL PROPERTIES:

PARTITION COEFFICIENT

Test Substance

Remarks

p-methylstyrene
Test Article ID: Paramethyl Styrene
Purity: Assume 100%
Additives: Unknown
Carrier solvent: Unknown
Contaminants: Unknown
Chemical formula: C9H10

Chemical Category

Method

Method/Guideline Followed

GLP Unknown

Year Study Performed

Remarks for Method The partition coefficient of p-methylstyrene between n-octanol and water was taken from the tabulation of Leo et al. (1971).

Results

Precision =

Value of Log Pow 3.35

Upper Value 0

Temperature

Results Remarks

Estimated water solubility of 55.3 ppm at 25 degrees C.
Measured water solubility in three types of freshwater dilution water used for toxicity testing was estimated to be between 25 and 40 ppm (T.R. Wilbury Laboratories, Inc. Correspondence letter, January 15, 2001).
Benzenes and alkenes are generally resistent to hydrolysis, therefore p-methylstyrene is not expected to undergo hydrolysis in the environment.
A log BCF of 1.50 in goldfish indicates p-methylstyrene is not expected to bioconcentrate in aquatic organisms.
BCFs of 4.9, 9.2 and 4.0 in channel catfish indicate that p-methylstyrene is not substantially accumulated in fish under the conditions of the study (ABC Laboratories, Inc. Study No. 1341, 1982).
The BCF of 1 IO in bluegill sunfish indicated that p-methylstyrene is not substantially accumulated in fish under the conditions of the study (ABC Laboratories, Inc. Study No. 1342, 1982).

Conclusions

Data Qualitv

Admin Record Number:

ROBUST SUMMARY

Styrene, p-methyl-

CAS# 622979

Study Number: 1

CHEMICAL AND PHYSICAL PROPERTIES:

PARTITION COEFFICIENT

Reliability Not determined

Data Reliability Remarks The method was not reviewed.

Reference

Remarks

Leo A, Hansch C, Elkins D (1971) Partition coefficients and their uses. Chem. Rev. 71:525-563.
Ogata, M., et al. Bull Environ. Contam. Toxicol. 33: 561.7.
Lyman, W.J., et al. Handbook of Chemical Property Estimation Methods NY: McGraw-Hill, p. 4-9 (1982).

General

Based on a log Kow of 3.35 and an estimated water solubility of 55.3 ppm at 25 deg C, the Koc has been calculated using various regression equations to range from 360 to 1585. An estimated Koc suggests p-methylstyrene may partition from the water column via adsorption to organic matter contained in sediments and suspended solids. According to a suggested classification scheme, these Koc values indicate a medium to low soil mobility. (Swann RL et al.; Res Rev 85:1-28, 1983.

Admin Record Number:

ROBUST SUMMARY

Styrene, p-methyl-

CAS# 622979

Study Number: 1

CHEMICAL AND PHYSICAL PROPERTIES:

VAPOR PRESSURE

Test Substance

Remarks

p-methylstyrene
Test Article ID: Paramethyl Styrene
Purity: Assume 100%
Additives: Unknown
Carrier solvent: Unknown
Contaminants: Unknown
Chemical formula: C9H10

Chemical Category

Method

Method/Guideline Followed Other

GLP Unknown

Year Study Performed 1984

Remarks for Method The vapor pressure (log Po) was calculated using the Antoine equation; log Po=A - B/(t + C) where t is the temperature and A, B, C are constants characteristic Of the substance and the given temperature range. The following constants were used: A = 6.16114; B = 1586.596; C = 209.846. The conversion is 101.325 kPa = 760 mm Hg.

Results

Precision =

Vapor Pressure Value 1.81

Upper Value 0

Unit mm Hg

Temperature 25 degrees C

Decomposition

Results Remarks The vapor pressure of para methylstyrene (1.81 mm Hg) based on the Antoine equation.

Conclusions

The vapor pressure was calculated using the Antoine equation. This study validated the equation by providing direct experimental data at different temperatures and comparing to the smoothed values of the pressure fitted to the Antoine equation with deviations (Of the smoothed pressures from the experimental data) and percent deviations. Examples are as follows:

Temp (deg C)	kPa (exptl)	kPa (calcl)	Dev	Percent
31.828	0.376	0.375	-0.001	-0.24
41.768	0.689	0.684	-0.005	-0.74
75.400	3.858	3.831	-0.027	-0.71
96.930	9.466	9.456	-0.008	-0.09

Admin Record Number: **ROBUST SUMMARY**

Styrene, p-methyl-

CAS# 622979 **Study Number: 1**

CHEMICAL AND PHYSICAL PROPERTIES:

VAPOR PRESSURE

Data Qualitv

Reliability Acceptable

Data Reliability Remarks The calculated vapor pressure at 25 deg C was determined using the Antoine equation validated at different temperatures.

Reference

Remarks Boublik, T., et.al. 1984. The Vapor Pressures of Pure Substances. Amsterdam: Elsevier. Antoine, C., C.R. Acad. Sci. Paris 107, 681,836, 1143, (1888).

General

Vapor pressure, mm Hg (ASTM D325)
@40 deg C = 4.5
60 deg C = 14.5
80 deg C = 35
100 deg C = 80
120 deg C = 170
140 deg C = 325
160 deg C = 580
170 deg C = 750
Reference: API Research Project 44.

Henry's Law constant = 3.01 x 10^{-3} atm-cu m/mole **at 25 deg C.**

Admin Record Number: ROBUST SUMMARY

Styrene, p-methyl-

CAS# 622979 **Study Number: 1**

CHEMICAL AND PHYSICAL PROPERTIES:

WATER SOLUBILITY

Test Substance

Remarks

p-methylstyrene (PMS)
Purity: 100% assumed for solution preparation
Additives: None reported
Carrier solvent: None
Contaminants: None reported
Chemical formula: C9H10

Chemical Category

Method

Method/Guideline Followed Other

GLP **Yes**

Year Study Performed **2001**

Remarks for Method

Solutions (4 liter) of PMS were prepared in each of three types of dilution water at three nominal concentrations, 10, 50, and 100 mg/L. A dilution water blank was included with each set of samples. These concentrations were chosen because at least the top two concentrations were above the water solubility limit, based on visual observations. Each solution was prepared in duplicate and sealed into amber glass bottles within two minutes. Each bottle contained a magnetic stir bar and head space was minimized. Each of the solutions was stirred on a magnetic stir plate with a vortex of approximately 10% of the total depth. Solutions were stirred for four hours and allowed to settle for one hour at room temperature. Insoluble material was observed floating at the water surface in each 50 and 100 mg/L solution after the mixing period, and no material was observed after mixing the 10 mg/L solutions.

Following the settling period, water samples were removed through a port at the bottom of each mixing vessel into sealed vials with no head space and analyzed immediately for PMS. An aliquot of each sample was removed from each vial using a Hamilton gas-tight syringe and immediately diluted into the appropriate range with deionized water. An aliquot of the diluted solution was then transferred to a purge and trap concentrator (O-I-Analytical Sample Concentrator fitted with a MPM-16 multiple purging module). Analyses were performed by gas chromatography (Hewlett Packard 5890 Series II Plus), flame ionization detection, The concentrations of PMS were determined using calibration curves constructed using seven standards. The first set of of PMS solutions was prepared in hard dilution water (160-I 80 mg/L as calcium carbonate; pH 7.2-7.4). This water is used for toxicity tests with the freshwater invertebrate, Daphnia magna. The second set of PMS solutions was prepared in soft dilution water (40-48 mg/L as calcium carbonate; pH 7.4-7.5). This water was used for toxicity tests with freshwater fish. The third set of PMS was prepared in AAP medium (160-I 80 mg/L as calcium carbonate; pH 7.2-7.4). This water is used for toxicity tests with freshwater algae.

Results

Precision range

Admin Record Number: **ROBUST SUMMARY**

Styrene, p-methyl-

CAS# 622979 **Study Number: 1**

CHEMICAL AND PHYSICAL PROPERTIES:

WATER SOLUBILITY

Water Solubility Value	25
Upper Value	40
Unit	mg/L
Temperature	ambient
Solubility Category	Slightly soluble
pH Vaue	
pKa Value	0
Results Remarks	Insoluble material was observed floating at the water surface in each 50 and 100 mg/L solutions. The mean (of duplicate samples) measured concentrations of 10, 50, and 100 mg/L in hard dilution water was 7.6, 32, and 27 mg/L, respectively. The mean measured concentrations of 10, 50, and 100 mg/L in soft dilution water was 7.7, 39, and 34 mg/L, respectively. The mean measured concentrations of 10, 50, and 100 mg/L in AAP medium was 9.5, 40, and 35 mg/L, respectively.

Conclusions

The water solubility limit of PMS in the three types of freshwater dilution water used for toxicity testing was estimated to be between 25 to 40 mg/L.

Data Quality

Reliability	Acceptable
Data Reliability Remarks	The results are acceptable for determining the water solubility PMS in three freshwater dilution waters used for toxicity testing of daphnids, fish and algae. The specific water quality conditions (pH, temperature, etc.) can be found in the referenced ecotoxicity reports.

Reference

Remarks

T.R. Wilbury Laboratories, Inc. Correspondence letter, January 15, 2001.
Growth and Reproductive Toxicity Test with Paramethyl Styrene and the Freshwater Alga, Selenastrum capricornutum. T.R. Wilbury Study Number 1922-UL.
Acute Toxicity of Paramethyl Styrene to the Daphnid, Daphnia magna. T.R. Wilbury Study Number 1921-UL.
Acute Toxicity of Paramethyl Styrene to the Fathead Minnow, Pimephales promelas. T.R. Wilbury Study Number 1920-UL.

General

An estimated water solubility of 55.3 ppm at 25 deg C was referenced in Lyman WJ, et al.; Handbook of Chemical Property Estimation Methods NY: McGraw-Hill p. 4-9, 1982.

Admin Record Number: 20113044B **ROBUST SUMMARY**

Styrene, p-methyl-

CAS# 622979 Study Number: 1

HUMAN HEALTH EFFECTS ELEMENTS:

ACUTE TOXICITY

Test Substance

Remarks	p-methylstyrene Test Article ID# : MCTR-86-79 Purity: 100% assumed for dosage calculations Additives: None reported Solvent carrier: Cottonseed oil Contaminants: None reported Chemical formula: C9H10
Chemical Category	

Method

Method/Guideline followed	Other
GLP	**Yes**
Year Study Performed	**1979**
Species	mouse
Strain	CD-1
Sex	Both
Number of Males per Dose	**5**
Number of Females per Dose	**5**
Vehicle	cottonseed oil, volume of 10.0 ml/kg
Route of Administration	Oral
Remarks for Method	The test substance was suspended in cottonseed oil and administered once by oral gavage to 4-hour fasted male and female rats (Charles River CD-I, approximately 2 1/2 months old). The concentrations were 215.0, 462.0, 993.0, 2135.0, and 4590.0 mg/kg and administered at a volume 10 ml/kg of body weight. Mice were observed for mortality frequently during the first 4 hours following dosing and twice daily (AM and PM) thereafter for a total of 14 days. Mice were observed for pharmacotoxic signs during the first 1/2 hour, at 1, 2 1/2, and 4 hours following dosing, at 24 hours and daily thereafter for a total of 14 days. Body weights were obtained immediately prior to test article administration (control weight) and at 7 and 14 days. All mice which died during the study were subjected to gross necropsy examinations as were all survivors at the end of the 14-day study period.

Results

Precision	=
Acute Lethal Value	1072
Unit	mglkg-bw
Deaths per Dose	0/10, 0/10, 4/10, 10/10, and 10/10 rats treated at 215, 462, 993, 2135, and 4590 mg/kg, respectively
Results Remarks	Deaths occurred as follows: 1/5 male rats between O-4 hours at 4590 mg/kg; 1/5, 5/5, and 4/4 male rats at day 1 in 993, 2135, and 4590 mg/kg, respectively; 2/5, 5/5, and 5/5 female rats at day 1 in 993, 2135, and 4590 mg/kg, respectively. Clinical signs of toxicity (Number, [hour], (day)) occurred as follows: Males at 215 mglkg exhibited the following, Wet, yellow-stained anogenitals 1[21/2,4], Clear moist stain around mouth 1[21/2, 4], piloerection 1(1). Males at 462 mg/kg exhibited the following, Clear moist stain around mouth 2[21/2,4], l(1). Males at 993 mg/kg exhibited the followig, Hypoactivity 3[21/2],4[4], 5(1), 2(2), 1(3), Wet, yellow-stained abdomen l(l), Ataxia 2(1), Low carriage l(l), Coarse body tremors 1 (I), Clear moist stain around mouth 1[21/2], 2[4], 2(1), 1(2), Clear ocular discharge 1(2), Tan stain around mouth l(l), Unkept appearance l(l), Ptosis l(l), l(3). Males at 2135 mglkg exhibited the following, Hypoactivity 5[1,21/2,4], l(l), Ataxia 2[1,21/2], 3[4], l(l), Low carriage 1 (I), Clear moist stain around mouth 1 [I ,21/2,4], Ptosis 2[1,21/2,4], l(l), Bradypnea l(1). Males at 4590 mg/kg exhibited the following, Hypoactivity 3[1/2],4[1,21/2,4], Wet, yellow-stained anogenitals 1[21/2], 2[4], Ataxia 2[1/2,1], 3[21/2], 4[4], Loss of limb tone 1 [I], 2[21/2,4], Low carriage 1[1/2,1,21/2,4], Clear moist stain around mouth 1[1,21/2,4], Bradypnea 2[1/2], 1[1,21/2,4], Prostration 1 [1/2,1,21/2,4]. Females at 215 mglkg exhibited the following, Hypoactivity l(l), Wet, Yellow-stained anogenitals 1[4], Clear moist stain around mouth 1[21/2,4], l(1). Females at 462 mg/kg exhibited the following, Hypoactivity 1[21/2], Clear moist stain around mouth 1[21/2,4], l(1). Females at 993 mg/lkg exhibited the following, Hypoactivity 2[21/2], 5[4], 3(1), Wet, yellow-stained anogenitals 1 (I), Ataxia 1 [I], Coarse body tremors l(l), Low carriage 2(1), Clear moist stain around mouth 1[21/2,4], l(1). Females at 2135 mg/kg exhibited the following, Hypoactivity 3[1], 4[21/2], 5[4], Wet, yellow-stained anogenitals 1[21/2,4], Ataxia 1[1,21/2,4], Loss of limb tone 1[4], Coarse body tremors 1[21/2,4], Clear moist stain around mouth 2[1,21/2,4], Prostration 1[4]. Females at 4590 mglkg exhibited the following, Hypoactivity 5[1/2,1,21/2,4], Ataxia 3[1/2,1,21/2,4], Low carriage 1 [1,21/2,4], Bradypnea 1 [1/2,1,21/2,4], Clear moist stain around mouth 3[1/2,1,21/2,4], Red stain around nose 1[21/2,4], Ptosis 1[21/2,4], Prostration 1 [I], Fine body tremors 1[4]. All surviving animals recovered from their respective signs by

the end of the study. No significant changes in body weights of surviving animals were reported. Apart from an increased incidence of gastro-intestinal hyperemia at the upper dose levels in the animals dying in the study, no specific changes were seen at necropsy.

Male mice LD50: 1072.2 (736.9-1559.9) mg/kg. Female mice LD50: 1072.2 (736.9-l 559.9) mg/kg. Both Slopes were 1 .0.

Conclusions

The combined 14-day single oral LD50 of male and female rats was calculated to be 1072.2 mg/kg of body weight, with 95% confidence limits of 835.0-l 376.7 mg/kg. Slope: 1 .O.

Data Quality

Reliability Acceptable

Data Reliability Remarks Experimental design and key parameters (number of animals/dose, concentrations, number of days of observations, etc.) are appropriate and described in the study.

Reference

Remarks Acute Oral Toxicity (LD50) Study in Mice with MCTR-86-79. International Research and Development Corporation #450-003, December 12, 1979. M862-79

General

Admin Record Number: 20113044B **ROBUST SUMMARY**

Styrene, p-methyl-

CAS# 622979 Study **Number: 2**

HUMAN HEALTH EFFECTS ELEMENTS:

ACUTE TOXICITY

Test Substance

Remarks	p-methylstyrene Test Article ID#: MCTR-112-79 Purity: 100% purity assumed for dosage calculations Additives: None reported Solvent carrier: Corn oil Contaminants: None reported Chemical formula: C9H10
Chemical Category	

Method

Method/Guideline followed	Other
GLP	**Yes**
Year Study Performed	1979
Species	rat
Strain	Fischer 344
Sex	Both
Number of Males per Dose	10
Number of Females per Dose	10
Vehicle	corn oil, volume of 20 ml/kg
Route of Administration	Oral
Remarks for Method	The test substance was dissolved in corn oil and administered once by oral gavage to fasted male and female albino rats (Fischer 344, body weights of males ranged from 195 to 225 grams and body weights of females ranged from 180 to 215 grams). The concentrations were 1260, 1780, 2510, 3550, and 5010 mg/kg and administered at a volume of 20 ml/kg of body weight. All rats were observed for mortality and signs of toxic and pharmacologic effects at one, two, and four hours postdose, and twice daily thereafter for fourteen consecutive days. Individual body weights were recorded prior to treatment, at seven days, and at termination. At termination (Day 14), all surviving rats were sacrificed by carbon dioxide asphyxiation, necropsied, and observations recorded. Necropsies were also performed on animals which died during the study. Mortality data were analyzed by a probit analysis method.

Results

Precision	=
Acute Lethal Value	2523
Unit	mg/kg-bw
Deaths per Dose	0/20, 2/20, 7/20, 20/20, and 20/20 rats treated at 1260, 1780, 2510, 3550, and 5010 mg/kg, resp.
Results Remarks	Deaths occurred as follows: l/l 0, 6110, 1 0/1 0, and 1 O/l 0 male rats dead at day 1 in 1780, 2510, 3550, and 5010 mg/kg, respectively; 1/9 male rats dead at day 2 in 1780 mg/kg; l/l 0 female rats dead at day 3 in 2510 mglkg; 8/10 female rats dead at day 1 in 3550 and 5010 mg/kg; 2/2 female rats dead at day 2 in 3550 mg/kg; 1/2 female rats dead at day 2 in 5010 mg/kg; 1/1 female rats dead at day 3 in 5010 mg/kg. Clinical observations consisted of soft feces, rough coat, depression, red stains on nose and/or eyes, and urine stains, noted at all levels; lacrimation and hunching, noted at the 1260, 1780, 2510, and 3550 mg/kg dose levels; slight depression, noted at the 1260, 1780, 2510, and 5010 mg/kg dose levels; and salivation, noted at the 1260, 2510, 3550, and 5010 mglkg levels. Animals dosed at 3550 mg/kg were noted to have tremors, and those dosed at 5010 mg/kg exhibited labored respiration, prostration, and ataxia. A common clinical sign of all animals, whether surviving or dying, was depression. All surviving animals were normal by day 7. In addition, all surviving animals gained weight during the end of the 14-day observation period. Gross pathology consisted of the following: 1260 mg/kg, no gross pathology observed; 1780 mglkg, 2/20 had one or more of the following: 1/20 lungs bright red, 1/20 intestines contain red material, 1/20 intestines contains yellowish-red material; 2510 mg/kg 7/20 had one or more of the following: 1/20 lungs bright red, 7/20 stomach contains compound, 6/20 stomach contains air, 1/20 stomach contains clear fluid, 1/20 stomach contains reddish-black material, 6/20 intestine contains red material, 1/20 intestine contains dark red or reddish fluid; 3550 mg/kg 20/20 had one or more of the following: 2/20 lungs bright red, 10/20 stomach contains compound, 18/20 stomach contains air, 2/20 stomach walls thin, 8/20 intestine contains red material, 10/20 intestine contains dark red or reddish fluid; 5010 mg/kg 20/20 had one or more of the following: 20/20 lungs bright red, liver dark, stomach contains compound, stomach contains clear yellow fluid, stomach walls thin, intestine contains dark red or reddish fluid. The acute oral LD50 in males was calculated to be 2255 mg/kg of body weight, with 95% confidence limits from 1981 to 2568 mg/kg. The acute oral LD50 in females was calculated to be 2724 mglkg of body weight, with 95% confidence limits from 1620 to 4580 mg/kg.

The 5010 mglkg dose level was not incorporated into the calculation of the LD50s for males, females or combined LD50 (below).

Conclusions

The combined 14-day single oral LD50 of male and female rats was calculated to be 2523 mglkg of body weight, with 95% confidence limits from 2311 to 2755 mglkg.

Data Quality

Reliability Acceptable

Data Reliability Remarks Experimental design and key parameters (number of animals/dose, concentrations, number of days observed, etc.) are appropriate and described in the study.

Reference

Remarks Acute Oral Toxicity Study in Male and Female Rats: MCTR-112-79. Hazleton Laboratories America, Inc. #230-1 96, December 21, 1979. Ml 122-79.

General

Admin Record Number: 20113044B **ROBUST SUMMARY**

Styrene, p-methyl-

CAS# 622979 Study Number: 3

HUMAN HEALTH EFFECTS ELEMENTS:

ACUTE TOXICITY

Test Substance

Remarks	p-methylstyrene Test Article ID# 2161801 Purity: 100% assumed for dosage calculations Additives: None reported Solvent carrier: Olive oil Contaminants: None reported Chemical formula: C9H10
Chemical Category	

Method

Method/Guideline followed	Other
GLP	**Yes**
Year Study Performed	1981
Species	rat
Strain	Sprague-Dawley
Sex	Both
Number of Males per Dose	**5**
Number of Females per Dose	**5**
Vehicle	Olive oil, volume 1 ml/100 gm body weight
Route of Administration	Oral
Remarks for Method	The test substance was dissolved in olive oil and administered once by oral gavage to fasted male and female albino rats (Sprague-Dawley, males and females approximately 8 and 14 weeks old, respectively). The concentrations were 1480, 2000, 2700, 3650, and 4935 mg/kg and administered at a volume of 1 ml/100 g of body weight. Rats were observed for signs of toxicity 1 and 4 hours after dosing and daily thereafter for 14 days. The animals were weighed on days 0, 7, and 14. Animals which died during the study were necropsied. Surviving rats were killed and discarded. Only 4 female rats were dosed at 3650 mg/kg. A standard test method of 1.2.3 was referenced in the study.

Results

Precision	>
Acute Lethal Value	4935
Unit	mg/kg-bw
Deaths per Dose	0110, 0110, 1/10, 1/9, and 5/10 rats treated at 1480, 2000, 2700, 3650, and 4935 mg/kg, respectively
Results Remarks	Deaths occurred as follows: 1/5 male rats dead at day 1 in 2700 and 4935 mg/kg, 1/4 male rats dead at day 2 in 4935 mglkg, 1/4 female rats dead at day 1 in 3650 mg/kg, 3/5 female rats dead at day 2 in 4935 mg/kg. Clinical signs of toxicity observed in male rats at 1480 mg/kg included decreased activity (1/5) and females exhibited lacrimation (1/5) and nasal discharge (1/5). Clinical signs in male rats at 2000 mglkg included nasal discharge (2/5) and decreased activity (2/5) and females exhibited lacrimation (1/5), diarrhea (1/5), nasal discharge (2/5), perianal discharge (1/5), and decreased activity (1/5). Clinical signs in male rats at 2700 mg/kg exhibited lacrimation (1/5) and decreased activity (2/5) and females exhibited lacrimation (4/5), oral discharge (1/5), nasal discharge (3/5) and decreased activity (3/5). All animals at 1480 to 2700 mg/kg were normal by day 2. Clinical signs in male rats at 3650 included diarrhea (1/4), oral discharge (3/4), nasal discharge (1/4), and decreased activity (4/4) and females exhibited dehydration (3/4), hunching (4/4), lacrimation (1/5), oral discharge (1/5), nasal discharge (4/5), perianal discharge (2/4), decreased activity (5/5) and hyperactive (1/5). All animals were normal by day 4. Clinical signs in male and female rats at 4935 mg/kg (exact number difficult to determine due to 5110 deaths) included hypothermia, tremors, dehydration, prostration, lacrimation, oral discharge, nasal discharge, perianal discharge, decreased activity and moribund. All surviving animals were normal by day 5 and gained weight normally. Necropsies of rats dying from the test material revealed red lungs and viscous reddish fluid in the gastrointestinal tract. Males and females exhibited similar mortalities at 4935 mglkg (males 2/5, females 3/5).

Conclusions

The acute oral LD50 of para-methyl styrene is greater than 4935 mglkg body weight. Since no dose had greater than 50% mortality, an exact calculation of an LD50 and 95% confidence limits is not appropriate.

Data Quality

Reliability	Acceptable
Data Reliability Remarks	Experimental design and key parameters (number of animals/dose, concentrations, number of days of observations, etc.) are appropriate and described in the study. An LD50 with confidence limits could not be

calculated because no dose had greater than 50% mortality. However, the purpose of the study was to compare the toxicity of para-methyl styrene with cosden vinyl toluene.

Reference

Remarks

The Acute Oral Toxicity of para-Methyl Styrene in Albino Rats, Mobil Environmental and Health Science Laboratory #691-81, June 2, 1981.

General

The purpose of the study was to compare the toxicity of para-methyl styrene with cosden vinyl toluene. There was essentially no difference in toxicity between the two compounds.

Admin Record Number: 201130448 **ROBUST SUMMARY**

Styrene, p-methyl-

CAS# 622979 Study Number: 4

HUMAN HEALTH EFFECTS ELEMENTS:

ACUTE TOXICITY

Test Substance

Remarks	Comparison of three p-methylstyrene samples: Edison (Reference) MEHSL Sample # 02298001; IRDC, MCTR-251-79, MEHSL Sample # 11067901; and Hazleton, MCTR-144-79, MEHSL Sample # 10037901. Purity: 100% assumed for dosage calculations Additives: None reported Solvent carrier: Corn oil or Olive oil Contaminants: None reported Chemical formula: C9H10
Chemical Category	

Method

Method/Guideline followed	Other
GLP	**Yes**
Year Study Performed	**1980**
Species	mouse
Strain	Swiss Webster
Sex	Both
Number of Males per Dose	10
Number of Females per Dose	**10**
Vehicle	corn oil and/or olive oil, volume 3.33 ml/kg
Route of Administration	Oral
Remarks for Method	The objective of this study was twofold: A) to observe the effect, if any, of corn oil or olive oil when used as a vehicle for PMS on its oral toxicity as measured by the LD50 when compared to the LD50 of the neat compound and, (B) to detect any difference in three PMS samples based on the mortality response at the calculated LD50 of the reference sample. In the first study, the test substance (PMS Edison reference sample) was used without vehicle (neat) or dissolved in corn oil or olive oil and administered once by oral gavage to approximately 12 weeks old fasted male and female mice (Swiss Webster,). The concentrations were 600, 810, 1095,

1480, and 2000 mg/kg and administered at a volume of 0.67, 0.91, 1.23, 1.66, and 2.25 ml/kg, respectively. In the second study, three samples Edison (Reference), IRDC (MCTR-251-79), and Hazleton (MCTR-144-79) were dissolved in olive oil and administered once by oral gavage to fasted male and female mice at a concentration of 1182 mglkg (volume was 3.33 ml/kg).

In both studies, mice were observed for mortality twice daily for a total of 7 days. Mice were observed for clinical signs during the first 5 minutes, at 1 and 4 hours following dosing and once daily thereafter for 7 days. Body weights were obtained immediately prior to test article administration and at 7 days. All surviving animals were killed after 7 days. No post mortem examinations were conducted.

Regression anaysis of percent death versus dose were used to calculate LD50s.

Results

Precision	=
Acute Lethal Value	1150
Unit	mg/kg-bw
Deaths per Dose	See in the Results Remarks

Results Remarks

Deaths in the Edison Reference sample (neat) occurred as follows: 1/10 male mice dead at day 1 in 1095 mg/kg, 8/10 male mice dead at day 1 and 2/2 dead at day 3 in 1480 mg/kg, 10/10 male mice dead at day 1 in 2000 mglkg, 1110 female mice dead at day 3 in 600 mg/kg, 4/10 female mice dead at day 1 and 1/6 dead at day 2 in 810 mg/kg, 6/10 female mice dead at day 1 in 1095 mglkg, 8/10 female mice dead at day 1 in 1480 mg/kg, 10110 female mice dead at day 1 in 2000 mg/kg. Deaths in the Edison Reference sample (corn oil vehicle) occurred as follows: 1/10 male mice dead at day 2 in 1095 mg/kg, 6/10 male mice dead at day 1 and 1/4 dead at day 2 in 1480 mg/kg, 8110 male mice dead at day 1 and 2/2 dead at day 2 in 2000 mglkg, 1/10 female mice dead at day 1 in 600 mg/kg, 3/10 female mice dead at day 1 and 3/7 dead at day 2 in 1095 mg/kg, 7/10 female mice dead at day 1 and 3/3 dead at day 2 in 1480 mglkg, 9/10 female mice dead at day 1 and 1/1 dead at day 2 in 2000 mg/kg. Deaths in the Edison Reference sample (olive oil vehicle) were as follows: 1 /1 0 male mice dead at day 3 in 600 mglkg, 1 /1 0 male mice dead at day 1 and 1/9 dead at day 4 in 1095 mg/kg, 4110 male mice dead at day 1 and 3/6 dead at day 2 in 1480 mg/kg, 8/10 male mice dead at day 1 and 2/2 dead at day 2 in 2000 mg/kg, 1 /1 0 female mice dead at day 3 in 600 mg/kg, 1 /1 0 female mice dead at day 2 in 810 mg/kg, 2/10 female mice dead at day 1 in 1095 mglkg, 7110 female mice dead at day 1 and 3/3 dead at day 2 in 1480 mg/kg, 6/10 female mice dead at day 1 and 3/4 dead at day 2 in 2000 mg/kg.

Deaths for the Edison Reference sample (1182 mg/kg) are as follows: 2110 male mice dead at day 1 and 2/8 male mice dead at day 2, 7/10 female mice dead at day 1. Deaths for the IRDC sample (MCTR-251-79)(1182 mg/kg) are as follows: 3/10 male

mice dead at day 1, 1/7 dead at day 2 and 1/6 dead at day 3, 6/10 female mice dead at day 1. Deaths for the Hazelton sample (MCTR-144-79)(1182 mglkg) are as follows: 1110 male mice dead at day 3, 5110 female mice dead at day 1.

Summary of total deaths

PMS Edison (neat): 1/20, 5/20, 7/20, 18/20, and 20/20 mice treated at 600, 810, 1095, 1480, and 2000 mglkg, respectively.

PMS Edison (corn oil vehicle): 1/20, 0/20, 7/20, 17/20, and 20/20 mice treated at 600,810, 1095, 1480, and 2000 mglkg, respectively.

PMS Edison (olive oil vehicle): 2/20, 1/20, 4/20, 17/20, and 19/20 mice treated at 600, 810, 1095, 1480, and 2000 mg/kg, respectively.

Total deaths of three samples using 1182 mg/kg

Edison Reference sample: 11/20

IRDC: 1 1/20

Hazleton: 6/20

Clinical signs observed in animals surviving treatment included the following: rough coat, decreased excreta, decreased activity, dehydration, anorexia, respiratory distress, prostration, emaciation, and hunching. Animals which died showed all the aforementioned signs, as well as convulsing, ataxia, ocular and oral discharge, and shivering. All surviving animals dosed with 1182 mg/kg were normal at the end of 7 days.

Conclusions

LD50s (95% confidence limits)
PMS Edison Reference sample: males 1200 mglkg (91 0-1500 mglkg), females 960 mg/kg (790-I 200 mg/kg), combined 1100 mglkg (950-1200 mg/kg).
PMS Edison Reference (corn oil vehicle): males 1300 mg/kg (1000-I 600 mg/kg), females 1100 mglkg (860-I 300 mglkg), combined (1000-I 300 mglkg).
PMS Edison Reference (olive oil vehicle): males 1200 mg/kg (1000-l 500 mg/kg), females 1100 mglkg (960-I 300 mglkg), combined 1200 mg/kg (1100-I 300 mglkg).

Inspection of the data show that LD50 (combined sexes) is not affected by corn oil or olive oil when used as a vehicle and that, when treated at the LD50 dose level, the biological response of mice (as measured by the number dying) cannot distinguish between the three samples (Edison, IRDC, and Hazleton).

The LD50 of PMS in Swiss-Webster mice is judged to be 1150 mg/kg (average of 1100 and 1200 mg/kg). Female

mice appear slightly more sensitive to the toxicity of PMS than male mice.

Data Quality

Reliability Acceptable

Data Reliability Remarks Experimental design and key parameters (number of animals/dose, concentrations, number of days of observations, etc.) are appropriate and described in the study.

Reference

Remarks Oral LD50 of Para-Methylstyrene (PMS) in Swiss-Webster Mice after a Single Exposure: Effect of Corn Oil/Olive Oil Vehicle and Comparison of Three PMS Samples. Mobile Environmental and Health Science Laboratory #441-80, December 18, 1980.

General

One purpose of the study was to compare three different PMS samples using 1182 mg/kg. No significant differences were observed (Chi Square).

Admin Record Number: 20113044B

ROBUST SUMMARY

Styrene, p-methyl-

CAS# 622979 Study Number: 5

HUMAN HEALTH EFFECTS ELEMENTS:

ACUTE TOXICITY

Test Substance

Remarks	p-methylstyrene Test Article ID#: MCTR-86-79 Purity: 100% assumed for dosing calculations Additives: None reported Solvent carrier: None Contaminants: None reported Chemical formula: C9H10
Chemical Category	

Method

Method/Guideline followed	Other
GLP	**Yes**
Year Study Performed	1979
Species	mouse
Strain	CD-1
Sex	Both
Number of Males per Dose	**5**
Number of Females per Dose	**5**
Vehicle	cottonseed oil, volume of 10.845 ml/kg
Route of Administration	Intraperitoneal
Remarks for Method	The test substance was suspended in cottonseed oil and administered once by intraperitoneal injection to male and female mice (Charles River CD-l, approximately 13 weeks old). The concentrations were 215, 462, 993, 2135, 4590, and 9869 mg/kg and administered at a volume of 10.845 ml/kg of body weight. Mice were observed for mortality during the first 1/2 hour, at 1, 21/2, and 4 hours following dosing and twice daily (AM and PM) thereafter for a total of 14 days. The mice were observed for pharmacotoxic signs during the first 1/2 hour, at 1, 21/2, and 4 hours following dosing and daily thereafter for a total of 14 days. Body weights were obtained immediately prior to test article administration (control weight) and at 7 and 14 days of the observation period. All mice which died during the study period were subjected to gross necropsy examination as

were all survivors at the end of the 14-day observation period.

Results

Precision	=
Acute Lethal Value	770
Unit	mg/kg-bw
Deaths per Dose	0110, 1/10, 8110, 9110, 10/10, and IO/IO mice treated at 215, 462, 993, 2135, 4590, and 9869, resp.
Results Remarks	Deaths occurred as follows: 2/5 male mice dead at day 1 and 1/3 dead at day 2 in 993 mglkg, 4/5 male mice dead at day 1 in 2135 mg/kg, 1/5 male mice dead between O-4 hours and 4/4 male mice dead at day 1 in 4590 mg/kg, 5/5 male mice dead between O-4 hours in 9869 mglkg, 1/5 female mice dead at day 4 in 462 mg/kg, 5/5 female mice dead at day 1 in 993 mg/kg, 5/5 female mice dead at day 1 in 2135 mglkg, 4/5 female mice dead between O-4 hours and 1/1 dead at day 1 in 4590 mg/kg, 5/5 female mice dead between O-4 hours in 9869 mg/kg. Clinical signs of toxicity (Number, [hour], (day)) occurred as follows: Males at 215 mglkg exhibited the following, Wet, yellow-stained anogenitals 1 [I ,21/2],[4], Soft stool 1 [I]. Males at 462 mg/kg exhibited Hypoactivity 2(1), Wet yellow-stained anogenitals 2(1). Males at 993 mg/kg exhibited Hypoactivity 1[21/2,4], 4(1), 1(2-3), Ataxia 2(1), Wet yellow-stained anogenitals 2(I), Clear moist-stained abdomen I(1), Coarse body tremors I(1). Males at 2135 mglkg exhibited Hypoactivity 4[1,21/2,4], 1 (I), Bradypnea 1[4], Ataxia 4[1,21/2,4], Loss of limb tone 3[1],4[21/2,4]. Males at 4590 mg/kg exhibited Hypoactivity 1 [I ,21/2,4], Bradypnea 3[21/2], 3[4], Gasping 1[4], Clonic convulsions 2[1],4[21/2], 3[4], Ataxia 1 [I ,21/2], Loss of limb tone 5[1,21/2], 4[4], Prostration 4[1,21/2,4]. Males at 9869 mg/kg exhibited Hypoactivity 5[1/2], 1 [I], No motor activity 5[1], 3[21/2], Bradypnea 3[1], 2[21/2], Gasping 1[1], Clonic convulsions 2[21/2]. Females at 215 mg/kg were normal. Females at 462 mglkg exhibited Hypoactivity I(1 -3), Bradypnea 1(2-3), Wet yellow-stained anogenitals 2(1), 1(2-3), Clear, moist-stained abdomen 4(1), 1(2-3), Coarse body tremors 1(1-3). Females at 993 mglkg exhibited Hypoactivity 1[1], 2[21/2,4]. Females at 2135 exhibited Hypoactivity 4[1,21/2], 2[4], No motor activity 1[4], Bradypnea 1[4], Ataxia 2[1,21/2], 1[4], Loss of limb tone 4[1,21/2], 2[4]. Females at 4590 mg/kg exhibited No motor activity 3[1], 1[4], 2[21/2], Bradypnea 3[21/2], Gasping 1[21/2,4], Clonic convulsions 1[1], 3[21/2], 1[4], Loss of limb tone 1[1,21/2], Prostration 5[1], 4[21/2], 1[4]. Females at 9869 exhibited Hypoactivity 5[1/2], No motor activity 5[1], 1[21/2], Bradypnea 5[1], 1[21/2], Clonic convulsions 1[21/2]. All surviving male and females were normal by day 3. No significant changes in body weights of surviving animals were observed. There were a variety of changes in the animals dying in the study, including congestion, but these were considered to be nonspecific and not attributable to an identifiable toxic effect

of the test article.

Male mice LD50: 1072.2 (660.8-l 739.8) mg/kg. Female mice LD50: 581.4 (428.1-789.6) mg/kg. Both slopes were 1 .O.

Conclusions

The combined male and female acute intraperitoneal (LD50) value and 95% confidence limits were 769.7 (567.2 - 1044.4) mg/kg. Combined slope was 0.9. Females appeared slightly more sensitive in terms of mortality when compared with males.

Data Quality

Reliability Acceptable

Data Reliability Remarks Experimental design and key parameters (number of animals/dose, concentrations, number of days of observation, etc.) are appropriate and described in the study.

Reference

Remarks Acute Intraperitoneal Toxicity (LD50) Study in Mice with MCTR-86-79. International Research and Development Corporation #450-004, December 3, 1979. M861-79.

General

Admin Record Number: 20113044B

ROBUST SUMMARY

Styrene, p-methyl-

CAS# 622979

Study Number: 6

HUMAN HEALTH EFFECTS ELEMENTS:

ACUTE TOXICITY

Test Substance

Remarks	p-methylstyrene Test Article ID#: MCTR-112-79 Purity: 100% assumed for dosage calculations Additives: None reported Solvent carrier: None Contaminants: None reported Chemical formula: C9H10
Chemical Category	

Method

Method/Guideline followed	Other
GLP	**Yes**
Year Study Performed	**1979**
Species	rat
Strain	Fischer 344
Sex	Both
Number of Males per Dose	**10**
Number of Females per Dose	10
Vehicle	None reported
Route of Administration	Intravenous
Remarks for Method	Appropriate amounts of the test material (based on density of 892 mg/ml) were administered once via the tail vein to male and female rats (Fischer 344, body weights of males ranged from 212 to 250 grams and body weights of females ranged from 185 to 230 grams). The concentrations were 316, 562, 1000 1780, and 3160 mg/kg. All rats were observed for mortality and signs of toxic and pharmacologic effects at 1, 2, and 4 hours postdose, and twice daily thereafter for 14 consecutive days. Individual body weights were recorded prior to treatment, on Day 7, and at death or termination (Day 14). At termination, all surviving rats were sacrificed by carbon dioxide asphyxiation and necropsied. Necropsies were also performed on those animals which died during the study.

Results

Precision	>
Acute Lethal Value	3160
Unit	mg/kg-bw
Deaths per Dose	0/20, 2/20, 6/20, 3/20, and 6/20 rats treated at 316, 562, 1000, 1780, and 3160 mg/kg, respectively
Results Remarks	Deaths occurred as follows: 1110, 4/10, and 1110 male rats dead at 1 hour in 562, 1000, and 3160 mglkg, respectively; 1/9 male rats dead at day 4 in 562 mg/kg; 1/10 male rats dead at day 2 in 1780 mg/kg; 1/9 and 1/8 male rats dead at day 3 and 4, respectively in 3160 mg/kg; 1/10 and 2/10 female rats dead at 1 hour in 1000 and 1780 mg/kg, respectively; 1/9 female rats dead at 2 hours in 1000 mg/kg; 1110, 1/9 and 1/8 female rats dead at days 1, 2, and 7, respectively in 3160 mg/kg. Clinical observations consisted of slight depression at 562, 1000, 1780, and 3160 mglkg, soft feces and rough coat at 562, 1780, and 3160 mglkg, depression, urine stains, red stains on nose and/or eyes, and posterior portion of tail missing at 1780 and 3160 mg/kg, prostration at 1000 and 3160 mg/kg, labored respiration at 1000 mglkg, and ataxia at 1780 mg/kg. All surviving animals exhibiting clinical signs (excluding rats with portion of tail missing) were normal by day 6, except 1/10 female rat at 3160 mg/kg (no recovery was observed). Of surviving animals, 21/83 gained weight, 59/83 lost weight and 3/83 were found to have the same weight at initiation and termination. Weight loss in surviving females was higher than in males. For example, all surviving females lost weight at the end of the study, except 1/10 in 316 mg/kg. The following males lost weight at the end of the study; 2110, 2/8, 1/6, 5/9 and 7/7 in 316, 562, 1000, 1780, and 3160 mglkg, respectively. Gross pathology consisted of the following: 316 mg/kg, no gross pathology was observed; 562 mg/kg, 1/20 stomach and intestine contains dark brown material; 1000 mglkg, no gross pathology was observed; 1780 mg/kg, 3/20 exhibited one or more of the following: 1120 dark liver and spleen, 1120 stomach contains yellow material and air, 1/20 intestine contains yellow or dark yellow fluid, 3/20 intestine contains air; 3160 mg/kg, 12/20 exhibited one or more of the following: 2/20 dark red areas in lungs, 1/20 dark liver, 1/20 dark stomach lining, 7/20 red stomach lining, 1/20 intestines contains yellow or dark yellow fluid, black fluid, or red fluid-like material, 2/20 intestines contains dark red fluid. LD50s could not be calculated due to a lack of mortality (<50%) at the highest dose level.

Conclusions

The acute intravenous LD50 in male and female rats was estimated to be greater than 3160 mg/kg of body weight.

Data Quality

Reliability

Acceptable

Data Reliability Remarks

The selection of doses did not allow for a better estimate of the LD50. However, only 30% of animals died at the highest dose (3160 mg/kg). Other experimental design and key parameters (number of animals/dose, number of days observed, etc.) are appropriate and described in the study.

Reference

Remarks

Acute Intravenous Toxicity Study in Male and Female Rats: MCTR-112-79. Hazleton Laboratories America, Inc. #230-198, December 21, 1979. MI 121-79.

General

The test material was administered by an intravenous route to provide a comparison with toxicity resulting from oral and intraperitoneal administration.

Admin Record Number: 20113044B **ROBUST SUMMARY**

Styrene, p-methyl-

CAS# 622979 Study Number: 7

HUMAN HEALTH EFFECTS ELEMENTS:

ACUTE TOXICITY

Test Substance

Remarks	p-methylstyrene Test Article ID#MCTR-86-79 Purity: 100% assumed for dosage calculations Additives: None reported Solvent carrier: None Contaminants: None reported Chemical formula: C9H10
Chemical Category	

Method

Method/Guideline followed	Other
GLP	**Yes**
Year Study Performed	**1979**
Species	mouse
Strain	CD-1
Sex	Both
Number of Males per Dose	**5**
Number of Females per Dose	**5**
Vehicle	None reported
Route of Administration	Intravenous
Remarks for Method	The test substance was administered once intravenously to male and female mice (Charles River CD-l, approximately 13 weeks old). The concentrations were 90, 126, 176, 247, 346, and 484 mg/kg. All dosages were calculated based upon a specific gravity of 0.90 g/ml. The mice were observed for mortality frequently during the first 4 hours following dosing and twice daily (AM and PM) thereafter for a total of 14 days. The mice were observed for pharmacotoxic signs the first 1/2 hour, at 1, 2 1/2, and 4 hours following dosing and once daily thereafter for a total of 14 days. Body weights were obtained immediately prior to test article administration (control weight) and at 7 and 14 days of the observation periods. All mice which died during the study period were subjected to gross necropsy observation as were all survivors at the end of the 14-day study period.

Results

Precision	=
Acute Lethal Value	310
Unit	mg/kg-bw
Deaths per Dose	O/l 0, 1/10, 1/10, 3/10, 5/10, and 1 0/10 mice treated at 90, 126, 176,247, 346, and 484 mglkg, resp.
Results Remarks	Deaths occurred as follows: 1/5 male rats dead at day 4 in 126 mg/kg; 1/5, 2/5, and 5/5 male rats dead between O-4 hours in 247, 346, and 484 mg/kg, respectively; 1/5, 2/5, 3/5, and 5/5 female rats dead between O-4 hours in 176, 247, 346, and 484 mg/kg, respectively. Clinical signs of toxicity (Number, [hour], (day)) occurred as follows: Males at 90 mg/kg were normal. Males at 126 mg/kg exhibited the following, Decreased activity 5[1/2,1], 3[21/2,4], 3(1), 2(2), 1(3), Wet, yellow-stained anogenitals 2[1,21/2,4], 1(3), Loss of limb tone 1 [l ,21/2,4], 1 (1-3), Ataxia 1 (1-3), Low carriage 1(1-3), Bradypnea 1(2-3), Tremors 1(2-3), Wet, yellow-stained abdomen l(3). Males at 176 exhibited the following, Decreased activity 3[1/2,1,21/2,4], 3(1), 1(2), Wet, yellow-stained anogenitals 1 [1,21/2,4], Ataxia 1[21/2,4], Low carriage 2[21/2,4], 1(1-2), Red-stained anogenital region 1[21/2,4]. Males at 247 mg/kg exhibited the following, Decreased activity 3[1/2,1,21/2,4], 3(1), Wet, yellow-stained anogenitals 3[1/2,1,21/2,4], 2(1), Ataxia 1[21/2,4], l(1). Males at 346 mg/kg exhibited the following, Decreased activity 2[1/2], 3[1,21/2,4], Wet-yellow-stained anogenitals 1 [1/2], 2[1,21/2,4]. Females at 90 mg/kg exhibited the following, Decreased activity 1 [l ,21/2,4]. Females at 126 mglkg exhibited the following, Decreased activity 2[1/2,1], 1[21/2,4], Red discharge from anogenital region 1[21/2,4]. Females at 176 mg/kg exhibited the following, Decreased activity 1[1/2], 2[1,21/2,4], 2(1), Ataxia 1[21/2,4], l(l), Red discharge from vagina 1 [1,21/2,4]. Females at 247 mglkg exhibited the following, Hyperactivity 1[21/2,4], l(1 -3). Females at 346 mglkg exhibited the following, Decreased activity 2[1/2], 1[1,21/2,4], 1(1-2), Ataxia 1[21/2,4], l(l), Low carriage 1[21/2,4], l(l-2). All surviving male and female mice were normal by day 4. No significant changes in body weights of surviving animals were observed. The changes in necropsied animals were non-specific and not directly attributable to administration of the test article. There was a dose related increase in lung congestion in animals dying during the study. The LD50s (95% confidence limits and slope) are as follows: Male 334.0 mg/kg (269.9-413.4 mg/kg, 2.3), Female 279.8 mglkg (205.1-381.7 mglkg, 1.8).

Conclusions

The combined male and female acute intravenous LD50 (95% confidence limits and slope) was 308.7 mg/kg (260.1-366.4 mg/kg, 2.0).

Data Quality

Reliability Acceptable

Data Reliability Remarks Experimental design and key parameters (number of animals/dose, concentrations, number of days observed, etc.) are appropriate and described in the study. All dosages were based on a specific gravity of 0.90 g/ml (should have been 0.89 g/ml). This small error does not affect the validity of the results.

Reference

Remarks Acute Intravenous Toxicity Study in Mice: MCTR-86-79. International Research and Development Corporation #450-005, December 28, 1979. M860-79.

General

Admin Record Number: 20113044B **ROBUST SUMMARY**

Styrene, p-methyl-

CAS# 622979 Study Number: 8

HUMAN HEALTH EFFECTS ELEMENTS:

ACUTE TOXICITY

Test Substance

Remarks	p-methylstyrene Test Article ID# MCTR-88-79 Purity: 99.85% active ingredient Additives: None reported Solvent carrier: Mazola corn oil Contaminants: None reported Chemical formula: C9H10
Chemical Category	

Method

Method/Guideline followed	Other
GLP	**Yes**
Year Study Performed	1979
Species	dog
Strain	Beagle
Sex	Both
Number of Males per Dose	**2**
Number of Females per Dose	**2**
Vehicle	corn oil
Route of Administration	Oral
Remarks for Method	The study was designed to evaluate the acute toxicity of the test substance when administered orally via intubation to Beagle dogs (8-12 months old, Groups I and II; 13-14 months old, Group III; 2/sex/group). The study consisted of three groups: Group I received a single dose of the test substance (4 g/kg); Group II (4 g/kg) and Group III (5 g/kg) received a single dose mixed with an equal volume of corn oil. Animals were observed for mortality and toxicological or pharmacological signs twice daily for a total of 14 (Group I), 15 (Group Ill), or 16 days (Group II). Body weights were obtained immediately prior to test article administration (control weight) and at 1, 2,4, 7, and 14 days of the observation periods. All animals were subjected to gross necropsy observation at the end of the study.

Results

Precision	>
Acute Lethal Value	**5000**
Unit	mg/kg-bw
Deaths per Dose	0/4, 0/4, and 0/4 dogs treated at 4 g/kg, 4 g/kg (corn oil), and 5 g/kg (corn oil), respectively.
Results Remarks	All animals survived the duration of the study. Groups I males and females experienced varying degrees of emesis and salivation within 90 minutes post dosing which lasted not more than 1 hour and 45 minutes. All Group I animals also experienced at least one episode of slight to moderate body tremors of varying duration within 1 hour after test substance administration. Group II females (test substance with equal volume of corn oil) vomited within minutes after dosing. Emesis was observed in all Group II animals approximately 20-22 hours post-dosing. Slight to extreme body tremors were observed in all Group II animals within 1 hour post-dosing. Salivation was not evident in any of the Group II animals. Group III males and females experienced varying degrees of emesis, salivation, and tremors within 1 hour of dosing. All animals recovered from these signs within l-2 days after dosing, except one male in Groups I and II experienced emesis twice during the 5-14 day interval. One male and two females from Group I, one male and one female from Group II, and all Group III animals exhibited small body weight losses (100-400 grams) during the post-dosing observation period. Food consumption values showed no patterns that could be attributed to the administration of either the test substance alone or the test substance mixed with an equal volume of corn oil. No gross changes **were** observed upon necropsy that could be attributed to the administration of the test substance. Tissues which showed incidental gross post-mortem findings were preserved for possible future examination.
Conclusions	The acute toxicity of a single oral dose of MCTR-88-79 administered in corn oil to male and female Beagle dogs was greater than 5 g/kg.

Data Quality

Reliability	Acceptable
Data Reliability Remarks	The experimental design and key parameters (number of animals/dose, number of observation days, etc.) were appropriate and described in the study.

Reference

Remarks

An Acute Toxicity Study with MCTR-88-79 in Dogs. Bio/dynamics Inc. #79-2406, November 15, 1979. M880-79.

General

A five-day pilot study of PMS was conducted to find a non-emetic dose in male and female beagle dogs (Five-day Pilot Study in Dogs, Hazleton Laboratories America, Inc., Project No. 230-230, July 1, 1981, M2141-80). The test material was administered orally, by capsule, to six groups of one male and one female dog each for five consecutive days. Dogs in Groups 1, 3, and 5, dosed at 3.0, 1.0, and 0.3 g/kg, respectively, were administered capsules containing the test material immediately after the morning examination and prior to feeding (referrred to as fasted). Dogs in Groups 2, 4, and 6, dosed at 3.0, 1 .0, and 0.3 g/kg, respectively, were administered capsules containing the test material one-half hour after the feed bowl was removed (referred to as fed). The criteria used to evaluate for compound effect included mortality, body weight change, food consumption, and clinical signs. The loss in body weight was greater during the treatment period than pretreatment period in the fed males and females dosed at 3.0 g/kg and in the fasted females dosed at 3.0 g/kg. The mean daily food consumption was decreased after treatment in the fed and fasted males and females dosed at 3.0 g/kg and possibly in the fasted males and fed females dosed at 1 .0 g/kg. Clinical signs considered the result of compound administration consisted of emesis and/or change in stool. Emesis was observed most frequently during the five days of study in the fed and fasted females dosed at 3.0 g/kg while changes in stool were observed most frequently in the fasted males dosed at 3.0 g/kg, in the fed males dosed at 0.3 g/kg, and in the fasted females dosed at 1 .O g/kg.

In conclusion, dogs were able to survive the administration of PMS daily for five days at levels up to 3.0 g/kg with changes in body weight, food consumption, and/or occurrences of emesis or soft stool.

This study is acceptable based on an appropriate test design.

Admin Record Number: 20113044B

ROBUST SUMMARY

Styrene, p-methyl-

CAS# 622979 Study Number: 9

HUMAN HEALTH EFFECTS ELEMENTS:

ACUTE TOXICITY

Test Substance

Remarks	p-methylstyrene Test Article ID# MCTR-142-79 Purity: 100% assumed for dosage calculations Additives: None reported Solvent carrier: None Contaminants: None reported Chemical formula: C9H10
Chemical Category	

Method

Method/Guideline followed	Other
GLP	**Yes**
Year Study Performed	1980
Species	rat
Strain	Sprague-Dawley
Sex	Both
Number of Males per Dose	**5**
Number of Females per Dose	**5**
Vehicle	Air
Route of Administration	Inhalation
Remarks for Method	A four-hour inhalation exposure was performed using Sprague-Dawley male and female rats (males, 241-258 grams; females, 21 O-230 grams) to determine the acute toxicity of the test substance. Test substance vapor was generated using a heated-flask flash evaporator. An FMI fluid metering pump was used to draw the test material from a 250-ml flask containing approximately 75 ml of test material. The test material was pumped through a coil of 1/8" teflon tubing which was immersed in a water bath (50-85 degrees C). The heated test material was then delivered to the flash evaporator through teflon tubing. Dry air, at a flow rate of 15 liters per minute, was passed through a coil of 1/4" copper tubing which was immersed in a water bath (90-96 degrees C). The dry air was heated to 30 degrees C through the coil and was delivered to the flash evaporator. The test material was vaporized by allowing the heated dry air to pass

over the tip of the teflon tubing containing the heated test material. The vaporization occurred in a 1 OOO-ml Erlenmeyer flask heated to approximately 65 degrees C using a heating mantle. The test atmosphere was directed, undiluted, into a 26.5-liter glass exposure chamber housing the animals. Chamber temperature range was 25-28 degrees C during the exposure. The exposure lasted for four hours. The generation flask, connecting tubing, stopper, and clamp were weighed before and after the exposure. The difference in weight represented the total amount of test material delivered into the chamber; this, divided by the total volume of air delivered yielded the nominal exposure concentration. During the exposure, a total of 61.85 grams of test material was delivered in a total volume of 3,600 liters of air, yielding a nominal exposure concentration of 17.18 mg/l (equivalent to 3,500 ppm; with a molecular weight of 118 for MCTR-142-79, 1 mg/l is equivalent to 207 ppm).

The test group consisted of five male and five female Sprague-Dawley (CD) rats. The animals were observed for abnormal signs before the exposure, every 15 minutes during the first hour of the exposure period, hourly through the termination of the exposure, upon removal from the chamber, hourly for four hours post-exposure, and daily thereafter for 14 days. Individual body weights for all rats were recorded on Day 0 (prior to exposure) and on Days 1, 2, 4, 7, and 14. On Day 14, all rats were exsanguinated under ethyl ether anesthesia and gross necropsy examinations were performed.

Results

Precision >

Acute Lethal Value 3500

Unit ppm(air)

Deaths per Dose No deaths were observed at 3,500 ppm.

Results Remarks No rats died during the exposure or subsequent 14-day observation period.

During the exposure all animals exhibited signs of reaction to the test material including respiratory abnormalities and increased ocular, nasal, and oral secretions commencing after 15 minutes. After removal from the chamber, neuromuscular abnormalities (weakness, loss of reflex activity, spontaneous muscular activity, incoordination, tremors, etc.) were also observed. The secretory responses decreased during the four hour observation period whereas the neuromuscular and respiratory impairment increased in severity. The signs had abated by Day 1 except rales which persisted up to Day 3.

Isolated incidences of slight dry rales were seen in all rats from Days 4-14, but this incidence and severity did not indicate any residual irritant or toxic effect of the test material. Other signs were seen transiently in isolated rats and were

not associated with exposure.

Although small, transient weight-losses were observed in all rats, body weights recovered to pre-exposure values in males and 3/5 females by Day 7. Body weight increments in the second week were within the limits of normal expectations except for 2 females which exhibited a slower than normal weight gain.

At necropsy, 2 male rats showed lung discoloration (foci). These are common pathological entities in Sprague-Dawley rats and are not associated with the test substance.

Conclusions

A four-hour acute inhalation exposure of Sprague-Dawley male and female rats to a vapor of MCTR-142-79 at a nominal concentration of 17.18 mg/l (3,500 ppm) did not produce mortality. Signs of irritation and neuromuscular were observed in the study, but animals recovered by Day 3 post-exposure. Slight depression of body weight increments in two females was observed. Necropsy findings were unremarkable.

Data Quality

Reliability Acceptable

Data Reliability Remarks Experimental design and key parameters (vapor generation, number of days observed, etc.) are appropriate and described in the study.

Reference

Remarks An Acute Inhalation Toxicity Study of MCTR-142-79 in the Rat. Bio/dynamics, Inc. # 79-7347, May 19, 1980. M1420-79.

General

HUMAN HEALTH EFFECTS ELEMENTS:

ACUTE TOXICITY

Test Substance

Remarks	Comparison of p-methylstyrene (MEHSL Sample No. RM070279) and PMS-free poly-PMS extract (MEHSL Sample No 12088002). Purity: 100% assumed for dosage calculations Additives: None reported Solvent Carrier: Olive oil Contaminants: None reported Chemical formula: C9H10
Chemical Category	

Method

Method/Guideline followed	Other
GLP	**Yes**
Year Study Performed	1980
Species	mouse
Strain	Swiss Webster
Sex	Both
Number of Males per Dose	**5**
Number of Females per Dose	**5**
Vehicle	Olive Oil
Route of Administration	Oral
Remarks for Method	This study was conducted to assess the acute oral toxicity of PMS-free poly-PMS extract in mice when compared to the acute oral toxicity of PMS in mice. Only the PMS study will be described here; see General Comments for the PMS-free poly-PMS extract results. The test substance was suspended in olive oil and administered once by oral gavage to 24-hr fasted male and female Swiss-Webster mice (approximately 6 weeks old). Test concentrations were 800 and 1182 mg/kg and administered at a volume of 10 ml/kg of body weight. Mice were observed for mortality and pharmacotoxic signs during the first 5 minutes, at 1 and 4 hours, and daily thereafter for a total of 5 days. The study was ended early due to time constraints and no body weights were obtained or gross necropsy examinations conducted.

Results

Precision	<=
Acute Lethal Value	1182
Unit	mg/kg-bw
Deaths per Dose	1 /10 and 8110 in mice treated at 800 and 1182 mg/kg, respectively
Results Remarks	Deaths occurred as follows: No male rats dead at 800 mg/kg; 3/5 male rats dead at day 1 in 1182 mg/kg; 1/5 female rats dead at day 5 in 800 mg/kg, 5/5 female rats dead at day 1 in 1182 mg/kg. Clinical signs in 800 mg/kg were as follows: all surviving females were normal, 1 male exhibited decreased activity on days 1-3, 1 male exhibited rough hair on days 1-5, 1 male exhibited shaking on day 5. One surviving male in 1182 mg/kg exhibited decreased activity on days 4-5.

Conclusions

The study was conducted to assess the acute oral toxicity of PMS-free poly PMS extract in mice when compared to the acute oral toxicity of PMS in mice.

Data Qualitv

Reliability	Unacceptable
Data Reliability Remarks	This purpose of this study was not to determine the acute oral LD50 of PMS in mice but to compare the oral toxicity of PMS-free poly-PMS extract with the acute oral toxicity concentrations of PMS already determined in previous studies. Therefore, only two concentrations of PMS were used, the study was ended by day 5, no body weights were obtained and no gross necropsy examinations were conducted.

Reference

Remarks	Preliminary Acute Oral Toxicity of PMS-Free Poly-PMS Extract as Compared to the Previously Determined PMS LD50 in Mice, Mobile Environmental and Health Science Laboratory Study No. 2451-80, December 18, 1980.

General

Mortality of PMS-free Poly-PMS extract was 0/8, 2/9, 4/8, and 8/10 for mice treated at 1580, 2812, 5000, and 8800 mg/kg, respectively.

HUMAN HEALTH EFFECTS ELEMENTS:

ACUTE TOXICITY

Test Substance

Remarks	p-methylstyrene Test Article ID#: MCTR-243-77 Purity: 100% assumed for dosage calculations Additives: None reported Carrier solvent: None Contaminants: None reported Chemical formula: C9H10
Chemical Category	

Method

Method/Guideline followed	Other
GLP	Unknown
Year Study Performed	1977
Species	rabbit
Strain	New Zealand white
Sex	M
Number of Males per Dose	**6**
Number of Females per Dose	**0**
Vehicle	None
Route of Administration	Dermal
Remarks for Method	The objective of this study was to evaluate the irritation and corrosivity potential of the test substance in rabbits following a single dermal exposure. Six New Zealand white rabbits (2.05 to 2.15 kg; sex not reported) were dosed dermally with a single application of 0.5 ml of the test substance. The site of application was clipped and abraded on all six rabbits. Observations were made at 24 and 72 hours following application for signs of irritation and corrosivity. Irritation scores were recorded and the primary index was calculated according to the Federal Hazardous Substances Act, September 27, 1973. The test substance was applied to the backs and an occlusive wrap was not used because the test substance reacted with the dental dam creating an unwanted variable. The rabbits were restrained by being put in a Newman harness for the 24-hour initial period.

The scale for rating skin reactions is as follows:
Erythema and eschar formation
O=no erythema
1 =very slight
2=well defined
3=moderate to severe
4=severe erythema (beet red) to slight eschar formation (injuries in depth)

Edema formation:
O=no edema
1 =very slight
2=slight edema
3=moderate edema
4=severe edema

Results

Precision	>
Acute Lethal Value	0
Unit	ml
Deaths per Dose	No deaths were observed.
Results Remarks	At 24 hours, the average erythema score was 1.5 for both intact and abraded animals, the average edema score was 0.3 for both intact and abraded animals and the corrosivity score was 0. At 72 hours, the average erythema score was 1 .0 for both intact and abraded animals, the edema score was 0.2 for both intact and abraded animals and the corrosivity score was 0. The total score was 6.0. The calculation of primary index was 6.014 = 1.5.

Conclusions

The primary index for MCTR-243-77 is 1.5. Therefore, MCTR-243-77 is classified as a nonirritant when applied dermally according to the Federal Hazardous Substances Act, September 17, 1973.

Data Quality

Reliability	Acceptable
Data Reliability Remarks	The key parameters (i.e., number of animals used, methodology) was appropriate and described in the study.

Reference

Remarks	Acute Skin Irritation and Corrosivity Study in Rabbits with MCTR-243-77, Welcome 4=severe edemalndependent Laboratories, Inc. Project No.: WIL-1060-77, 1977. M2434-77.

General

A sample of p-methylstyrene (MCTR-109-77) was submitted to Consumer Product Testing for primary dermal irritation (M1091-77) and ocular irritation (M1092-77) studies. The

results of these tests are as follows: Primary Dermal; score 1 .1, rating, not a primary skin irritant. Ocular irritation; score, 5/1 hr, 4/1, 2/2, 1/3, 1/4, 0/7 days, rating, no effects.

A brief method is as follows: Primary Dermal Irritation; six rabbits, 1/2 male, 1/2 female, each abraded and non-abraded, 0.5 ml single application under occluded patch, 24 and 72 hour observation. Ocular Irritation; six rabbits,mixed sex, 1.8 to 2.4 kg, 0.1 ml single administration, all with no wash and up to seven day observation. Draize score was 4.7, 3.7, 2.0, 0.7, 0.7, and 0.3 for 1 hr, 1, 2, 3, 4, and 7 days, respectively.

Admin Record Number: 201130448 **ROBUST SUMMARY**

Styrene, p-methyl-

CAS# 622979 Study Number: 12

HUMAN HEALTH EFFECTS ELEMENTS:

ACUTE TOXICITY

Test Substance

Remarks	p-methylstyrene Test Article ID#: MCTR-243-77 Purity: 100% assumed for dosage calculations Additives: None reported Solvent carrier: None Contaminants: None reported Chemical formula: C9H10
Chemical Category	

Method

Method/Guideline followed	Other
GLP	Unknown
Year Study Performed	1977
Species	rat
Strain	Sprague-Dawley
Sex	M
Number of Males per Dose	10
Number of Females per Dose	0
Vehicle	None
Route of Administration	Oral
Remarks for Method	The purpose of the study was to evaluate the oral LD50 of the test substance following a single oral dose. Forty male Sprague Dawley CD rats (225.8 to 261 .0 g) were randominzed into four groups of ten rats per group and given a single oral dose of the test substance. Concentrations were 0.6, 1.3, 2.5, and 5.0 ml/kg. The rats were observed for 14 consecutive days following the day of dosing for signs of toxicity, changes in behavior, and mortality. Following the observation period, all surviving rats were weighed, sacrificed and a gross necropsy was performed.

Results

Precision	=
Acute Lethal Value	4

Unit	ml/kg
Deaths per Dose	1/10, 0/10, 3/10, and 7/10 for animals treated at 0.6, 1.3, 2.5, 5.0 ml/kg, respectively.
Results Remarks	All mortalities were observed by day 1, except one rat in 2.5 ml/kg died on day 14. All surviving rats in 0.6 and 1.3 ml/kg did not have any signs of toxicity throughout the 14-day observation period. On the day of dosing, all rats in 2.5 ml/kg and 7/10 rats in 5.0 ml/kg exhibited an increase in respiration, slight trembling throughout the body and were slightly listless. The other 3 rats in 5.0 ml/kg were also lying on their side very listlessly. On days 1 and 2, the surviving rats in 2.5 and 5.0 ml/kg were slightly listless and had a slight increase in respiration with the exception of one rat in 5.0 ml/kg which was still very listless on days 1 and 2 and slightly listless on day 3. All surviving rats were normal thereafter until day 14 when one of the rats in 2.5 ml/kg died. Gross necropsy revealed an enlarged lobe of the lung which contained a reddish-yellow, foamy fluid. All other rats were normal on day 14. All surviving rats gained weight normally by day 14.

Conclusions

The calculated oral dose of MCTR-243-77 is 3.68 ml/kg with 95% confidence interval of 2.07 ml/kg to 6.52 ml/kg. MCTR-243-77 is classified as being toxic when administered by the oral route according to the Federal Hazardous Substances Act, September 27, 1973. The one death in 0.6 ml/kg was due to improper dosing technique and therefore the death was not included in the LD50 calculation.

Data Quality

Reliability	Unnacceptable
Data Reliability Remarks	The LD50 was based on ml/kg and not mg/kg. No detailed description of the test substance, purity, handling, storage or dosage preparation was included.

Reference

Remarks	Acute Oral Toxicity Study in Rats with MCTR-243-77, Welcome Independent Laboratories, Inc. Project No. WIL-1060-77. M2435-77.

General

fluid. All other rats were normal on day
14. All surviving rats gained weight normally by day 14.

Admin Record Number: 201 130448 **ROBUST SUMMARY**

Styrene, p-methyl-

CAS# 622979 Study Number: 13

HUMAN HEALTH EFFECTS ELEMENTS:

ACUTE TOXICITY

Test Substance

Remarks	p-methylstyrene Test Article ID#: MCTR-243-77 Purity: 100% assumed for dosage calculations Additives: None reported Carrier solvent: None Contaminants: None reported Chemical formula: C9H10
Chemical Category	

Method

Method/Guideline followed	Other
GLP	Unknown
Year Study Performed	1977
Species	rabbit
Strain	New Zealand white
Sex	Both
Number of Males per Dose	2
Number of Females per Dose	2
Vehicle	None
Route of Administration	Dermal
Remarks for Method	The objective of this study was to evaluate the potential lethal effects of MCTR-243-77 when applied to the backs of rabbits for a single dermal exposure. Five groups of four New Zealand white rabbits (two males and two females per group, 2.10 to 2.50 kg) were randomized and given a single dermal application of MCTR-243-77. Test concentrations were 0.5, 1 .0, 2.0, 4.0, and 5.0 ml/kg. The skin of two rabbits per group was abraded while the skin of the remaining two rabbits was left intact. The test substance was applied to the site and remained in contact with the skin for 24 hours during which time the rabbits were restrained in harnesses. An occlusive wrap of dental dam was not used because the test material reacted with the dental dam causing an unknown parameter. Following the 24-hour exposure period, each rabbit was removed from the harness and the unabsorbed test material

washed off the skin with a damp towel. All rabbits were observed for dermal irritation, gross signs of systemic toxicity indicative of percutaneous absorption and mortality once daily for the next 14 days following the day of dosing. After the observation period, all rabbits were weighed, sacrificed, and gross necropsies performed for signs of morphologic toxicity. All procedures were conducted according to methods described in the Federal Hazardous Substance Act, September 27, 1973, Federal Register.

Results

Precision	>
Acute Lethal Value	5
Unit	ml/kg
Deaths per Dose	No deaths were observed.
Results Remarks	No mortality occurred in any of the rabbits during the study and no signs of systemic toxicity or behavioral changes were noted. Incidental findings of skin changes were observed at the site of application. Slight to moderate erythema and very slight edema were noted in 0.5 to 4.0 ml/kg, and moderate responses occurred in 5.0 ml/kg. These skin irritation responses returned to normal during the second week in all groups with the exception of 5.0 ml/kg where they persisted throughout the study. Slight fissuring was noted occasionally in two rabbits in 0.5 ml/kg and one rabbit in 1 .0 ml/kg and was classified as being slight to moderate in the group dosed with 5.0 ml of the test substance. A dose-related occurrence of coriaceous skin was observed in most rabbits with slight responses in the four lowest dose levels which returned to normal by the end of the second week of the study. Moderate responses of coriaceous skin were noted in 5.0 ml/kg. Atonia and desquamation were also observed in 5.0 ml/kg.

Conclusions

The dermal LD50 is greater than 5.0 ml/kg. Compound MCTR-243-77 produced no lethality from dermal applications of 0.5, 1 .0, 2.0, 4.0, or 5.0 ml/kg when applied according to methods described in the Federal Hazardous Substances Act of September 27, 1973.

Data Quality

Reliability	Acceptable
Data Reliability Remarks	The key parameters (i.e., doses, number of animals, duration of exposure, etc.) were appropriate and described in the study.

Reference

Remarks	Acute Dermal Toxicity Study in Rabbits with MCTR-243-77,

Welcome Independent Laboratories, Inc. Project No. WIL-1060-77. M2438-77.

General

Admin Record Number: 201130448 **ROBUST SUMMARY**

Styrene, p-methyl-

CAS# 622979 Study Number: 14

HUMAN HEALTH EFFECTS ELEMENTS:

ACUTE TOXICITY

Test Substance

Remarks	p-methylstyrene Test Article ID#: MCTR-161-79 Purity: 100% assumed for dosage calculations Additives: None reported Carrier solvent: None Contaminants: None reported Chemical formula: C9H10
Chemical Category	

Method

Method/Guideline followed	Other
GLP	Unknown
Year Study Performed	**1980**
Species	rat
Strain	Sprague-Dawley
Sex	Both
Number of Males per Dose	**5**
Number of Females per Dose	**5**
Vehicle	None
Route of Administration	Inhalation
Remarks for Method	Two four-hour inhalation exposures were performed to determine the acute toxicity of MCTR-161-79 in rats. During the first exposure, a total of 728.98 grams of the test material was delivered in a total volume of 45,000 liters of fresh air, yielding a nominal concentration of 16.2 mg/l or 3,300 ppm. Mean chamber concentration was 1,960 ppm. During the second exposure, a total of 505.20 grams of test material was delivered in a total volume of 45,000 liters of air, yielding a nominal exposure concentration of 11.2 mg/l or 2,280 ppm. Mean chamber concentration was 1,510 ppm. In each exposure, the test material was placed in a 1,000 ml nipple bottom bubbler and suspended in a waterbath maintained at 72 degrees C by a Thermomix 1420. A 13 foot coil of copper tubing was also placed in the waterbath. Nitrogen, at flow rates of 20 and 13 liters per minute for Groups I and II,

respectively, was passed through the copper coil and into the bubbler to create a vapor. The vapor-laden airstream then passed through a kjeldahl trap and through a 1,000 ml round bottom trap flask before entering the 760-liter glass and stainless steel exposure chamber housing the test animals. Oxygen was pumped into the chamber at a rate of 4 liters per minute to maintain the oxygen content of the chamber air at approximately 20%. The chamber flow was 125 liters per minute. Additional compound was delivered into the nipple bottom bubbler from a 1,000 ml Erlenmeyer reservoir flask when needed using an FMI lab pumb. A similar FMI pump was used to drain compound from the 1,000 ml trap flask and deliver it into the Erlenmeyer reservoir flask.

The test material, 1,000 ml nipple bottom bubbler, three necked round bottom flask, and Erlenmeyer reservoir flask, clamps, tubing, and stoppers were weighed before and after each exposure period. The difference in weight represented the amount of test material delivered during each exposure. The nominal concentration was calculated by dividing the amount of material delivered by the total air flow through the chamber during each exposure period. Chamber air concentration was monitored continuously during each exposure using a Miran IA Ambient Air Analyzer and recorded once each hour. Waterbath temperature, nitrogen flow rate, oxygen flow rate, and chamber air flow were also recorded hourly.

The test animals for both groups consisted of five male and five female Sprague-Dawley rats. On the day of the exposure in Group I, the body weights ranged from 235-294 g (males) and 217-236 g (females). On the day of exposure in Group II, the body weights ranged from 244-294 g (males) and 238-258 g (females). The animals in both groups were observed prior to exposure to ascertain their basic health status. Observations for abnormalities were made at 15-minute intervals during the first hour of exposure, hourly through the termination of the exposure, upon removal from the chamber, hourly for two hours post-exposure, and daily thereafter for 14 days. Individual body weights for both groups were scheduled to be recorded on day 0, 1, 2, 4, 7, and 14. Any animals dying spontaneously were necropsied as soon as possible after death. On day 14, all surviving animals were sacrificed and gross necropsy examinations were performed.

Results

Precision	>
Acute Lethal Value	1960
Unit	ppm(air), measured
Deaths per Dose	Only one death was observed (male) at a mean chamber concentration of 1,960 ppm.
Results Remarks	During the first exposure (1960 ppm), all animals exhibited

excessive lacrimation, reduced activity, and closed eyes. Other signs included gasping, chromodacryorrhea, ataxia, rapid breathing, shallow breathing, prostration, moist rales, loss of muscle tone and yellow staining of the ano-genital fur. One male rat was found dead on day 1. The majority of the signs were not present after day 2. Although small transient weight-losses were seen in all rats, the body weights recovered to pre-exposure values in males by day 7 and in three females by day 7. Body weight increments in the second week were within normal expectations for both sexes except one female which showed a slower than normal weight gain. At necropsy, two male and three female rats showed foci or areas of lung discoloration. These are common pathological entities in Sprague-Dawley rats and are not ascribed to exposure to the test material.

During the second exposure (1510 ppm), all animals exhibited decreased activity, and closed eyes. Similar signs in the first exposure were also observed in the second exposure. All animals returned to normal by day 1. Although small transient weight-losses were observed in all rats, the body weights recovered to pre-exposure values in males by day 7 and in females by day 14. Body weight increments in the second week were within the limits of normal expectation for the male animals. Three female rats showed weight gains lower than normal. At necropsy, four males and one female showed foci on areas of lung discoloration. These were not ascribed to exposure to the test material.

Conclusions

Two groups of five male and five female Sprague-Dawley rats were exposed to concentrations of MCTR-161-79 for four hours at nominal concentrations of 3,300 and 2,280 ppm. Chamber concentrations measured using the Miran IA Ambient Air Analyzer yielded mean concentrations of 1,960 and 1,510 ppm, respectively.

Ocular and nasal irritation and neuromuscular impairment were seen at both levels but these signs had abated within two days of exposure at the high level and within one day at the low level. One rat died after exposure to 3,300 ppm. Slight depressions of body weight increments in some females were seen at both levels. Necropsy findings were unremarkable.

Data Quality

Reliability Acceptable

Data Reliability Remarks All key parameters (i.e., number of animals, test exposure system) were appropriate and described in the study.

Reference

Remarks An Acute Inhalation Toxicity Study of MCTR-161-79 in the Rat, Bio/dynamics, Inc., Project

No. 79-7312, March 18, 1980. M1610-79.

General

Admin Record Number: ROBUST SUMMARY

Styrene, p-methyl-

CAS# 622979 **Study Number: 1**

HUMAN HEALTH EFFECTS ELEMENTS:

DEVELOPMENTAL TOXICITY/TERATOGENICITY

Test Substance

Remarks	p-methylstyrene Test Article ID#: MCTR-92-78 Purity: 100% assumed for dosage calculations Additives: None reported Carrier solvent: Corn oil (1 mg/kg) Contaminants: None reported Chemical formula: C9H10
Chemical Category	

Method

Method/Guideline Followed	Other
GLP	Unknown
Year Study Performed	1978
Species	rat
Strain	Sprague-Dawley
Sex	F
Number of Males per Dose	0
Number of Females per Dose	20
Route of Administration	Oral
Days of Gestation	6-15
Frequency of Treatment	once daily, 7 daystwk
Doses	60, 190, and 600 mglkglday
Control Group	Yes
Statistical Method	Incidences of occurrence (expressed as %) were analyzed using 95% confidence intervals for proportions or by computation of exact probabilities. Body weights analyzed by ANOVA and least significance difference test was used for comparisons.
Remarks for Method	Sexually mature female Sprague Dawley rats (220 to 230 g body weight) were mated 1 :1 with males in sufficient numbers to assign a minimum of 20 pregnant animals per group. Beginning on day 6 of gestation and continuing daily through day 15 of gestation, the test substance **was** suspended in corn oil and administered by oral intubation (volume, 1 .O ml/kg) to the pregnant females at a single daily dose of 60, 190, and 600 mg/kg/day. A negative control (corn oil, 1 ml/kg/day) and positive control (aspirin, 250 mg/kg/day) were also employed. On day 20 of gestation, all females were killed by a 5-l 0 minute exposure to chloroform vapors. The uterine contents of each were removed and the reproductive performance recorded. The urogenital tract of each female was examined for normal morphology. Body weights of all females were

HUMAN HEALTH EFFECTS ELEMENTS:

DEVELOPMENTAL TOXICITY/TERATOGENICITY

recorded on days 0, 6, 11, 15, and 20 of gestation. All animals were observed daily for signs of toxicity and a record maintained. At the time of sacrifice on day 20, the following observations were recorded for each female: numbers of corpora lutea, implantation sites, resorption sites, live and dead fetuses, sex of fetuses, and body weights of fetuses.

At the time of uterine examination, all fetuses were examined grossly for the presence of external congenital abnormalities. One-third of the fetuses from each litter were randomly selected and placed in Bouin's solution for detailed visceral examination employing Wilson free-hand slicing technique. Any fetus showing external abnormalities was selected for examination by this technique. The remaining fetuses were eviscerated, fixed in 70% isopropyl alcohol, macerated in a 2% potassium hydroxide solution, stained with Alizarin-Red S dye, cleared in glycerine, and examined under low power magnification for skeletal anomalies and ossification variations. Each fetus was processed, examined and stored for possible further examination in a manner retaining the identity of both dam number and uterine position.

Results

Maternal Precision/NOAEL	>
Maternal NOAEL Dose	600
Unit Used	mg/kg-bw
Maternal NOAEL Effect	Mortality, body weight, pregnancy
Maternal Precision/LOAEL	>
Maternal LOAEL Dose	600
Unit Used	mg/kg-bw
Maternal LOAEL Effect	Mortality, body weight, pregnancy
Developmental Precision/NOAEL	>
Developmental NOAEL Dose	**600**
Unit Used	mg/kg-bw
Developmental NOAEL Effect	Skeletal or soft tissue abnormalities
Developmental Precision/NOAEL	>
Developmental LOAEL Dose	**600**
Unit Used	mg/kg-bw
Developmental LOAEL Effect	Sketetal or soft tissue abnormalities

Admin Record Number:

ROBUST SUMMARY

Styrene, p-methyl-

CAS# 622979 **Study Number: 1**

HUMAN HEALTH EFFECTS ELEMENTS:

DEVELOPMENTAL TOXICITY/TERATOGENICITY

Actual Dose	Not available
Maternal Data with Dose Level (with NOAEL value).	There were no differences in body weights, pregnancy, implantation, numbers of live or dead fetuses, or numbers of resorptions per dam at any test level.
Fetal Data with Dose Level (with NOAEL value).	An increase in litters with rudimentary ribs at 60 mglkglday and extra ribs at 190 mg/kg/day were observed. In addition, fetuses exhibited an increase in incomplete ossification of vertebrae.
Statistical Results	Statistical significance (see below) was observed at $p<0.05$ in several parameters in the positive controls and a few in the 60 and 190 mg/kg/day groups.
Results Remarks	There were no significant differences in pregnancy, implantation, numbers of live fetuses, numbers of dead fetuses, or numbers of resorptions per dam between any test level and negative control. The test substance showed no dose-related effects on reproductive performance or fetal weight. Females exhibited body weights and weight gains no different from the negative control animals throughout gestation. The abnormalities noted in fetuses whose dams received 60, 190, or 600 mg/kg/day were generally variations rather than malformations. The data showed an increase in litters with rudimentary ribs at 60 mg/kg/day and an increase in extra ribs at 190 mglkglday. In addition, fetuses exhibited an increase in the incomplete ossification of vertebrae at 60 and 190 mg/kg/day. However, fetuses from dams treated at 600 mglkglday showed skeletal effects no different from the vehicle control. No dose-related skeletal effects were attributed to treatment with the test substance. Soft tissue examinations revealed no significant differences in type or frequency of anomalies between the control and any test group.

Conclusions

When orally administered to pregnant Sprague-Dawley rats from day 6 through day 15 of gestation at levels of 0, 60, 190, and 600 mg/kg/day, MCTR-92-78 had no dose-related effects on reproduction, gestation, nor on skeletal or soft tissue anomalies of fetuses.

Data Quality

Reliability	Acceptable
Data Reliability Remarks	The key parameters (i.e., number of doses, animals, observations, etc.) were appropriate and described in the study.

Reference

Remarks	Teratologic Evaluation of MCTR-92-78 in Sprague-Dawley Rats, Food and Drug Research Laboratories, Inc. No. 5924, January 19, 1979. M0922-78.

General

Admin Record Number: **ROBUST SUMMARY**

Styrene, p-methyl-

CAS# 622979 **Study Number: 2**

HUMAN HEALTH EFFECTS ELEMENTS:

DEVELOPMENTAL TOXICITY/TERATOGENICITY

Test Substance

Remarks	p-methylstyrene Test Article ID#: MCR-302-79 (01188001) Purity: 100% assumed for dosage calculations Additives: None reported Carrier solvent: Olive oil (5 ml/kg) Contaminants: None reported Chemical formula: C9H10
Chemical Category	

Method

Method/Guideline Followed	Other
GLP	Yes
Year Study Performed	1980
Species	rat
Strain	CD-I
Sex	F
Number of Males per Dose	0
Number of Females per Dose	**25**
Route of Administration	Oral
Days of Gestation	6-19
Frequency of Treatment	once daily, 7 days/wk
Doses	50, 300, and 600 mg/kg/day
Control Group	**Yes**
Statistical Method	Chi-square and/or Fisher's exact for the number of litters with malformations; Mann-Whitney U-test for early/late resorptions; ANOVA with Dunnett's for viable fetuses, implantations, corpora lutea, body weights
Remarks for Method	Sexually mature, virgin female Charles River COBS CD rats (approximately 12 weeks old) were mated 1 :1 with males in sufficient numbers to assign a minimum of 25 pregnant rats to the treatment groups. Beginning on day 6 of gestation and continuing through day 19 of gestation, the test substance was suspended in olive oil and administered by oral gavage (volume of 5 ml/kg) to pregnant females at a single daily dose of 50, 300, and 600 mg/kg/day. A control group received the vehicle only on a comparable regimen at a volume of 5 ml/kg. Prior to treatment, the dams were observed daily for mortality and overt changes in appearance and behavior. They were observed for mortality and clinical signs of toxicity on days 6 through 20 of gestation. Individual maternal body weights were recorded on gestation days 0, 6, 9, 12, 16, and

HUMAN HEALTH EFFECTS ELEMENTS:

DEVELOPMENTAL TOXICITY/TERATOGENICITY

20. On gestation day 20, all females were sacrificed by carbon dioxide inhalation. Immediately following sacrifice, the abdominal cavity was opened to expose the uterus and ovaries. The uterus was excised and weighed prior to removal of the fetuses. The number and location of viable and nonviable fetuses, early and late resorptions and the number of total implantations and corpora lutea were recorded. The abdominal and thoracic cavities and organs of the females were grossly examined for pathological changes and the carcasses discarded. Uteri from females that appeared nongravid were opened and placed in a 10% ammonium sulfide for confirmation of pregnancy status.

All fetuses were individually weighed and examined for external malformations and variations, including the palate and eyes. Each fetus was externally sexed and individually numbered and tagged for identification. Approximately one-half of the fetuses were placed in Bouin's fixative for subsequent visceral examination by razor-blade sectioning. The remaining one-half of the fetuses were fixed in alcohol, macerated in potassium hydroxide and stained with Alizarin Red S for subsequent skeletal examination.

Results

Maternal Precision/NOAEL	>
Maternal NOAEL Dose	600
Unit Used	mg/kg-bw
Maternal NOAEL Effect	Mortality, pregnancy
Maternal Precision/LOAEL	>
Maternal LOAEL Dose	600
Unit Used	mglkg-bw
Maternal LOAEL Effect	Mortality, pregnancy
Developmental Precision/NOAEL	>
Developmental NOAEL Dose	600
Unit Used	mglkg-bw
Developmental NOAEL Effect	Skeletal or soft tissue abnormalities
Developmental Precision/NOAEL	>
Developmental LOAEL Dose	600
Unit Used	mglkg-bw
Developmental LOAEL Effect	Skeletal or soft tissue abnormalities

Admin Record Number: **ROBUST SUMMARY**

Styrene, p-methyl-

CAS# 622979 **Study Number: 2**

HUMAN HEALTH EFFECTS ELEMENTS:

DEVELOPMENTAL TOXICITY/TERATOGENICITY

Actual Dose

Not available

Maternal Data with Dose Level (with NOAEL value).

A dose-related reduction in mean maternal body weight gain was observed in all treated groups when compared to the control group. Apparently, these decreases were not statistically significant.

Fetal Data with Dose Level (with NOAEL value).

A statistically significant reduction in mean fetal body weight was observed in all treated groups compared to controls. Mean number of corpora lutea was significant at 600 mg/kg/day.

Statistical Results

Mean fetal body weight for all treatment groups significant at $p<0.05$ or $p<0.01$ (600 mglkglday), Mean number of corpora lutea for 600 mglkglday significant at $p<0.05$.

Results Remarks

No biologically meaningful differences in appearance or behavior were noted in any of the treatment groups. At necropsy all animals were found to be internally normal with the exception of two controls animals (yellow fluid in intestines, 2 mm hydrocele on the left oviduct) and one animal in 600 mg/kg/day (hydronephrosis). A dose-related reduction in mean body weight gain was noted over the entire treatment period for all treated groups compared to control. A similar pattern was also observed for the adjusted body weight gain (female weight exclusive of uterus and contents). Statistical significance was not reported.

A statistically significant reduction in mean fetal body weight was observed in all treated groups when compared with the control. However, the reductions may not be indicative of a true compound effect as the reported values for all treated groups exceeded the historical control value, and the test control value was unusually high. There was a statistically significant reduction in the mean number of corpora lutea in the 600 mglkglday group when compared with the control group. However, this was not considered treatment-related because ovulation and implantation occur prior to test article administration.

There were no biologically meaningful or statistically significant differences in the mean number of viable fetuses, early or late resorptions, postimplantation loss, total implantations or the fetal sex distribution in any of the treated groups when compared to the control group. The mean number of corpora lutea in the 50 and 300 mglkglday groups was also comparable to the control value. Nonviable fetuses were not observed in any of the study groups including the controls.

No malformations were observed in the control, 50 or 300 mg/kg/day groups. One malformation, meningocele, was observed in one fetus from one litter in the 600 mg/kg/day. The number of fetuses and litters with genetic or developmental variations in all treated groups was comparable to the control group.

Conclusions

A dose related reduction in mean maternal body weight gain was noted over the entire treatment period for all treated groups when compared with the control group. A similar pattern was observed for the adjusted body weight gain (female weight exclusive of uterus and contents). The most notable reduction in mean maternal body weight gain occurred in the 300 and 600 mg/kg/day groups during the first three days of the treatment period (gestation days 6 through 9) and the last four days of the study period

HUMAN HEALTH EFFECTS ELEMENTS:

DEVELOPMENTAL TOXICITY/TERATOGENICITY

(gestation days 16 through 20). However, these results are apparently not statistically significant since the report does not indicate statistical significance. A reduction of mean fetal body weight occurred in all treated groups when compared with the control group. However, the importance of this reduction is unclear since the mean fetal body weights of all groups including the control were above the average as reported by the mean historical control of this laboratory. The exceptionally high control value (outside the range of historical control) may have contributed to the statistical significance between treated and control groups.

Treatment with Sample-01 188001 (p-methylstyrene) did not produce a teratogenic response when administered orally to pregnant rats at a dosage level of 600 mg/kg/day or less.

Data Quality

Reliability Acceptable

Data Reliability Remarks All key parameters (i.e., doses, number of animals, observations, etc.) were appropriate and described in the study.

Reference

Remarks Teratology Study in Rats (MCTR-302-79), International Research and Development Corporation Study No.: 450-025, October 8, 1981. M3020-79.
Pilot Teratology Study in Rats (MCTR-309-79), International Research and Development Corporation Study No.: 450-024, January 14, 1981. M3090-79.

General

Pregnant Charles River COBS CD rats were used to establish dosage levels of Sample 01188001 for a teratology study. Dosage levels of 0, 50, 200, 400, 800, and 1200 mglkglday were administered orally by gavage as a single daily dose on days 6 through 19 of gestation, at a constant vehicle volume of 5 ml/kg. The control group received the vehicle only, olive oil, on a comparable regimen. Uterine examinations were performed on all surviving females on gestation day 20. Survival in the control, 50, 200, 400, and 800 mglkglday dosage group was 100%. One female in the 1200 mg/kg/day treatment group died on gestation day 10. A cause of death could not be determined at necropsy. There were no biologically meaningful differences in appearance and behavior attributable to treatment with Sample 01188001 in the 50, 200, 400, or 800 mg/kg/day treatment groups when compared to the control group. Increases in yellow staining around the mouth and anogenital region and excessive salivation were observed in a few rats in the 1200 mg/kg/day dosage group. A slight decrease in mean maternal body weight gain in the 50 and 200 mglkglday treatment groups, a moderate decrease in the 400 and 800 mg/kg/day dosage groups and a severe reduction in the 1200 mg/kg/day group were noted. These effects were dose related. When compared to the control group, there were no biologically meaningful differences in mean uterine examination values in any of the Sample 01188001 treatment groups.

Based on these results, a dosage level of 800 mg/kg/day would be considered excessive for a teratology study in rats with Sample 01188001.

This pilot study was acceptable for determining doses for a teratology study.

Admin Record Number: **ROBUST SUMMARY**

Styrene, p-methyl-

CAS# 622979 **Study Number: 3**

HUMAN HEALTH EFFECTS ELEMENTS:

DEVELOPMENTAL TOXICITY/TERATOGENICITY

Test Substance

Remarks	p-methylstyrene Test Article ID#: MCTR-303-79 (01188001) Purity: 97% Additives: None reported Carrier solvent: None Contaminants: None reported Chemical formula: C9H10
Chemical Category	

Method

Method/Guideline Followed	Other
GLP	**Yes**
Year Study Performed	1980
Species	rabbit
Strain	Dutch
Sex	F
Number of Males per Dose	**0**
Number of Females per Dose	16
Route of Administration	Oral
Days of Gestation	6-27
Frequency of Treatment	once daily, 7 days/wk
Doses	50, 100, and 150 mg/kg/day
Control Group	**Yes**
Statistical Method	Chi-square test and/or Fisher's exact test for male to female fetal sex distribution and number of litters with malformations, Mann-Whitney U-test for early and late resorptions and postimplantation loss, ANOVA with Dunnett's test for other endpoints
Remarks for Method	Sexually mature virgin female Dutch Belted rabbits (6 months of age, 2.194 to 2.970 kg) were inseminated and ovulation was induced by an injection of chorionic gonadotropin. Prior to insemination, females were randomly assigned to one control group and three treatment groups consisting of 16 rabbits each. The test substance was dispensed daily and administered undiluted orally by gavage, as a single daily dose. The test article was administered under a ventilation hood at dosage levels of 50, 100, and 150 mg/kg/day at total dosage volumes of 0.056, 0.112, and 0.169 ml/kg, respectively. The test article administration began on day 6 and continued up to and including day 27 of gestation. The control group received distilled water only on a comparable regimen at a total dosage volume equivalent to that of the highest dosage group (0.169 ml/kg).

HUMAN HEALTH EFFECTS ELEMENTS:

DEVELOPMENTAL TOXICITY/TERATOGENICITY

Prior to treatment, the females were observed daily for mortality and overt changes in appearance and behavior. The females were observed daily for mortality and clinical signs of toxicity on days 6 through 28 of gestation. A gross necropsy was performed on all rabbits not surviving to the scheduled sacrifice in an attempt to determine the cause of death. Tissues were preserved in 10% neutral buffered formalin only as deemed necessary by gross findings. The fetuses from these dams were examined externally and preserved in 10% neutral buffered formalin. Individual maternal body weights were recorded on gestation days 0, 6, 12, 18, 24, and 28.

On gestation day 28, all surviving females were sacrificed by injection of an overdose of sodium pentobarbital. Immediately following sacrifice, the uterus was exised and weighed and the fetuses were removed. The number and location of viable and nonviable fetuses, early and late resorptions and the number of total implantations and corpora lutea were recorded. The abdominal and thoracic cavities and organs of the dams were examined for grossly evident morphological changes and the carcasses discarded. Uteri from females that appeared nongravid were opened and placed in a 10% ammonium sulfide solution for confirmation of pregnancy status.

All fetuses were individually weighed and examined for external malformations and variations, including the palate and eyes. Each fetus was dissected, internally sexed and examined for visceral malformations and variations, including the brain by a mid-coronal slice. The eviscerated, skinned fetuses were fixed in alcohol, macerated in potassium hydroxide and stained with Alizarin Red S for subsequent skeletal examination. Fetal findings were classified as malformations or genetic or developmental variations.

Results

Maternal Precision/NOAEL	>
Maternal NOAEL Dose	150
Unit Used	mg/kg-bw
Maternal NOAEL Effect	Reproductive parameters
Maternal Precision/LOAEL	>
Maternal LOAEL Dose	150
Unit Used	mg/kg-bw
Maternal LOAEL Effect	Reproductive parameters
Developmental Precision/NOAEL	>
Developmental NOAEL Dose	150
Unit Used	mg/kg-bw
Developmental NOAEL Effect	Skeletal or soft tissue abnormalities

Admin Record Number:

ROBUST SUMMARY

Styrene, p-methyl-

CAS# 622979 **Study Number: 3**

HUMAN HEALTH EFFECTS ELEMENTS:

DEVELOPMENTAL TOXICITY/TERATOGENICITY

Developmental Precision/NOAEL	>
Developmental LOAEL Dose	150
Unit Used	mg/kg-bw
Developmental LOAEL Effect	Skeletal or soft tissue abnormalities
Actual Dose	Not available
Maternal Data with Dose Level (with NOAEL value).	None observed
Fetal Data with Dose Level (with NOAEL value).	None observed
Statistical Results	None
Results Remarks	There were no biologically meaningful differences in mean maternal body weight gain in any of the treated groups when compared to controls. A slight reduction in mean maternal body weight gain was noted in the 100 mg/kg/day group. However, this was considered due to random occurrence as no corresponding trend was noted in the 150 mg/kg/day group. There were no biologically meaningful differences in the mean numbers of corpora lutea, total implantations, early or late resorptions, postimplantation loss, viable fetuses, the fetal sex distribution or mean fetal body weight in any treatment groups compared to controls. A slight reduction in the mean number of total implantations with a corresponding decrease in the mean number of viable fetuses was observed at the 150 mg/kg/day level. These reductions were probably the result of a decrease in the mean number of corpora lutea which was also seen at this level. However, this was not considered treatment-related as ovulation occurred prior to test article administration. Nonviable fetuses were not observed in any of the study groups. There were no biologically meaningful or statistically significant differences in the number of litters with malformations in any treatment group compared to controls. No malformations were observed in the 150 mg/kg/day group. In addition, there were no biologically meaningful differences in the number of fetuses (and litters) with genetic or developmental variations in any treatment group compared to controls.
Conclusions	Ten rabbits died prior to scheduled sacrifice between days 9 and 21 of gestation. An intubation error was cited as the probable cause of death of four rabbits; one each in the 50 and 150 mg/kg/day group and two in the 100 mg/kg/day group. Pneumonia was established as the cause of death for five animals; two each in the 50 and 100 mg/kg/day groups and one in the 150 mg/kg/day group. One animal at the 100 mg/kg/day group died without apparent cause. Treatment with Sample 01188001 (p-methylstyrene) did not produce a teratogenic response when administered to pregnant rabbits at a dosage level of 150 mg/kg/day or less.

Admin Record Number: **ROBUST SUMMARY**

Styrene, p-methyl-

CAS# 622979 **Study Number: 3**

HUMAN HEALTH EFFECTS ELEMENTS:

DEVELOPMENTAL TOXICITY/TERATOGENICITY

Data Quality

Reliability Acceptable

Data Reliability Remarks All key parameters (i.e., number of doses, animals, observations, etc.) were appropriate and described in the study.

Reference

Remarks Teratology Study in Rabbits (MCTR-303-79), international Research and Development Corporation Study No.: 450-029, January 6, 1982. M3030-79.
Pilot Teratology Study in Rabbits (MCTR31 1-79), International Research and Development Corporation Study No.: 450-028, January 21, 1981. M3110-79.

General

A pilot study was conducted to determine the dosage levels **for** the teratology study. The methods were similar to those previously described, except 5 animals were used per treatment group. Pregnant Dutch Belted rabbits were orally dosed as a single daily dose on days 6 through 27 of gestation. The doses were 50, 200, 400, 800, and 1200 mg/kg/day. Survival in the control and 50 mg/kg/day was 100%. All rabbits in the 800 and 1200 mg/kg/day groups died between gestation 6 and 11. In the 400 mglkglday group, three rabbits died between gestation days 7 and 17 and one rabbit in the 200 mg/kg/day died on gestation day 19. There were no biologicaly meaningful differences in appearance or behavior or mean maternal body weights in the 50 mg/kg/day. A reduction in the amount of fecal material beneath the cages of a few rabbits in 200 and 400 mg/kg/day groups was observed. At postmortem examination, erosions of the stomach mucosa were observed in **a few** rabbits in 200 and 400 mg/kg/day and in a majority of rabbits in 800 and 1200 mg/kg/day. A mean maternal body weight loss occurred in the 200 mglkglday group during the first six days of treatment. However, weight gains were comparable to the control group over the entire treatment period. Prior to death, maternal body weight losses were noted in **a** majority of rabbits in 400 mglkglday. Mean uterine examination values for all treatment groups were comparable to the controls. There were no biologically meaningful differences in the mean numbers of corpora lutea, total implantations, postimplantation loss, early resorptions or viable fetuses in any treatment groups compared to controls. Nonviable fetuses and late resorptions were not observed in any group.

Based on these results, a dosage level of 200 mg/kg/day would be considered excessive for **a** teratology study in rabbits with the test substance.

This pilot study was acceptable for determining doses for a teratology study.

Admin Record Number: 20113044B ROBUST SUMMARY

Styrene, p-methyl-

CAS# 622979 Study Number: 1

HUMAN HEALTH EFFECTS ELEMENTS:

REPEATED DOSE TOXICITY

Test Substance

Remarks	p-methylstyrene Test Article ID#: MCTR-144-79 Purity: A value of 99.7% was assumed for dosage calculations Additives: None reported Carrier solvent: Olive oil (1 ml/kg) Contaminants: None reported Chemical formula: C9H10
Chemical Category	

Method

Method/Guideline Followed	Other
GLP	Yes
Year Study Performed	1980
Species	rat
Strain	Fischer 344
Sex	Both
Number of Males per Dose	15
Number of Females per Dose	15
Route of Administration	Oral
Exposure Period	90
Frequency of Treatment	once daily, 7 dayslwk
Doses	50, 100, 300,700, and 1500 mg/kg
Control Group	Yes
Post Observation Period	Terminated after 13 weeks of dosing
Statistical Method	ANOVA with Scheffe's or Games and Howell's test
Remarks for Method	The test substance was suspended in olive oil and administered once daily by oral gavage for 90 consecutive days to male and female rats (Fisher-344; approximately 6 weeks old). The concentrations were 50, 100, 300, 700, and 1500 mg/kg and the volume of olive oil administered to animals was 1 ml/kg. A vehicle control (1 ml/kg) was also employed. All rats were observed for mortality and moribundity twice daily, and body weight, food consumption, and signs of toxic and pharmacologic effects were recorded weekly during the study. Clinical laboratory studies were performed on all surviving animals at week 14 at the time of terminal sacrifice. Blood samples for hematology and clinical chemistry determinations were collected from the orbital sinuses of animals. Urine samples were collected by housing the rats individually

HUMAN HEALTH EFFECTS ELEMENTS:

REPEATED DOSE TOXICITY

overnight in metabolism cages with water ad libitum.

Necropsies were performed on all animals found dead or sacrificed moribund during the study. After thirteen weeks of treatment all surviving animals were sacrificed, necropsied, and gross observations recorded. Organ weights were also obtained. The following preserved tissues from all animals were examined microscopically: lungs, liver, kidneys, testes or ovaries, and prostate or uterus.

All statistical analyses were evaluated at the 5.0% probability level.

Results

NOAEL Precision	=
NOAEL Dose	300
Unit	mglkg-bw
NOAEL Effect	Decrease in body weight, organ histopathology
LOAEL Precision	=
LOAEL Dose	700
Unit	mglkg-bw
LOAEL Effect	Decrease in body weight, organ histopathology
Actual Dose Received by Dose Level by Sex	Not available
Toxic Response	The survival rate of the 1500 mg/kg/day group was significantly lower than controls. A significantly lower growth rate through week 13 was observed in 700 and 1500 mg/kg/day. A significant increase in mean hematocrit and erythrocyte values was observed in 1500 mg/kg/day. Several significant differences in various organ weights were observed in 700 and 1500 mglkglday. Compound-related histopathology was observed in lung sections from animals treated at 700 and 1500 mg/kg/day.
Statistical Results	All statistical significance was observed at $p<0.05$.
Results Remarks	The survival rate of males and females in 1500 mg/kg/day were signigicantly lower than that of controls. Total deaths were as follows: 0130, 0130, 0/30, 1130, 3/30, and 19/30 for control, 50, 100, 300, 700, and 1500 mg/kg/day, respectively. A significantly lower body weight was observed in 100,700 and 1500 mg/kg/day in males at week 13 and in females in 700 mg/kg/day at initiation. The mean body weights in the male treated groups were consistently lower than those of the control throughout the study, and the growth rate through week 13 for 700 and 1500 mg/kg/day was significantly lower than that of the controls. No treatment-related clinical signs were apparent during the study. No significant differences in food consumption in the treated groups were noted.

HUMAN HEALTH EFFECTS ELEMENTS:

REPEATED DOSE TOXICITY

Although several statistically significant differences were noted in hematocrit, erythrocyte counts, blood urea nitrogen, total protein and albumin, all of these values were within acceptable laboratory limits. The results of urinalyses were generally unremarkable.

No apparent compound-related gross pathology findings were noted. The following organ weight alterations were considered the result of compound administration at 700 and 1500 mg/kg/day: increase in relative brain weight (male); increase in relative and decrease in absolute heart, kidney, and testes weight (male); increase in relative liver weight (male, and at 300 mg/kg/day); increase in relative and absolute liver weight (female, and at 300 mg/kg/day); and increase in relative kidney weight (female).

Compound-related histopathology was observed in lung sections from treated animals. At 700 and 1500 mg/kg/day groups, there was evidence of severe irritation of the bronchial and bronchiolar epithelium. An addition compound effect in all treatment groups was the exacerbation of multifocal chronic pneumonitis and focal hyperplasia of bronchial and bronchiolar epithelium. These lesions were primarily limited to animals receiving 700 or 1500 mg/kg/day.

Conclusions

The NOAEL and LOAEL for male and female rats orally dosed for 13 weeks with MCTR-144-79 was not reported in the study. However, it appeared to be 300 and 700 mg/kg/day, respectively. These values are based on significant differences in body weights and organ histopathology. In the high dose group (1500 mg/kg/day), 63% mortality was observed.

Data Quality

Reliability Acceptable

Data Reliability Remarks All key parameters (i.e., doses, number of animals, observations, etc.) were appropriate and described in the study.

Reference

Remarks

Subchronic Toxicity Study in Rats, MCTR-144-79. Hazleton Laboratories Project No. 230-217, July 25, 1980. M1440-79.
Fourteen-day Repeated Dose Study in Male and Female Rats, Hazleton Laboratories Project No. 230-218, November 28, 1979. M1441-79.
Chronic Toxicity of PMS in Rats, Mobile Environmental and Health Science Laboratory, Study No.: 2151-80, July 16, 1984.

General

MCTR-144-79 was evaluated in a fourteen-day repeated dose (by oral gavage) study in male and female rats at the dose levels of 200, 560, 920, 1280, 1640, and 2000 mg/kg of body weights. This study was designed to obtain mortality data in order to establish the high dose to be used in the 90-day oral gavage study. Nine of ten animals in 2000 mg/kg died within five days of dosing. In the five remaining groups, all but two animals survived the 14-day observation period. The incidence of clinical observations increased from 200 to 1640 in a dose-related fashion. Clinical observations included depression, rough coat, urine stains, soft feces, red stains on nose and/or eyes, hunching, and thinness. A NOAEL was not reported in the study, but based on the clinical observations, it appears to be 200 mg/kg/day.

Admin Record Number: 20113044B **ROBUST SUMMARY**

Styrene, p-methyl-

CAS# 622979 **Study Number: 1**

HUMAN HEALTH EFFECTS ELEMENTS:

REPEATED DOSE TOXICITY

A chronic toxicity study was conducted. PMS was administered by oral gavage, as solutions in olive oil, to 3 groups of 60 male and 60 female weanling Fischer 344 rats for 16 months, at doses of 67, 200, and 600 mg/kg/day. Another group of 60 males and 60 females, dosed with only olive oil, served as controls. Each rat received 0.5 ml/kg of olive oil daily, with or without PMS. The assessment of the toxic effects of PMS was based on clinical observations, body weights, food consumption, hematologic, serum chemical and enzymologic measurements, urine analyses, organ weights, and gross and microscopic evaluation of organs/tissues. The administration of PMS at a dose of 600 mg/kg/day resulted in increased mortality, decreased body weights, and, predominately in females, increased liver weights. At a dose of 200 mg/kg/day, there were increased mortality, decreased body weight in males, and, in females, increased liver weight. During the progress of the 12 month scheduled sacrifice, projections (nodules) on the craniad surface of the liver were seen in some rats, distributed in both sexes in all experimental groups including the controls. Neither the cause(s) nor the possible biological fate and role of these liver nodules could be determined, although the test material clearly was not a factor in their development. Because of the high incidence of this confounding factor in the primary target organ for PMS toxicity, it was decided to terminate the study at 16 months.

Based on the evaluation of all the test parameters, the NOAEL in this study was 67 mg/kg/day.

This study is acceptable based on the description of appropriate key parameters.

Admin Record Number: 20113044B ROBUST SUMMARY

Styrene, p-methyl-

CAS# 622979 Study Number: 2

HUMAN HEALTH EFFECTS ELEMENTS:

REPEATED DOSE TOXICITY

Test Substance

Remarks	p-methylstyrene Test Article ID#: 02298001 Purity: p-MS 97%, m-MS 3% Additives: None reported Carrier solvent: None Contaminants: None reported Chemical formula: C9H10
Chemical Category	

Method

Method/Guideline Followed	Other
GLP	Unknown
Year Study Performed	1980
Species	rat
Strain	Sprague-Dawtey
Sex	Both
Number of Males per Dose	15
Number of Females per Dose	15
Route of Administration	Inhalation
Exposure Period	90
Frequency of Treatment	6 hr/wk, 5 day/wk
Doses	100,500, 1600 (reduced to 1300 in week 7) ppm
Control Group	Yes
Post Observation Period	Terminated after 13 weeks of dosing
Statistical Method	ANOVA, Dunnett's test or Kruskal-Walks, Dunn
Remarks for Method	Male and female Sprague-Dawley rats (approximately 48 days old) were exposed six hours per day, five days per week for 13 weeks at target concentrations of 100, 500, and 1600 ppm (reduced to 1300 ppm in week 7 due to excess mortality). Measured concentrations were 101, 505, 1583 ppm (reduced to 1313 after 27 exposures), respectively. The exposure concentrations were generated by flash evaporation of the test material. The stainless steel and glass chamber in which the animals were exposed had a total volume of one cubic meter with an effective exposure volume of 760 liters. They were operated dynamically at an air flow rate of 190 liters/minute. This flow rate provided one complete air change every 5.3 minutes and a 99% equilibrium time of 24.2 minutes. At least three samples were drawn from each

HUMAN HEALTH EFFECTS ELEMENTS:

REPEATED DOSE TOXICITY

exposure chamber each day.

There were 15 male and female animals per dose. A control group of rats exposed to air alone was also employed. Hematology, clinical chemistry, and urinalysis parameters were measured for five animals/sex/group during week 5 and all surviving animals during week 13. Ophthalmoscopic examinations were performed pretest and at termination. Gross post mortem examinations were performed and tissues saved for all animals. At the time of necropsy on sacrificed animals, the following organ weight/body weight ratios were calculated: gonads (ovaries or testicles, with epididymides, paired), kidneys (separately), liver and lungs.

Results

NOAEL Precision	=
NOAEL Dose	505
Unit	ppm(air)
NOAEL Effect	Mortality, body weights, physical signs, hematology and clinical chemistry
LOAEL Precision	=
LOAEL Dose	1313
Unit	ppm(air)
LOAEL Effect	Mortality, body weights, physical signs, hematology and clinical chemistry
Actual Dose Received by Dose Level by Sex	101, 505, 1583 (reduced to 1313 after 27 exposures
Toxic Response	No significant effects were observed at 100 ppm. Observations of the rats during the study revealed a slight (but not statistically significant) increase in physical signs (excessive lacrimation, chromodactyorrhea) for 500 ppm. There were lower hematocrit values for males at 500 ppm. At 1600 ppm, excess mortality was observed. In addition, marked increase in physical signs, decreased in body weights and hematocrit values, decreases in mean liver and ovarian weights, and increases in serum glutamate pyruvate transaminase and alkaline phosphatase activities were observed.
Statistical Results	Several parameters were statistically significant in 1600 ppm at $p<0.01$ or $p<0.05$.
Results Remarks	Based on infrared analysis, the animals in 100 ppm and 500 ppm nominal concentrations, received exposure to cumulative mean concentrations of 101 and 505 ppm, respectively. Animals in 1600 ppm were exposed to a cumulative mean concentration of 1583 for 27 exposure days. At this time, due to mortality and overt responses to treatment, exposures were terminated for a five day period and exposures at a nominal concentration of 1300 ppm (measured 1313 ppm) were recommenced and continued until termination. During the first week of exposure, two female rats in 1600 ppm died and were replaced

HUMAN HEALTH EFFECTS ELEMENTS:

REPEATED DOSE TOXICITY

with those of equivalent weight from the same shipment. Subsequently, three other female rats and a male from 1600 ppm died on days 8, 16, 88, and 36, respectively and were not replaced. These six deaths were attributed to treatment.

Observations revealed a slight increase in the number of signs for 500 ppm including excessive lacrimation, chromodacryorrhea, yellow ano-genital staining, and swollen eyes/eyelids. Animals in 1600 ppm exhibited a marked increase in the above signs and additional signs of matted fur, increased activity, uncoordinated body movement, general body tremors, loss of balance, slow righting reflex, lack of support, prostration, and hunched appearance. These signs were most prominant during the first four to five weeks while the animals were exposed to 1600 ppm. Following reduction of the level to 1300, these signs decreased or abated with the exception of matted fur.

Mean body weights for 1600 ppm males were significantly decreased ($p<0.01$) on weeks 1 through 7, and significantly decreased ($p<0.05$) on weeks 8 and 9. The reduced mean body weights were more marked during the 1600 ppm exposure. The body weight increments showed evidence of normalization following reduction of exposure concentration to 1300 ppm.

The interim and terminal hematology examinations revealed the mean total leukocyte values for 1600 ppm females to be significantly elevated ($p<0.05$) compared to controls. There were no similar findings in males. At termination, the mean hematocrit values for males in 500 and 1300 were lower than controls ($p<0.01$). This small reduction is of dubious toxicological significance. Mean serum glutamate pyruvate transaminase (both sexes) and alkaline phosphatase (females) activities were increased at 1600 ppm after five weeks. The GPT were comparable to controls at termination. Slight reductions in blood glucose at the high level (both sexes) were seen after five weeks and at termination although only in females at termination did this difference from controls attain statistical significance.

The mean absolute and relative liver and ovarian weights in 1600 ppm females were significantly elevated ($p<0.01$) compared to controls. In males at this level, the absolute liver weights were comparable to controls although the relative weights were slightly higher (8%) than controls. The absolute and relative liver weights in females at 100 ppm were lower than controls ($p<0.05$). This is not considered treatment related.

No ocular abnormalities were observed which were related to the exposure of the test substance. No macroscopic or microscopic lesions were detected that was related to the test substance.

Conclusions

Six rats died (1 male, 5 females) at 1600 ppm during the first eight weeks of the study. This exposure was reduced to 1300 after 27 exposures. Because of this change, the LOAEC cannot be reliably estimated. However the NOAEL remains at 500 ppm. Body weight increments were decreased in males at the 1600 ppm but after reduction to 1300 ppm showed signs of normalization. Increased leukocyte numbers, serum glutamate pyruvate transaminase and alkaline phosphatase activities and reduced blood glucose were observed at 1300/1600 ppm.

Data Qualitv

Reliability Acceptable

Admin Record Number: 20113044B **ROBUST SUMMARY**

Styrene, p-methyl-

CAS# 622979 **Study Number: 2**

HUMAN HEALTH EFFECTS ELEMENTS:

REPEATED DOSE TOXICITY

Data Reliability Remarks All key parameters (i.e., methods of exposure, number of animals and observations) were appropriate and described in the study.

Reference

Remarks A 13-week Inhalation Toxicity Study of para-methylstyrene in the rat, Bio-dynamics Inc. Project No. 79-7327, November 11, 1980. 1 I-80
A two-week Inhalation Toxicity Study of MCTR-241-79 in the Rat, Bio-dynamics, Inc. Project No. 79-7348, June 30, 1980. M2410-79
Subacute Inhalation Study in Rats with MCTR-243-77, WIL Research Laboratories, Inc., Project Number: WIL-1060-77. M2431-77

General A two-week inhalation study (MCTR-241-79) in rats was conducted to find concentrations for a 13-week inhalation study. Test concentrations were 125, 250, 500, 1000, and 1600 ppm. During the exposure, rats in 1600 ppm showed increased grooming activity, ocular and oral secretions, respiratory abnormalities, prostration, yellow staining of the ano-genital area, neuromuscular impairment and in some cases, tremors. Less severe or frequent responses were seen in 1000 ppm. No abnormalites were detected at lower levels. Increased liver weights were observed in rats of both sexes in 1600 ppm and for males in 1000 ppm.

MCTR-243-77 was administered to ten male and ten female Sprague-Dawley rats for one hour per day, five days a week for two weeks. One group was exposed to an average of 35.6 ml/hr of the test substance; the average exposure level was 3470 ppm. Another group was exposed to an average of 32.8 ml/hr of the test substance; the average exposure level was 3200 ppm. Excessive secretion from the mouth and nose, trembling and jerking movements, shallow breathing and little or no response to stimuli were observed during or following exposures. However, a few hours after each exposure, the rats all appeared normal. The histopathological evaluation of tissues from all rats exposed to the test substance revealed no distinct or consistent treatment related changes. No treatment-related mortality was observed.

Admin Record Number: 20113044B **ROBUST SUMMARY**

Styrene, p-methyl-

CAS# 622979 **Study Number: 3**

HUMAN HEALTH EFFECTS ELEMENTS:

REPEATED DOSE TOXICITY

Test Substance

Remarks	p-methylstyrene Test Article ID#: MCTR-243-77 Purity: 100% assumed for dosage calculations Additives: None reported Carrier solvent: None Contaminants: None reported Chemical formula: C9H10
Chemical Category	

Method

Method/Guideline Followed	Other
GLP	Unknown
Year Study Performed	1977
Species	rabbit
Strain	New Zealand white
Sex	Both
Number of Males per Dose	3
Number of Females per Dose	3
Route of Administration	Dermal
Exposure Period	21
Frequency of Treatment	6 hrs/day, 7 days/wk
Doses	0.5 and 2.0 ml/kg
Control Group	Yes
Post Observation Period	Terminated after 21 days of dosing
Statistical Method	None
Remarks for Method	The test substance was applied to the skin of albino New Zealand rabbits (three males and three females; weighing from 2.05 kg to 2.55 kg) for 6 hours/day for a period of 21 consecutive days. The test concentrations were 0.5 and 2.0 ml/kg and distilled water (2 ml/kg) served as controls. An application site of approximately 25% of the total body surface was clipped free of hair 24 hours prior to the start of the study and once weekly thereafter to prevent an accumulation of test substance on the site and to permit observation for skin irritation. The rabbits were weighed once a week and the dosage adjusted accordingly. One rabbit per sex per dose level was abraded at the test site before each application. The test substance was measured and delivered from a glass syringe and spread evenly over the test site. Each rabbit was then placed

HUMAN HEALTH EFFECTS ELEMENTS:

REPEATED DOSE TOXICITY

in a Newman harness to prevent oral ingestion and returned to its cage for a six-hour exposure period. After the exposure period, the harness was removed and the area was rinsed with tepid water and thoroughly dried. The rabbits were observed daily for any signs of toxicity or changes in behavior. Skin irritation was scored before application and after rinsing each day.

The rabbits were bled from the ear artery with a needle and blood was collected for hematological evaluation and serum was collected for clinical chemistry. At the end of the 21-day period, all rabbits were weighed and sacrificied. A necropsy to observe gross lesions was performed and tissues were taken and evaluated histomorphologically.

Results

NOAEL Precision	c
NOAEL Dose	1
Unit	ml/kg
NOAEL Effect	Moderate to severe skin irritation, decrease in body weight and increases in white blood cell and neutrophile counts
LOAEL Precision	<
LOAEL Dose	1
Unit	ml/kg
LOAEL Effect	Moderate to severe skin irritation, decrease in body weight and increases in white blood cell and neutrophile counts
Actual Dose Received by Dose Level by Sex	Not available
Toxic Response	The test substance produced moderate to severe skin irritation at 0.5 and 2.0 ml/kg
Statistical Results	None reported
Results Remarks	No signs of systemic toxicity or changes in behavior attributable to the test substance were observed during the study and no rabbits died during the period of dermal application with the test substance. Skin irritation was observed in all rabbits dosed with 0.5 or 2.0 ml/kg. The irritation was first observed on day one of the study and was characterized as very slight etythema and edema in 0.5 ml/kg and coriaceous skin and atonia were observed in 2.0 ml/kg. The skin irritation was generally more severe in 2.0 ml/kg throughout the study and the irritation was usually slightly more severe when observed approximately six hours following application than during the 24-hour observation. Following application on the fifth day of the study, moderate to marked fissuring of the skin at the application site occurred in the 0.5 ml/kg group and marked fissuring was observed in all but one of the rabbits in the 2.0 ml/kg group. The irritation was generally observed to be moderate in 0.5 ml/kg and severe in 2.0 ml/kg during the final

HUMAN HEALTH EFFECTS ELEMENTS:

REPEATED DOSE TOXICITY

week of the study with marked coriaceous skin, fissuring and sloughing of the skin at the site of application in both groups.

There was a dose related reduction in body weight gain which was considered to be related to the pain and severe irritation at the site of test substance application. The skin irritation appeared to have very little effect on the hematology values with the exception of the elevated white blood cell counts observed in most rabbits in 2.0 ml/kg and a dose-related increase in segmented and non-segmented neutrophiles in both treatment groups which indicate an inflammatory process. The only effect on the clinical chemistry parameters was an elevation in SGOT which probably is related to the skin cell damage at the application site.

There was no evidence of systemic toxicity in the tissues observed microscopically. The prominent collecting tububles in the kidneys observed grossly were within the limits of normal for rabbits. The skin lesions observed from both treatments consisted of thickening of the epidermis, congestion, edema, and inflammatory cellular infiltration in the dermis, and serum exudation and necrotic cellular debris on the surface.

Conclusions

MCTR-243-77 produced no systemic toxicity or changes in behavior when applied topically to albino rabbits for 21 consecutive days at the dose levels of 0.5 or 2.0 ml/kg. The test material produced moderate to severe skin irritation which caused a reduction in body weight gain and elevations in the white blood cell and neutrophile counts. The purpose of this study was not to obtain a NOAEL or LOAEL.

Data Quality

Reliability Acceptable

Data Reliability Remarks All key parameters (i.e., doses, number of animals and observations) were appropriate and described in the study. The data were not analyzed statistically, but the observations of skin irritation were unambiguous.

Reference

Remarks Subchronic Percutaneous Toxicity Study in Rabbits (21-day) with MCTR-243-77, WIL Research Laboratories, Inc,. Project Number: WIL-1060-77. M2436-77.

General

Admin Record Number: 20113044B

ROBUST SUMMARY

Styrene, p-methyl-

CAS# 622979

Study Number: 4

HUMAN HEALTH EFFECTS ELEMENTS:

REPEATED DOSE TOXICITY

Test Substance

Remarks	p-methylstyrene Test Article ID#: MCTR-251-79 Purity: 100% assumed for dosage calculations Additives: None reported Carrier solvent: Olive oil (2.0 ml/kg/day) Contaminants: None reported Chemical formula: C9H10
Chemical Category	

Method

Method/Guideline Followed	Other
GLP	Yes
Year Study Performed	1979
Species	mouse
Strain	Charles River CFW
Sex	Both
Number of Males per Dose	10
Number of Females per Dose	10
Route of Administration	Oral
Exposure Period	28
Frequency of Treatment	once/day, 7 days/wk
Doses	250, 500, 1000, 2000, and 4000 mg/kg/day
Control Group	No
Post Observation Period	Terminated after 4 weeks of dosing
Statistical Method	ANOVA with Dunnett's multiple comparison tables
Remarks for Method	The test substance was suspended in olive oil and administered once daily for 28 days by oral intubation to male and female Charles River CFW mice (males, 24 to 29 g; females, 17 to 23 g) at concentrations of 250, 500, 1000, 2000, and 4000 mg/kg/day. fresh solutions were prepared weekly and the standard quantity of olive oil was 2.0 ml/kg/day. There were 10 male and 10 female rats per concentration. All animals were observed three times daily (Monday through Friday) and twice daily (weekends and holidays) for signs of overt toxicity, moribundity and mortality. Individual body weights were recorded weekly beginning with the pretest period. After 4 weeks of test substance administration, all surviving mice were sacrificed and

HUMAN HEALTH EFFECTS ELEMENTS:

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necropsied. At necropsy, organ weights were recorded for liver, kidneys, and brain. All mice which died during the course of the study were discarded.

Results

NOAEL Precision	<
NOAEL Dose	250
Unit	mg/kg-bw
NOAEL Effect	Clinical signs: reduced motor activity, labored breathing, reddish eyelids, and ventral abdomen masses
LOAEL Precision	<
LOAEL Dose	250
Unit	mg/kg-bw
LOAEL Effect	Clinical signs: reduced motor activity, labored breathing, reddish eyelids, and ventral abdomen masses
Actual Dose Received by Dose Level by Sex	Not available
Toxic Response	All animals died in 1000 to 4000 mg/kg/day within 24 hours following dosing. Two male animals died in 250 and 500 mg/kg/day and 5 female animals died in 500 mg/kg/day. Compound-related signs were observed at 250 and 500 mg/kg/day.
Statistical Results	Female liver weights at 500 mglkglday were significantly different from the low dosage group at p<0.01.
Results Remarks	Death occurred in most of the mica at 1000, 2000, and 4000 mglkglday within 24 hours after dosing. Observations just prior to death included reduced motor activity, labored breathing, loss of righting response, tremors, excessive salivation, hunched posture and prostration. Moist yellow substance around ventral neck and thorax was observed in most male and female mice at the 500 mglkglday and somewhat less frequently for male and female mice at 250 mglkglday. Compound related signs seen at 250 and 500 mg/kg/day included reduced motor activity, labored breathing, reddish eyelids and ventral abdomen masses. During week 1 of the study, deaths occurred in the lower dosage levels; 8/10 males in both 250 and 500 mg/kg/day and 5/10 females in 500 mg/kg/day. Group mean body weight gains for male and female mice at the 250 and 500 mglkglday were similar. No compound-related gross pathologic lesions were observed in any of the mice which were sacrificed at study termination. No biologically significant differences were noted in the comparison of the liver, kidney or brain weights when (in the absence of a control group) the 500 mg/kg/day group was compared to the 250 mglkglday group.

Conclusions

The test article, p-methylstyrene was toxic at all levels of administration. With mortality occurring among the low dose (250 mg/kg/day) males, as well as apparent signs of toxicity in both sexes, this level would be considered too high to be used for the high level in the 90-day study.

Admin Record Number: 20113044B

ROBUST SUMMARY

Styrene, p-methyl-

CAS# 622979

Study Number: 4

HUMAN HEALTH EFFECTS ELEMENTS:

REPEATED DOSE TOXICITY

Data Quality

Reliability Unacceptable

Data Reliability Remarks This study is not acceptable for establishing a 28-day repeated dose NOAEL or LOAEL. Clinical effects were noted at the low concentration. In addition, no controls were employed in the study. However, the purpose of this study was to determine a high dose for a 90-day study.

Reference

Remarks 4-Week Range-Finding Study in Mice, International Research and Development Corporation Project No.: 450-021, April 24, 1980. M3280-79.
Evaluation of Oncogenic Potential of Para-methylstyrene (PMS) in Mice, Mobil Environmental and Health Science Laboratory, Study No:291-80, July 13, 1984. 290-80.

General

An oncogenicity study of PMS was conducted in Swiss mice (approximately 6 weeks old). The test subsutance was suspended in olive oil and given to male and female mice (60 each per dose) once daily, 5 days/week for 83 weeks by oral gavage. The test concentrations were 10, 50, and 250 mglkglday, and a vehicle control (0.2 ml/100 g) was employed. Measurements of individual body weight, food and water consumption were performed weekly for the first 13 weeks and then bi-weekly. Observations for group behavior and mortality were done 3 times a day. A complete necropsy was performed on each animal and histopathology examination was carried out on all tissues and organs taken at necropsy, in the highest dose and control group. The experiment was conducted in compliance with GLPs, 43FR59986, December 22, 1978, and Proposed Chronic Health Effects Test Standards of the EPA, 44FR27334, May 9, 1979. Appropriate statistical tests, i.e., ANOVA, Duncan's, Fisher's exact test, were employed.

The purity of the test substance measured for four consecutive shipments was in excess of 99.75%. A high resolution GC analysis to identify contaminants revealed the presence of a variety of single ring aromatics, none present at a concentration greater than 0.067%.

No effects on survival, behavior or weight occurred among the animals of a 3-week range-finding test using dose rates of 10, 50 or 250 mg/kg/day.

In the oncogenic study, no significant effect on survival was observed. At 79 weeks, percent survival ranged from 81 to 87%, with no relationship between dose rate and response. In male mice, reduced survival in treated groups was first observed after approximately 4 months of dosing. Maximal differences (-25%) between treated and control survival rates occurred during the second year. However, dose-response relations appeared to be complex. High mortality levels occurred in the 10 mg/kg/day group. Based on the mortality data alone, a clear picture of the dose-survival relationship does not emerge for male mice. Based on other considerations, it was concluded that the reduced mortality in mice mice was the result of interaction between treatment, a naturally occurring disease (primarily amyloidosis) and perhaps other factors as well.

In females, body weight gain was depressed at 250 mg/kg/day; no effects on weight

HUMAN HEALTH EFFECTS ELEMENTS:

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gain were observed at 50 mg/kg/day or less. In males, there was no effect of treatment on body weight.

No specific or unique neoplasm arose as a result of treatment. Hyperplasias and tumors, benign and malignant, were observed in hemolymphoreticular tissues, lung, liver, mammary gland and sporadically in other tissues. The incidence of the individual neoplasms were within the ranges historically observed in Swiss mice. Considering the tumor types individually, there ware no oncologically significant differences between treated and control groups in either sex. Considered in the aggregate, there were no increases in either the total numbers of tumors or the proportions of tumor-bearing animals in treated animals or either sex.

It is concluded, therefore, that ingestion of PMS at rates of 250 mg/kg/day or less did not induce the formation of neoplasms, nor did it influence the natural rates of neoplasia in Swiss mice.

The NOAEL of PMS, after lifetime ingestion by female Swiss mice, is 50 mg/kg/day. The lifetime ingestion of PMS by male Swiss mice at rates of IO-250 mg/kg/day appears to reduce survival via complex interaction with disease(s) found naturally in the population.

This study was acceptable based on an appropriate test design.

Admin Record Number: 20113044B **ROBUST SUMMARY**

Styrene, p-methyl-

CAS# 622979 **Study Number: 5**

HUMAN HEALTH EFFECTS ELEMENTS:

REPEATED DOSE TOXICITY

Test Substance

Remarks	p-methylstryene Test Article ID#: MCTR-92-78 Purity: Unknown Additives: None reported Carrier solvent: None Contaminants: None reported Chemical formula: C9H1 0
Chemical Category	

Method

Method/Guideline Followed	Other
GLP	Yes
Year Study Performed	1978
Species	rat
Strain	Sprague-Dawley
Sex	Both
Number of Males per Dose	15
Number of Females per Dose	15
Route of Administration	Oral
Exposure Period	90
Frequency of Treatment	once daily, 5 dayslwk
Doses	0.1, 0.3, and 0.6 ml/kg
Control Group	Yes
Post Observation Period	Terminated on the morning after the 64th dose
Statistical Method	Student's t-test
Remarks for Method	The test substance was administered daily, 5 days/week for 90 days by oral gavage to male and female Sprague-Dawley CD rats (15 each per concentration) at concentrations of 0.1, 0.3, and 0.6 ml/kg. A control group was administered 0.6 ml/kg of distilled water. Individual body weights were taken initially and weekly thereafter. All animals were observed for clinical signs and mortality daily. On the morning after the 64th dose (90 days after study initiation), the animals were sacrificed and gross necropsied. Tissues were preserved for histology. Blood and urine was obtained on days 0, 30, 60 and 90 for hematology, clinical chemistry and urinalysis.

Results

Admin Record Number: 20113044B

ROBUST SUMMARY

Styrene, p-methyl-

CAS# 622979

Study Number: 5

HUMAN HEALTH EFFECTS ELEMENTS:

REPEATED DOSE TOXICITY

NOAEL Precision	<
NOAEL Dose	0
Unit	ml/kg
NOAEL Effect	Changes in several organ weights
LOAEL Precision	<
LOAEL Dose	0
Unit	ml/kg
LOAEL Effect	Changes in several organ weights
Actual Dose Received by Dose Level by Sex	Not available
Toxic Response	There were significant decreases in body weights in 0.3 (male) and 0.6 (male and female) ml/kg. Significant changes in organ weights were observed at all test concentrations.
Statistical Results	All statistical significance noted below was at $p<0.05$.
Results Remarks	Only 2/30 mortalities were observed in each treatment group. Significant decreases ($p<0.05$) in body weight ware observed for males at 0.3 ml/kg and both males and females at 0.6 ml/kg. In general, control and test group food consumption were similar. All groups were within normal limits for hematology values. No trends in blood chemistry were observed for the treatment groups compared to controls. There were no consistent differences in urinalysis data between test groups and controls. With the exception of the first day of dosing, all test animals exhibited increased motor activity or salivation or both immediately after dosing. These were not seen on days when no dosing occurred (weekends). The only consistent change upon necropsy was an area of excoriation near the side of the mouth where the dosing needle was inserted. Significant organ weights were as follows: 0.1 ml/kg; female heart (increased), group liver (increased), female spleen (increased), female and group kidneys (increased), female adrenals (decreased), 0.3 ml/kg; female heart (increased), group heart (increased), female and group liver (increased), female and group spleen (increased), female and group kidneys (increased), and male adrenals (increased), 0.6 ml/kg; female heart, liver, spleen and kidneys (increased), male kidneys (decreased).
Conclusions	Test article MCTR-29-78 was administered by oral gavage for 5 days per week over a 90 day period to rats at levels of 0.1, 0.3, and 0.6 ml/kg. Overt symptoms of toxicity were primarily increased motor activity and increased salivation. The test article caused a bald area around the mouth due to physical contact with the test material. The males exhibited a dose-dependent decrease in weight gain without a decrease in food consumption. There were no obvious compound related effects on hematology, urinalysis, or blood chemistry values. Histopathologically, the only compound related lesion was the excoriation around the mouth and, when it occurred, direct-contact damage to the lungs. There were changes in several organ weights in all treatment

HUMAN HEALTH EFFECTS ELEMENTS:

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groups. The NOAEL and LOAEL were not reported but was below 0.1 ml/kg, the lowest concentration tested.

Data Quality

Reliability Unacceptable

Data Reliability Remarks There was no description of the purity or specific gravity of the test substance. The dosage was reported as "ml/kg" and not "mg/kg" making comparison with other toxicity data difficult. Significant changes were seen in organ weights at the lowest concentration and the NOAEL and LOAEL could not be determined.

Reference

Remarks 90-Day Gavage Toxicity Study of MCTR-92-78 in Rats, Springborn, Project No. 3046.22. M0921-78.
Evaluation of Chronic Toxicity and Oncogenic Potential of para-Methylstyrene (PMS) in Rats, Bologna Institute of Oncology, Study No.: BT106bis and BT106. 41-80 and 43-80.

General

An oncogenicity study of PMS was conducted in Sprague-Dawley rats (approximately 6 weeks old). The test substance was suspended in olive oil and given to male and female rats once daily, 5 days/week for 108 weeks by oral gavage. There were 30 males/group and 30 females/group in the chronic study and 60 males/group and 60 females/group in the oncogenic study (except 90 males and females/group were used in the high dose). The test concentrations for the chronic study were 10, 50, and 500 mg/kg/day. The test concentrations for the oncogenic study were 10, 50, 250, and 500 mg/kg/day. A vehicle control (2.0 ml/kg/day) was also employed. Measurements of individual body weight, food and water consumption were performed weekly for the first 13 weeks and bi-weekly thereafter. Observations for group behavior and mortality were done 3 times a day. Blood samples for limited hematological and serum chemistry analyses and samples for urine analysis were collected at 26 and 78 weeks of the study, from the first 10 surviving animals in numerical order per sex per group. Additional parameters were measured at 54 and 107 weeks. A complete necropsy was performed on each animal and histopathological examinations was carried out on all tissues and organs taken at necropsy, in the highest dose and control group. The experiments were conducted in compliance with GLPs, 43FR59986, December 22, 1978, and Proposed Chronic Health Effects Test Standards of the EPA, 44FR27334, May 9, 1979. Appropriate statistical tests, i.e., ANOVA, Duncan's, Kruskal-Wallis test, were employed.

The purity of the test substance measured for four consecutive shipments was in excess of 99.75%. A high resolution GC analysis to identify contaminants revealed the presence of a variety of single ring aromatics, none present at a concentration greater than 0.067%.

No deaths occurred among the animals in a range-finding study. At an oral gavage dose rate of 250 mg/kg/day for 21 days, PMS did not produce effects on the survival, behavior or weight of the animals.

Concurrent chronic toxicity (CT) and oncogenicity (ONCO) studies were conducted. The CT study, conducted at dose levels of 0,10, 50, and 250 mg/kg/day, was

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terminated at 108 weeks. The ONCO study was conducted at dose rates of 0, 10, 50, 250, and 500 mg/kg/day, and was designed such that certain data from the high dose group would be intercomparable with the CT study. Dosage in the ONCO study was terminated at 108 weeks; the study was terminated at 122 weeks.

Survival was reduced in males at 250 mg/kg/day in the CT study, and in males at 250 and 500 mglkglday in the ONCO study. In both studies, differences between treated and control groups were readily discernible at 6 months, and reached a maximum difference of -20% during the second year of treatment. Thereafter, survival rates tended to converge. Female survival was not affected by treatment in either study. Body weights, body weight gain, food consumption and water consumption were not affected by treatment in either the CT or ONCO study.

Chemical analyses of three serum enzymes (SGOT, SGPT and AP) were conducted at 26, 54, 78, and 107 weeks, as were hematologic evaluations of red blood cell and white blood ceil parameters. Statistically significant differences between treated and control groups were sporadic, and of no toxicological significance. A variety of standard blood chemical analyses and plasma clotting factors were evaluated at 54 and 107 weeks. Statistically significant differences between treated and control groups were randomly distributed and were interpreted as stochastic variations in the data.

No lesions were associated with the increased mortality observed at dose rates of 250 and 500 mg/kg/day in male rats. There was no evidence of any specific organ or system toxicity resulting from the ingestion of PMS at dose rates of 500 mg/kg/day or less, in males and females.

No specific or unique neoplasm arose as a result of PMS treatment. Hyperplasias and tumors, benign and malignant, were observed in almost all system and organs in the test population as a whole. Greater number of neoplasms were observed in the mammary gland, pituitary, adrenal, uterus and hemo-reticular tissues. Lesser numbers appeared in the pancreas, forestomach, Zymbal gland, brain, subcutis and other sites. The incidence of the neoplasms were within ranges commonly observed in the Sprague-Dawley rat. Taking the tumor types individually, there were no toxicologically significant differences between treated and control groups. Taken in aggregate, there were no treatment-related increases in either the total number of tumors or the proportion of tumor-bearing animals in each group.

In conclusion, the ingestion of para-methylstyrene at rates of 500 mg/kg/day or less did not include the formation of neoplasms, nor did it influence the natural rates of neoplasia in Sprague-Dawley rats.

The NOAEL of PMS, after lifetime ingestion by Sprague-Dawley rats, occur at dose rates of 50 mglkglday in males and 500 mg/kg/day in females.

The study was acceptable based on an appropriate test design.

Admin Record Number: 20113044B ROBUST SUMMARY

Styrene, p-methyl-

CAS# 622979

Study Number: 6

HUMAN HEALTH EFFECTS ELEMENTS:

REPEATED DOSE TOXICITY

Test Substance

Remarks	p-methylstryene Test Article ID#: 11218001 Purity: 100% assumed for dosage calculations Additives: None reported Carrier solvent: gelatin capsules Contaminants: None reported Chemical formula: C9H10
Chemical Category	

Method

Method/Guideline Followed	Other
GLP	Yes
Year Study Performed	1981
Species	dog
Strain	Beagle
Sex	Both
Number of Males per Dose	2
Number of Females per Dose	2
Route of Administration	Oral
Exposure Period	28
Frequency of Treatment	once daily, 7 days/wk
Doses	30, 100, 300, and 1000 mg/kg/day
Control Group	Yes
Post Observation Period	Terminated after 28 days of dosing
Statistical Method	ANOVA with Scheffe's multiple pairwise comparison
Remarks for Method	The purpose of the one-month study was to determine the maximum dose tolerated by capsule administration in male and female beagle dogs and to provide data to be used to set doses for a long-term study. The test material was drawn into a glass syringe and immediately placed into a one-fourth ounce gelatin capsules and administered once daily for 28 days to two male and two female beagle dogs (approximately 4 months of age) at concentrations of 30, 100, 300, and 1000 mglkglday. The volume to be dosed was based on individual body weights and a density of 0.89 mg/ml. A control group received one empty capsule/dog/day. The capsules were administered one-half hour after removal of the food.

Admin Record Number: 20113044B **ROBUST SUMMARY**

Styrene, p-methyl-

CAS# 622979 **Study Number: 6**

HUMAN HEALTH EFFECTS ELEMENTS:

REPEATED DOSE TOXICITY

All animals were observed once daily for mortality or moribundity, and once daily for appearance, behavior, excretory function, and discharges. Clinical signs were recorded twice daily, food consumption was recorded daily beginning two weeks prior to treatment, and body weights were recorded weekly beginning nineteen days prior to treatment. Several hematology, clinical chemistry and urinalysis endpoints were measured in all animals at initiation and week 4. Following 28-days of treatment, all animals were weighed and sacrificed and gross necropsies were performed. At the time of sacrifice, the following organs from each animal were weighed: heart, lung, liver, kidneys, adrenals, thyroid/parathyroids and gonads. Tissues were prepared for histological examination.

Results

NOAEL Precision	=
NOAEL Dose	100
Unit	mg/kg-bw
NOAEL Effect	Clinical signs - tremors
LOAEL Precision	=
LOAEL Dose	300
Unit	mg/kg-bw
LOAEL Effect	Clinical signs - tremors
Actual Dose Received by Dose Level by Sex	Not available
Toxic Response	Apparent treatment-related changes in clinical observations consisted of tremors observed primarily in 300 and 1000 mg/kg/day. Body weights were slighly lower in 1000 mg/kg/day.
Statistical Results	Statistical significance ($p<0.05$) was observed in a few clinical chemistry data and the relative liver weight at the high dose.
Results Remarks	There were no deaths among the test animals during the 28-day period. Treatment-related clinical observations consisted of tremors, beginning on day 1 in one or both males in 1000 mglkglday for 25 out of 28 days, and in one or both females in 1000 mg/kg/day for 22 our of 28 days. From days 9-28, tremors were observed on 13 days in 300 mg/kg/day males and on 11 days in females. There were sporadic incidences of tremors in 30 and 100 mg/kg/day. Changes in stool were noted primarily in the treated groups. No statistically significant changes were noted in the mean body weights or body weight changes; however, the high dose group gained less weight than controls. No apparent compound-related trends were noted in daily food consumption Analysis of hematology data revealed no statistically significant differences. Although still within normal limits, slight decreases were noted in the erythrocyte count,

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hemoglobin and hematocrit in 1000 mg/kg/day. Mean SGOT values were significantly lower in 300 and 1000 mg/kg/day at week 3. These findings, as well as the remaining clinical chemistry data were within normal limits and did not appear to be compound related.

Analysis of mean terminal body weights, organ weights, and organ/body weight ratios revealed a slight increase in the absolute and relative liver weight of males and females in 1000 mglkglday (significant in the relative weight). Also, the testes weight of one male in 30, 100, and 1000 mg/kg/day and both males in 300 mglkglday was noted to be about one-third the testes weight of males in the control group.

Conclusions

Apparent treatment-related changes in clinical observations consisted of tremors observed primarily in male and females at 300 and 1000 mg/kg/day. Body weights and body weight changes were slightly lower in males and females at 1000 mglkglday. Although slight decreases were noted in erythrocyte count, hemoglobin and hematocrit in males and females in 1000 mg/kg/day and in SGOT levels in male and females in 300 and 1000 mg/kg/day, all these values were within normal limits. Alterations in relative and absolute organ weights were noted for livers in the males and females in 1000 mglkglday and testes in males. No treatment-related trends were noted in food consumption, gross pathology or urinalysis.

The NOAEL and LOAEL were not reported. However, based on the clinical findings of tremors, the NOAEL is 100 mg/kg/day and the LOAEL is 300 mg/kg/day.

Data Quality

Reliability Acceptable

Data Reliability Remarks All key parameters (i.e., doses, observations, exposure period) was appropriate and described in the study.

Reference

Remarks One-Month Oral Toxicity Study in Dogs with PMS, Mobil Environmental and Health Science Laboratory Project No.: 230-230, September 3, 1981.
Twelve-Month Oral (Capsule) Toxicity Study of p-Methylstyrene (PMS) in Dogs, Mobile Environmental Health Science Laboratory Project No.: 2142-80, May 28, 1985. M2142-80.

General

PMS was administered orally (by capsule) to groups of 6 male and 6 female beagle dogs at doses of 62.5, 250, and 1000 mg/kg/day for 12 months. An additional group of 6 males and 6 females served as controls; they received empty capsules daily. The high-dose animals exhibited decreased body weight gain, and a generally poor appearance; the mid-dose dogs also had slightly decreased weight gains. Tremors were also observed in males and females at 1000 mg/kg/day, mainly during weeks 14 and 15; however, neurologic and neuropathologic examinations at the termination of the study revealed no differences between the controls and exposed animals. Slight adverse effects were observed in the liver of the high-dose animals; these included slight decreases in the levels of serum total protein and albumin, a slight increase in the liver weight and a slight retention of bile pigment in the liver cells and canaliculi, but no increase in serum bilirubin. The mid-dose animals had slightly increased liver weight-to-body weight ratios, which were judged not to be of toxicological significance.

Admin Record Number: 20113044B ROBUST SUMMARY

Styrene, p-methyl-

CAS# 622979 Study Number: 6

HUMAN HEALTH EFFECTS ELEMENTS:

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There was a consistent, slight effect throughout the study on the red blood cells of the high-dose animals. After 13 weeks of treatment, the number of red blood cells, the hemoglobin concentration, and the hematocrit of the dogs treated at 1000 mg/kg/day were slightly below the normal range for control dogs; throughout the remainder of the study, there were no changes indicative of a worsening of the effect. When the hematologic values were analyzed by Dunnet's test after appropriate correction for baseline values, there were no significant differences between the control and 250 or 1000 mg/kg/day group.

The NOAEL was 62.5 mg/kg/day. This study is acceptable based on an appropriate test design.

Admin Record Number:

ROBUST SUMMARY

Styrene, p-methyl-

CAS# 622979

Study Number: 1

HUMAN HEALTH EFFECTS ELEMENTS:

TOXICITY TO REPRODUCTION

Test Substance

Remarks	p-methylstyrene Test Article ID#: p-methylstyrene Purity: 100% assumed for dosage calculations Addivites: None reported Solvent carrier: Olive oil (1 .O ml/kg) Contaminants: None reported Chemical formula: C9H10
Chemical Category	

Method

Method/Guideline Followed	Other
Test Type	Two generation study
GLP	**Yes**
Year Study Performed	1980
Species	rat
Strain	CD-I
Sex	Both
Number of Males per Dose	**25**
Number of Females per Dose	**25**
Route of Administration	Oral
Days of Gestation	404
Frequency of Treatment	once daily, 7 dayslwk
Doses	25, 200, 500, and 600 mg/kg/day
Control Group	**Yes**
Premating Exposure Period for Female	P, 14 weeks; F1, 17 weeks
Premating Exposure Period for Male	P, 14 weeks; F1, 17 weeks
Statistical Method	ANOVA, Kruskal-Wallis test
Remarks for Method	P-methylstyrene (PMS) was administered by daily oral gavage in olive oil (volume of 1 .0 ml/kg) to male and female Sprague-Dawley CD rats for 2 generations at daily doses of 25, 200, and 500 mglkglday. PMS was administered to another group of rats at 600 mglkglday for 1 generation (500 mg/kg/day plus controls were added when excess mortality was seen in 600 mg/kg/day). Another group of rats which received olive oil

HUMAN HEALTH EFFECTS ELEMENTS:

TOXICITY TO REPRODUCTION

without PMS for 2 generations served as controls. In the P generation (FO), rats were 5 weeks old at the initiation of dosing (males 45 to 65 grams, females 40 to 60 grams). The number of males and females in controls and 500 mg/kg/day were 30 each, and the number of males and females in 25, 200, and 600 mglkglday were 25. After 14 weeks of dosing of P rats, males and females were placed in cohabitation, one male per each female. The rats continued to receive PMS during cohabitation, gestation, delivery, and lactation. At weaning, 5 male and 5 female pups from each treatment group were selected for gross necropsy; after fixation and processing, tissues from the pups from the control and 500 mg/kg/day were examined microscopically. Forty male and forty female Fl weanling pups from each treatment group were selected for continued treatment with PMS (20 males and 20 females were obtained for controls). Treatment groups were administered PMS for 17 weeks (25,200, and 500 mglkglday) before cohabitation, during cohabitation, gestation, delivery, and lactation. At weaning, 5 male and 5 female F2 pups from each litter were selected for gross necropsy; after fixation and processing, tissues from the pups from the control and 500 mglkglday were examined microscopically. F1 and F2 generation litters were examined as soon as possible after natural delivery. Pup viability and body weights of live pups were recorded at birth (day 1) and when pups were 4, 7, 14, and 21 days old. Litters were evaluated for maternal and pup behavior during the lactation period, when pup body weights were recorded. Ten adult males and 25 adult females killed by design from each dose were also necropsied, plus animals found dead, and tissues from the control and 500 mg/kg/day were examined microscopically. The rats in all treatment groups were observed daily for mortality and clinical signs of toxicity; body weight and food consumption were measured weekly.

Results

Parental Precision/NOAE	=
Parental NOAEL Dose	200
Parental NUnit Used	mg/kg-bw
Parental NOAEL Effect Assessed	Mortality
Parental Precision/LOAEL	=
Parental LUnit Used	mglkg-bw
Parental LOAEL Dose	500
Parental LOAEL Effect Assessed	Mortality
F1 Precision/NOAEL	=
F1 NOAEL Dose	200
F1 NUnit Used	mglkg-bw
F1 NOAEL Effect Assesse	Mortality
Fl Precision/LOAEL	=

Admin Record Number: **ROBUST SUMMARY**

Styrene, p-methyl-

CAS# 622979 **Study Number: 1**

HUMAN HEALTH EFFECTS ELEMENTS:

TOXICITY TO REPRODUCTION

F1 LOAEL Dose	500
F1 LUnit Used	mg/kg-bw
F1 LOAEL Effect Assesse	Mortality
F2 Precision/NOAEL	>
F2 NOAEL Dose	500
F2 NUnit Used	mg/kg-bw
F2 NOAEL Effect Assesse	Mortility or reproductive parameters
F 2 Precision/LOAEL	>
F2 LOAEL Dose	500
F2 LUnit Used	mglkg-bw
F2 LOAEL Effect Assesse	Mortality or reproductive parameters
Actual Dose Received by Dose Level by Sex	25.5, 196.0, 508.0, and 887.2 mg/ml
Parental/ F1 Data	Deaths were 1/80, 2/80, 4/80, and 27/80 for control, 25, 200, and 500 mglkglday.
Offspring Data	Administration of 500 mg/kg/day to F1 generation male and female rats resulted in a small increase (not significant) in the incidence of stillborn pups and in a slight increase in pup mortality.
Statistical Results	All statistically significant results (below) were at p<0.05.
Results Remarks	Mortality in the Fo generation for male rats was as follows: 2/54, 0/25, 2/25, 10/30, and 12/25 for control, 25, 200, 500, and 600 mg/kg/day, respectively. Mortality in the Fo generation for female rats was as follows: 2/55, 1/25, 3/27, 7/30, and 14/25 for control, 25, 200, 500, and 600 mglkglday, respectively. Physical signs observed in Fo generation rats in the 25, 200, 500, and 600 mg/kg/day dosage groups and attributed to treatment with PMS generally occurred in a dosage-related pattern and included excess salivation and hyperactivity/vocalization. Excess urination during handling, "tip-toe" walk, chromorrhinorrhea/nasal discharge and pilo-erection also occurred in a dosage-related pattern in male rats in these four PMS-treated groups, and in female rats in the 200, 500 and 600 mg/kg/day dosage groups. Rales/dyspnea and urine-stained abdominal fur were considered dose-related in 500 and 600 mg/kg/day dosage group rats. During the 14 week precohabitation period, dose-related inhibition of average body weight gain was observed in Fo generation male and female rats administered 200, 500, and 600 mg/kg/day, compared with controls. The effect was slightly more severe in male than in female rats and gradually disappeared in rats in the 200 and 500 mg/kg/day groups. Average weekly body weights in rats administered 25 mglkglday were comparable to controls during the first 14 weeks. During the postcohabitation period in Fo generation male rats, inhibition of body weight gain was observed in 500 and 600 mg/kg/day groups, compared to controls. Dose-

HUMAN HEALTH EFFECTS ELEMENTS:

TOXICITY TO REPRODUCTION

related inhibition of average body weight gain during gestation and lactation occurred in Fo generation female rats administered 600 mglkglday, compared to controls.

During the precohabitation period, dose-related inhibition of average weekly food consumption occurred in Fo generation male rats administered 500 and 600 mg/kg/day, compared with controls. Later in the study, male rats administered 200 mg/kg/day, and female rats administered 600 mg/kg/day had significantly higher average weekly food consumption values than controls; the biological importance of this observation is not presently known. Average weekly food consumption of rats in the 25 mg/kg/day group was comparable to vehicle controls.

During the first week of the postcohabitation period, Fo generation male rats in 200, 500, and 600 mg/kg/day consumed more food than controls. This effect disappeared after week 1 and may have been associated with the return of the rats from cohabitation to individual housing.

During gestation in Fo generation female rats, decreased average food consumption was observed in 500 and 600 mg/kg/day, compared with controls. The effect gradually disappeared. During the lactation period, a minimal decrease in average food consumption was observed in 500 and 600 mg/kg/day rats, compared with controls.

Compared with controls, treatment of Fo generation male and female rats with 600 mglkglday resulted in a decrease in the average number of liveborn pups/litter, and with 500 and 600 mg/kg/day in a small increase in pup mortality and decrease in average pup body weight per litter. Postweaning and during the period of PMS-administered evaluation, PMS was considered the cause of death of male and female rats in the 200 and 500 mg/kg/day groups.

A summary of mating and fertility data for the Fo generation is as follows (presented for control I, control II, 25, 200, 500, and 600 mglkglday, respectively): Fertility Index (%, pregnant/total); 63.6, 93.1, 88.0, 91.7, 90.9, and 86.7. Duration of gestation (mean days), 23.5, 23.0, 23.6, 23.2, 23.2, and 23.2. Gestation index (live litters day 1, %), 85.7, 96.3, 95.4, 100, 89.5, and 91.7. Implantations (mean), 11.4, 12.9, 11.6, 12.0, 12.2, and 11 .O.

Mortality in the male F1 generation was as follows: 0/40, 0/40, 1/40, and 19/40 for controls, 25, 200, and 500 mg/kg/day, respectively. Mortality in the female Fl generation was as follows: 1140, 2/40, 3/40, and 8/40 for controls, 25, 200, and 500 mg/kg/day, respectively. Physical signs observed in Fl generation rats were similar to those observed in the Fo generation rats. Dose-related signs observed generally occurred in a dose-related pattern. Signs observed in male and female rats which were considered related to administration of 25, 200, and 500 mglkglday, compared with controls, included excess salivation, hyperactivity/vocalization, excess urination during handling, “tip-toe” walk, chromorrhinorrhea/nasal discharge and hypersensitivity to touch. Male rats in all dose groups had pilo-erection and ungroomed coat; in female rats this sign was associated only with the 500 mg/kg/day group. Thin appearance in male rats administered 200 and 500 mg/kg/day, and in female rats administered 25, 200, and 500 mg/kg/day was attributed to the test substance. Urine-stained abdominal fur in 500 mglkglday group male rats, and in 200 and 500 mg/kg/day group female rats was also considered dose-related. Decreased motor activity and rales/dyspnea in both male and female rats were considered effects of the 500 mglkglday group.

HUMAN HEALTH EFFECTS ELEMENTS:

TOXICITY TO REPRODUCTION

During the 17-week precohabitation period, dose-related inhibition of average body weight gain was observed in F1 generation male rats in 25, 200, and 500 mg/kg/day, and in F1 generation female rats in 500 mg/kg/day, as compared with controls. F1 generation female rats in 25 mg/kg/day had slightly smaller average body weight gains than controls. This observation was not attributed to the test substance as a dose-response was not observed. Administration of 200 mg/kg/day, compared with controls, frequently resulted in greater average weekly body weight gains in F1 generation female rats. The biological significance of this observation is currently unknown.

Dose-related inhibition of average body weight gain persisted during the postcohabitation period, for F1 generation male rats in the 25, 200, and 500 mg/kg/day groups, compared with controls.

For F1 generation female rats, dose-related lower average body weights persisted for the 500 mg/kg/day group, compared with controls, during the gestation, lactation, and postweaning periods.

During the precohabitation period, weeks 1 through 17 of the study, and the postcohabitation period, weeks 21 through 26 of the study, dose-related decrease in average weekly food consumption occurred in 500 mg/kg/day group F1 generation male rats, as compared with controls. In F1 generation female rats in 25 and 200 mg/kg/day, average weekly food consumption was increased, as compared with controls, during the precohabitation and gestation period. The observations disappeared during the lactation period. Average weekly food consumption of 500 mg/kg/day group female rats was similar to controls during the precohabitation, gestation, and lactation periods.

Administration of 500 mg/kg/day to F1 generation male and female rats, compared to controls, resulted in a small increase in the incidence of stillborn pups and in a slight increase in pup mortality. Differences were not significant at $p<0.0.5$.

Treatment of F1 generation rats with 25,200, and 500 mg/kg/day, compared to controls, did not result in alteration of the percentage of rats which mated, the number of days required to mate, the duration of gestation, the fertility index, the gestation index, pup mortality per litter, pup sex ratio or pup average body weight per litter.

A summary of mating and fertility data for F1 generation is as follows (presented as control I, control II, 25, 200, and 500 mg/kg/day, respectively); Fertility Index (pregnant/total, %), 88.9, 84.2, 91.4, 86.5, and 83.9. Duration of gestation (mean days), 22.2, 22.1, 22.2, 22.4, and 22.4. Gestation Index (live litters day 1, %), 100, 100, 100, 100, and 92.3. Implantations (mean), 11.0, 12.2, 10.9, 10.4, 10.5.

A summary of the pathology report is as follows:
Dosing observations, gross necropsy findings, and extensive microscopic examination all support the conclusion that many deaths were caused by aspiration or intubation accidents. From the microscopic evaluation it is certain that nearly all animals in the F1 high-level group (possibly all PMS groups) were unintentionally subjected to periodic (perhaps frequent) small aspirations of PMS/oil, with rapid or overnight death following the aspiration or intubation of a larger amount.

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Essentially all the tracheobranchial lesions are regarded as part of one spectrum of changes induced by unintended periodic local exposure of the tracheobronchial mucosa to small amounts of PMS/olive oil, the exposure occurring via repetitive tiny aspirations or intubation of test material. Once developed, some primary lesions caused secondary changes: 1) mucus retention was accentuated by the simultaneous loss of cilia in the upper bronchi and trachea, and hypertrophy/hyperplasia of mucous cells in the lower bronchi; 2) segmental occlusion/obliteration of bronchioles was associated with an increased incidence of areas of atelectasis; and 3) all primary changes may have contributed to the development of a fatal severity of acute bronchitis and bronchopneumonia in seven of the animals found dead.

Except for the local effects on trachea and lungs of unintended repetitive aspiration/intubation of test article, there was no morphologic evidence of any effect of PMS/olive oil in Fo and F1 adult rats dosed daily by gavage at a level of 500 mg/kg/day. In F1 and F2 weanling pups, there was no morphological evidence of any effect of PMS. Histopathologic examinations revealed no neoplastic or preneoplastic changes.

Specifically, morphologic examinations of Fo adults (gross only) and F1 adults and F1 and F2 pups (gross and microscopic) revealed no evidence of any treatment effect on the organs of reproduction.

Conclusions

When PMS was administered to rats at a dose of 600 mg/kg/day, there were adverse effects on both the parents (increased mortality, reduced weight gain, excitability) and on the pups (slightly smaller litter size, slightly more mortality during the first 4 days after birth, and slightly less weight gain). When PMS was administered at 500 mg/kg/day, similar adverse effects (mortality, reduced weight gain, excitability) were seen in the parents, but less pronounced effects on pups (slightly smaller litter size and slightly more mortality during the first 4 days after birth in the first generation only). When PMS was administered at 200 mg/kg/day, there were no effects on the success of reproduction or health of offspring, and only a slight effect on the behavior of the parents (excitability).

There was no effect of treatment with PMS on the viability of pups from dams dosed at 25 or at 200 mg/kg/day. In addition, there was no effect on the mating, fertility, gestation, delivery of pups, or lactation index (survival to weaning of pups alive at day 4). The growth rate of pups from dams dosed at 25, 200, or 500 mg/kg/day was not different from the pups from the control dams. Pups from dams dosed at 600 mg/kg/day showed a 10% reduction in body weight, both at birth and at weaning, as compared to the controls.

The effects observed in the offspring of the rats dosed at 500 or 600 mg/kg/day probably resulted from maternal toxicity, not from effects of PMS directly on the reproductive process. It is concluded that at doses which are not maternally toxic, PMS does not interfere with the reproductive process in rats.

Data Quality

Reliability Acceptable

Data Reliability Remarks The key parameters (doses, number of animals, observations, etc.) were appropriate and described in the study.

Admin Record Number:

ROBUST SUMMARY

Styrene, p-methyl-

CAS# 622979

Study Number: 1

HUMAN HEALTH EFFECTS ELEMENTS:

TOXICITY TO REPRODUCTION

Reference

Remarks

Reproductive Effects of p-Methylstyrene Administered Orally Via Gavage to Crl:COBSCD (SD)BR Rats for Two Generations, Mobil Environmental and Health Science Laboratory Study No: 2160-80, July 16, 1984. M2161-80.

General

Admin Record Number: **ROBUST SUMMARY**

Styrene, p-methyl-

CAS# 622979 **Study Number: 1**

HUMAN HEALTH EFFECTS ELEMENTS:

TOXICITY IN VITRO (GENE MUTATIONS)

Test Substance

Remark	p-methylstyrene Test Article ID#: MCTR-243-77 Purity: 100% assumed for dosage calculations Additives: None reported Solvent carrier: Dimethylsulfoxide (DMSO) Contaminants: None reported Chemical formula: C9H10
Chemical Category	

Method

Method/Guideline Followed	Other
Test Type	Ames test
System of Testing	Bacterial
GLP	Unknown
Year study performed	1977
Species	Salmonella typhimurium
Metabolic Activation	A 9,000 x g supernatant prepared from Sprague-Dawley adult male rat liver induced by Aroclor 1254
Concentration	0.001, 0.01, 0.1, 1.0, and 5.0 microliters/plate
Statistical Method	Computer program used was not referenced
Remarks for Method	The test design was a standard Ames overlay method. Approximately xl 0.E8 cells from an overnight culture of five indicator strains each of Salmonella typhimurium (strains TA-1535, TA-1537, TA-1538, TA-98, and TA-100) were added to separate test tubes containing 2.0 ml of molten agar supplemented with biotin and a trace of histidine. For both nonactivation and activation assays, 0.001, 0.01, 0.1, 1 .0, and 5.0 microliter per plate were poured over the surfaces of the selective agar plates (1 plate per dose level). In the activation assays, an aliqout of reaction mixture (0.5 ml containing the 9,000 x g liver homogenate) was added to each of the activation overlay tubes (prior to test substance administration), which were then mixed, and the contents poured over the surface of the minimal agar plate and allowed to solidify. The plates were incubated for 48 hours at 37 degrees C, and scored for the number of colonies growing on each plate. Chemicals used for positive controls are as follows: Nonactivation assay; methylnitrosoguanidine (used for TA-1535 and TA-100, 10 micrograms/plate, solvent was water or saline), 2-nitrofluorene (used for TA-1538 and TA-98, 100 micrograms/plate, solvent was DMSO), quinacrine mustard (used for TA-1537, 10 micrograms/plate, solvent was water or saline). Activation assay; 2-anthramine (used for

HUMAN HEALTH EFFECTS ELEMENTS:

TOXICITY IN VITRO (GENE MUTATIONS)

TA-1535 and TA-100, 100 micrograms/plate, solvent was DMSO), 2-acetylaminofluorene (used for TA-1538 and TA-98, 100 micrograms/plate, solvent was DMSO), 8-aminoquinoline (used for TA-1537, 100 micrograms/plate, solvent was DMSO). The solvent control for both nonactivation and activation assays was DMSO (50 micrograms/plate).

The numbers of colonies on each plate were counted, recorded and analyzed in a computer program. The results are presented as revertants per plate for each indicator strain employed in the assay. The positive and the solvent controls are provided as reference points.

Because the test substance and the cells are incubated in the overlay for 2 to 3 days, and a few cell divisions occur during the incubation period, the test is semi-quantitative in nature. The criteria used to determine positive effects are inherently subjective and are based primarily on a historical data base. Most data sets are evaluated using the following criteria: Strains TA-1535, TA-1537, and TA-1538--If the solvent control value is within the normal range, a chemical that produces a positive dose response over three concentrations with the lowest increase equal to twice the solvent control value is considered to be mutagenic; Strains TA-98, TA-1 00--If the solvent control value is within the normal range, a chemical that produces a positive dose response over three concentrations with the highest increase equal to twice the solvent control value for TA-100 and two to three times the solvent control value for strains TA-98 is considered to be mutagenic. For these strains, the dose response increase should start at approximately the solvent control value.

Results

Result	Negative
Cytotoxic Concentration	The compound was toxic to the strain TA-1537 at 5 microliter dose level (without activation)
Genotoxic Effects	With metabolic activation
Statistical results	All results were negative
Results Remarks	The number of revertants per plate for each assay is as follows (all results will be presented in the following sequence, solvent control, positive control, 0.001, 0.01, 0.1, 1 .0, and 5.0 microliters): Nonactivation; TA-1535, 14, >1000, 12, >1000, 23, 29, and 8; TA-1537, 15, >1000, 14, 10, 11, 15, and 0; TA-1538, 16, >1000, 24, 30, 17, 15, and 13; TA-98, 44, 938, 43, 34, 25, 34, and 19; TA-100, 89, >1000, 128, 119, 132, 125, and 41. Activation; TA-1535, 15, 303, 25, 17, 12, 15, and 9; TA-1537, 16, 167, 13, 4, 11, 9, and 3; TA-1538, 27, 904, 29, 26, 26, 28, and 12; TA-98, 49, 767, 21, 33, 45, 34, and 19; TA-100,107, >1000, 104, 66, 57, 57, and 58.

Conclusions

The test compound, MCTR-243-77, did not demonstrate mutagenic activity in any of the assays conducted in this evaluation and was considered not mutagenic under these test conditions.

Admin Record Number: **ROBUST SUMMARY**

Styrene, p-methyl-

CAS# 622979 **Study Number: 1**

HUMAN HEALTH EFFECTS ELEMENTS:

TOXICITY IN VITRO (GENE MUTATIONS)

Data Qualitv

Reliability Acceptable

Data Reliability Remarks The key parameters (i.e., dose levels, strains, listing of appropriate negative and positive controls) are appropriate and described in the study.

Reference

Remarks Mutagenicity Evaluation of MCTR-243-77, Litten Bionetics, Inc. Project No. 2683, September 1977, M2437-77.
Ames et al., Mutation Research 31:347, 1975.

General

Saccharomyces cerevisiae, strain D4, was also used as the indicator microorganism in this assay. The test design was identical to that previously described. The results (revertants per plate) are as follows: Nonactivation; 24, 302, 49, 26, 27, 29, and 0 for solvent control (DMSO, 50 microliters/plate), positive control (methylnitrosoguanidine in water or saline, 10 micrograms/plate), 0.001, 0.01, 0.1, 1 .0, and 5.0 microliters, respectively. Activation; 41, 168, 59, 55, 46, 48, and 16 for solvent control (DMSO, 50 microliters/plate), postive control (DMNA, 100 micromoles/plate), 0.001, 0.01, 0.1, 1 .0, and 5.0 microliters, respectively.

The test compound did not demonstrate mutagenic activity in these assays under these test conditions.

Admin Record Number: **ROBUST SUMMARY**

Styrene, p-methyl-

CAS# 622979

Study Number: 2

HUMAN HEALTH EFFECTS ELEMENTS:

TOXICITY IN VITRO (GENE MUTATIONS)

Test Substance

Remark	p-methylstyrene Test Article ID#: MCTR-243-77 Purity: 100% assumed for dosage calculations Additives: None reported Carrier solvent: Dimethylsulfoxide (DMSO; 1%) Contaminants: None reported Chemical formula: C9H10
Chemical Category	

Method

Method/Guideline Followed	Other
Test Type	Mouse lymphoma assay
System of Testing	Non-bacterial
GLP	Unknown
Year study performed	1977
Species	L5178Y Mouse Lymphoma cells
Metabolic Activation	A 9,000 x g supernatant prepared from adult male mice livers (not induced)
Concentration	0.02, 0.03, 0.04, 0.06, and 0.08 microliters/ml
Statistical Method	Not reported
Remarks for Method	The purpose of this study was to evaluate MCTR-243-77 for specific locus forward mutation induction in the L5178Y Thymidine Kinase (TK) mouse lymphoma cell assay. Cells were maintained in Fischer's Medium for Leukemic Cells of Mice with 10% horse serum and sodium pyruvate. Cloning medium consisted of Fischer's medium with 20% horse serum, sodium pyruvate, and 0.37% agar. Selection medium was made from cloning medium by the addition of 5.0 mg of bromodeoxyuridine (BUdR) to 100 ml of cloning medium. Dimethylsulfoxide (DMSO) was used as the carrier solvent. Growth medium without the addition of solvent was used as the negative control. Ethylmethanesulfonate (EMS) was dissolved in culture medium and used as a positive control for the nonactivation studies at a final concentration of 0.5 microliters/ml. Dimethylnitrosamine (DMN) was used as a positive control for the activation studies at a final concentration of 0.5 microliters/ml. The solubility, toxicity, and doses for all chemicals were determined prior to screening. The concentrations used in the assays were 0.02, 0.03, 0.04 0.06, and 0.08 microliters/ml.

HUMAN HEALTH EFFECTS ELEMENTS:

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The procedure for the nonactivation assay is as follows: Prior to **each** treatment, cells were cleansed of spontaneous TK-/- by growing them in a medium containing thymidine, hypoxanthine, methotrexate, and glycine to permit the survival of only those cells that produce the enzyme thymidine kinase, and can therefore utilize the exogenous thymidine from the medium. The test compound was added to the cleansed cells in growth medium for four hours. The mutagenized cells were washed, fed, and allowed to express in growth medium for three days. At the end of this expression period, TK-/- mutants were detected by cloning the cells in the selection medium for ten days. Surviving cell populations were determined by plating diluted aliquots in nonselective growth medium. The procedure for the activation assay differs from the nonactivation assay in the following manner only. Two mls of the reaction mixture (microsomes prepared from un-induced male mice livers were added to the reaction mixture) were added to 10 ml of growth medium. The desired number of cleansed cells was added to this mixture, and the flask was incubated on a rotary shaker for four hours. The incubation period was terminated by washing the cells twice with growth medium. The washed mutagenized cells were then allowed to express for three days and were cloned as indicated for the nonactivated cells. In both assays, one replicate per concentration was used, except two replicates were used for the solvent control.

A mutation index was derived by dividing the number of clones formed in the BUdR-containing selection medium by the number found in the same medium without BUdR. The ratio was then compared to that obtained from other dose levels and from positive and negative controls. Colonies were counted on an electronic colony counter that resolves all colonies greater than 200 microns in diameter.

The criteria for considering if a compound is mutagenic in the Mouse Lymphoma Assay is as follows: A dose response relationship is observed over three of the doses employed; the minimum increase at the high level of the dose response curve is at least 2.5 times greater than the solvent control value; the solvent control data are within the normal range of the spontaneous background for the TK locus.

All evaluations of mutagenic activity are based on the concurrent solvent control value run with the experiment in question. Positive control values are not used as reference points, but are included to ensure the current cell population responds to direct and promutagens under the appropriate treatment conditions.

Results

Result	Negative
Cytotoxic Concentration	Concentrations of 0.12 microliters and higher were completely cytotoxic (without activation)
Genotoxic Effects	With metabolic activation
Statistical results	All results were negative
Results Remarks	Preliminary solubility studies indicated that 0.16 ul/ml (in growth medium with DMSO as carrier solvent) and higher were slightly cloudy. DMSO (1%) was used as the solvent

HUMAN HEALTH EFFECTS ELEMENTS:

TOXICITY IN VITRO (GENE MUTATIONS)

control and growth medium without the addition of solvent was included as a negative control compound. No genetic effects were attributable to the presence of solvent. The known mutagens, EMS and DMN, were included as reference mutagens. EMS showed a weak response while DMN was positive. The reason for this difference was not determined.

The results will be presented in the following sequence: solvent control, negative control, positive control (EMS for nonactivation and DMN for activation assays), 0.02, 0.03, 0.04, 0.06, and 0.08 microliters test substance/ml. The total mutant clones for the nonactivation assay was 49.5, 27, 81, 17, 36, 43, 87, and 20. The total viable clones for the nonactivation assay was 307, 328, 251, 210, 341, 275, 362, and 306. The mutant frequencies (mutant clones/viable clones xl O.E-4) for the nonactivation assay was 0.161, 0.0823, 0.3227, 0.0810, 0.1056, 0.1564, 0.2403, and 0.0654. The total mutant clones for the activation assay was 81, 70, 137, 62, 52, 13, 44, and 55. The total viable clones for the activation assay was 394.5, 338,241, 510,404, 395,321, and 365. The mutant frequencies for the activation assay was 0.2053, 0.2071, 0.5685, 0.1216, 0.1287, 0.0329, 0.1371, and 0.1507.

Conclusions

No dose-related patterns or trends indicative of induced mutagenesis were observed in either the absolute number of mutants or in the mutation frequency. Variations in these parameters did occur, but were not related to dose or to toxicity and were of insufficient magnitude with respect to the spontaneous levels to be considered positive.

Data Quality

Reliability Acceptable.

Data Reliability Remarks The key parameters (i.e., dose levels, positive and negative controls, etc.) were appropriate and described in the study. Although a weak response was seen with the EMS positive control, the sample conducted at the same time with the **DMN** positive control in the activation assay was clearly positive. The current cell population did respond, although weakly, to known mutagens under the treatment conditions.

Reference

Remarks

Mutagenicity Evaluation of MCTR-243-77 in the Mouse Lymphoma Assay. Litton Bionetics, Inc. Project No. 20839, October, 1977. MCTR-2432-77.
Clive and Spector, Mutation Research, 31:17-29, 1975.

General

Admin Record Number: **ROBUST SUMMARY**

Styrene, p-methyl-

CAS# 622979 **Study Number: 3**

HUMAN HEALTH EFFECTS ELEMENTS:

TOXICITY IN VITRO (GENE MUTATIONS)

Test Substance

Remark	p-methylstyrene Test Article ID#: MCTR-136-79 Purity: 100% assumed for dosage calculations Additives: None reported Carrier solvent: Dimethylsulfoxide (DMSO; 1%) Contaminants: None reported Chemical formula: C9H10
Chemical Category	

Method

Method/Guideline Followed	Other
Test Type	Sister chromatid exchange **assay**
System of Testing	Non-bacterial
GLP	**Yes**
Year study performed	**1980**
Species	Primary cultures • human lymphocytes
Metabolic Activation	None
Concentration	25, 50, 65, 80, and 100 nl/ml
Statistical Method	two-tailed t-test (Finney, 1971).
Remarks for Method	The objective of this assay was to evalute the ability of the test substance to induce sister chromatid exchange (SCE) in cultured human lymphocytes. Human venous blood was added to culture medium containing phytohaemagglutinin (PHA) and incubated at 37 degrees C in the dark for 24 hours. After this time, solutions of the test compound, positive control (ethylmethanesulfonate, EMS; 0.1 microliter/ml final concentration) or the DMSO solvent (1%) were then added to appropriate cultures, followed by 5-bromodeoxyuridine (BrdU) (final concentration 25 micromolar) and cultures were re-incubated for a further 46-48 hours (total culture time 70 - 72 hours). Colcemid was added 2.5 hours before harvest of dividing lymphocytes. Two replicate cultures were used for **each** treatment in the assay. Three trials **were** conducted. Test substance concentrations in Trial 1 were 1, 10, 25, 50, and 100 nl/ml. In Trials 2 and 3, the following concentrations were used; 25, 50, 65, 80, and 100 nl/ml. In Trial 3, BrdU was added at initiation of cultures instead of after the test compound at 24 hours. The solubility of the test compound was determined prior to screening. The compound was dissolved in DMSO and a series of dilutions performed so that the final concentrations in cultures were achieved by adding 0.1 ml of test solution (1% DMSO).

Admin Record Number: **ROBUST SUMMARY**

Styrene, p-methyl-

CAS# 622979 **Study Number: 3**

HUMAN HEALTH EFFECTS ELEMENTS:

TOXICITY IN VITRO (GENE MUTATIONS)

The preliminary range of doses treated was based on toxicity data from the rat hepatocyte UDS assay. Doses selected for the lymphocyte assay included the dose that produces approximately a 50% reduction in rat cell survival, along with four lower doses.

The cell suspensions were centrifuged, the supernatant discarded, and cells treated with hypotonic KCL (0.075M) for 3 minutes to swell the cells and eliminate red blood cells. Cells were then washed three times with fixative (methanol:glacial acetic acid, 3:1) and dropped onto slides to air dry.

Staining for detecting SCE was accomplished by a modified fluorescent plus Giemsa (FPG) technique. Slides were stained for 10 minutes with Hoechst 33258 (5 micrograms/ml) in phosphate buffer (pH 6.8), mounted in the same buffer and exposed at 55-60 degrees C to "black-light" from a 15 Watt tube for the amount of time required for differentiation between chromatids (about 15 minutes). Finally, slides were stained with 5% Giemsa for 10 to 20 minutes and air dried.

M2 cells were scored for the frequency of SCE per cell and per chromosome. Fifty cells were scored per dose. For control of bias, all slides were coded prior to scoring and scored "blind".

If an increase in SCE is observed, one of the following criteria must normally be met to assess the compound **as** positive: i.) Two-fold increase: approximately a doubling in SCE frequency over the "background" (solvent and negative control) levels, at a minimum of three doses; ii.) Dose response: a positive assessment may be made in the absence of a doubling if there is a statistically significant increase at a minimum of three doses and evidence for a positive dose response.

In some cases, statistically significant increases are observed with neither a doubling or a dose response. These results are assessed according to repeatability, the magnitude of the response, and the proportion of the dose levels affected. Statistical analysis employs a Students t-test.

Results

Result	Positive
Cytotoxic Concentration	All treatment levels
Genotoxic Effects	Dose-response
Statistical results	T-test results and appropriate p values are presented below for all Trials.
Results Remarks	In Trial 1, SCEs/Cell (standard error) [46 Chromosomes] were 8.9 (0.4), 31* (0.8). 9.0 (0.4), 10.2 (0.5), 9.7 (0.4), 12.5* (0.5), and 16.4* (1.3) for solvent control, positive EMS control, 1, 10, 25, 50, and 100 nl/ml, respectively. * indicates significant difference from solvent controls at p<0.001 . The negative control was not done due to laboratory error. In Trial 2, SCEs/Cell (standard error) were 8.8 (0.4), 9.4 (0.4), 28.3' (0.8), 10.0 (0.5), 13.6* (0.5), 10.9** (0.5), 13.1* (0.5), and 10.5 (0.5) for negative control, solvent control,

HUMAN HEALTH EFFECTS ELEMENTS:

TOXICITY IN VITRO (GENE MUTATIONS)

positive EMS control, 25, 50, 65, 80, and 100 nl/ml, respectively. *indicates significance at p<0.001; **indicates significance at p<0.25. In Trial 3, SCEs/Cell (standard error) were 7.3 (0.4) 8.8 (0.4). 27.3* (0.8), 9.3 (0.4), 9.7 (0.4), 12.8* (0.5), 11.8* (0.5), and 13.6* (0.5) for negative control, solvent control, positive EMS control, 25, 50, 65, 80, and 100 nl/ml, respectively. *indicates significance at p<0.001. The mitotic index (% 500 cells) was 1.2, 0.4. and 0.6% for the solvent control, 25, and 100 nl/ml, respectively.

The results in Trial 1 (above) showed an increase in SCE at the two highest doses (50 and 100 nl/ml) with some evidence for a dose response. The assay was repeated in Trial 2 using a series of doses to include more in the positive range. There was some evidence for an increase, but the highest SCE frequency was 13.6 per cell at 50 nl/ml, compared with 16.4 per cell at 100 nl/ml in Trial 1. In this second trial there was no clear evidence for a dose response. Because the highest SCE frequency was lower in Trial 2 than in Trial 1, the question arose whether the test sample might have lost some activity during storage at room temperature. There was no visible change in the test compound to suggest polymerization, however, since it remained clear and colourless. A fresh sample of MCTR-136-79 was obtained and stored at -20 degrees C until use in Trial 3. In Trial 3, the same range of doses used in Trial 2 was employed. In the third trial, there was once again a slight but significant increase in SCE in the cultures treated with 65, 80, and 100 nl/ml. There was some evidence for a positive dose response. The SCE frequencies in the solvent control and positive control demonstrated reproducibility and that the cells were sensitive to SCE induction by EMS.

Dose related cell toxicity was observed at all treatment levels, demonstrated by a reduction in the frequency of dividing cells (mitotic index) and cell cycle delay.

Conclusions

The test compound caused a small but reproducible increase in SCE. The maximum response was about 84% over the solvent control level (Trial 1). This compound is given a “weak positive” rating in the test for SCE in human lymphocytes.

Data Qualitv

Reliability Acceptable

Data Reliability Remarks The key parameters (i.e., number of concentrations, statistical methods, etc.) are appropriate and described in the study.

Reference

Remarks Mutagenicity Evaluation of MCTR-136-79 in the Sister Chromatid Exchange Assay in Human Lymphocytes, Litton Bionetics, Inc. Project No. 20990, March 1980, M1360-79.

General

Admin Record Number: **ROBUST SUMMARY**

Styrene, p-methyl-

CAS# 622979 **Study Number: 4**

HUMAN HEALTH EFFECTS ELEMENTS:

TOXICITY IN VITRO (GENE MUTATIONS)

Test Substance

Remark	p-methylstyrene Test Article ID#: MCTR-141-79 Purity: 100% assumed for dosage calculations Additives: None reported Solvent carrier: Dimethylsulfoxide (DMSO) Contaminants: None reported Chemical formula: C9H10
Chemical Category	

Method

Method/Guideline Followed	Other
Test Type	DNA damage and repair assay
System of Testing	Bacterial
GLP	**Yes**
Year study performed	**1979**
Species	Salmonella typhimurium
Metabolic Activation	A 9,000 x g supernatant prepared from Sprague-Dawley adult male rat liver induced by Aroclor 1254
Concentration	0.01, 0.03, 0.1, and 0.3 microliters/plate
Statistical Method	None referenced
Remarks for Method	MCTR-141-79 was tested in a bacterial DNA damage/repair suspension assay at doses of 0.01, 0.03, 0.1, and 0.3 microliters/plate in E. Coli tester strains WP2/WP100 and Salmonella tester strains TA1978/TA1538. Doses were selected on the basis of the specific test article toxicity curve exhibited in a preliminary toxicity experiment. Test material and bacteria were incubated with and without metabolic activation for 90 minutes with shaking prior to plating. Rat liver homogenate was used in the activation mixture. At the end of the incubation period, the incubation mixtures were added to 2.5 ml of appropriately supplemented top agar and plated on Vogel Bonner bottom agar in triplicate to determine viable counts. The plates were incubated for 48 hours at 37 degrees C. Positive controls were 4-nitroquinoline-1-oxide (4NQO; 0.075 microgram/ml) without activation and 2-aminofluorene (2FA; 200 microgram/ml) with activation. The negative control was penicillin (15 micrograms/ml). All colonies were counted with a BioTrans II automated colony counter when possible. Colony counts were made by hand when automated counting was not possible. The

HUMAN HEALTH EFFECTS ELEMENTS:

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percent survival of each tester strain was calculated by comparing the number of treated survivors to the solvent treated survivors. The survival index is then determined by dividing the percent survival of the repair deficient strain with that of the repair proficient strain (i.e., TA1538/TA1978 or WP100/WP2). For data comparison purposes, any repair deficient strain colony average equal to zero will be treated as a colony average of one. This allows the generation of a maximum survival index. Decreasing survival indices with increasing concentrations of test chemical are considered indicative of genotoxicity in this assay.

Results

Result Ambiguous

Cytotoxic Concentration 0.1 and 0.3 microliters/ml (without activation); 0.3 microliters/ml (with activation)

Genotoxic Effects Equivocal

Statistical results None reported

Results Remarks When the test material (0.3 microliters) was added to the reaction mixture with and without microsomal activation, the reaction mixture appeard cloudy. Following the 90 minute incubation period with shaking, the reaction mixture appeared to be clear. No precipitate was present when an overlay containing an aliquot of reaction mixture was plated.

Only a very weak preferential killing of the DNA repair deficient strains WP1 00 and TA1538 was observed at one dose level each without metabolic activation. Weak preferential killing of WP1 00 was observed at one dose level with metabolic activation. In the case of the TA1538 response, the penicillin negative control response was essentially of the same magnitude. No dose related preferential kill was observed.

The penicillin negative control theoretically should yield a survival index of approximately 1 .0. In the spot test, this is routinely observed. In the suspension test, however, there was a marked tendency to selectively kill the wild type strain, especially in the case of the E. Coli strain. The response is variable; however, it is probably a function of the rate of growth of the tester strains during the exposure period that contributes to the variability, given the mechanism of action of penicillin.

The results will be presented in the following sequence: penicillin (0 micrograms/ml), penicillin (15 micrograms/ml), 4NQ0 (0 micrograms/ml), 4NQO (0.075 micrograms/ml), 0, 0.01, 0.03, 0.1, and 0.3 microliters test substance/ml, respectively. Nonactivation assay, average % survivors, WP2; 100, 3, 100, 87, 100, 104. 100, 37, and 0. Nonactivation, % survivors, WP100; 100, 54, 100, 0, 100, 96, 97, 30, 0. Nonactivation, Survival index (WP100/WP2); 1.00, 18.0, 1.00, <0.1, 1.00, 0.92, 0.97, 0.81, 0. Nonactivation assay, % survivors, TA1978; 100, 38, 100, 106, 100, 98, 99, 1, 0. Nonactivation assay, % survivors, TA1538; 100, 31, 100, 3, 100, 93, 83, 0, 0. Nonactivation assay, Survival index (TA1538/TA1978); 1 .00, 0.82, 1 .00, 0.03, 1 .00, 0.95, 0.84, <0.1, 0. Activation assay, average % survivors, WP2; 100, 24, 100, 64, 100, 102,

HUMAN HEALTH EFFECTS ELEMENTS:

TOXICITY IN VITRO (GENE MUTATIONS)

84, 85, 3. Activation assay, % survivors, WP100; 100, 51, 100, 1, 100, 98, 87, 66, 0. Activation assay, Survival index (WP100/WP2); 1.00, 2.13, 1.00, 0.02, 1.00, 0.96, 1.04, 0.78, <0.01. Activation assay, % survivors, TA1978; 100, 39, 100, 23, 100, 97, 87, 72, 0. Activation assay, % survivors, TA1538; 100, 44, 100, 3, 100, 114, 97, 83, 0. Activation assay, Survival index (TA1538/TA1978); 1 .00, 1.13, 1 .00, 0.13, 1.00, 1.18, 1.11, 1.15, 0.

Conclusions

MCTR-141-79 is probably not a mutagen in this test system. No preferential kill was seen in Salmonella strain TA1538 and the experiment with E. Coli WP2/WP100 appears to be invalid since a preferential killing of WP2 was observed with penicillin, a compound used for the negative control. Penicillin is believed to kill bacteria by mechanisms which are independent of effects on DNA.

Data Quality

Reliability Unacceptable

Data Reliability Remarks A preferential killing of WP2 was observed with the negative control compound, penicillin. The results should be evaluated only in conjunction with other in vitro genetic tests. Uncorraborated results from this test system should not be heavily weighed.

Reference

Remarks Bacterial DNA Damage/Repair Suspension Assay of MCTR-141-79. M1410-79.

General

Admin Record Number: **ROBUST SUMMARY**

Styrene, p-methyl-

CAS# 622979 **Study Number: 5**

HUMAN HEALTH EFFECTS ELEMENTS:

TOXICITY IN VITRO (GENE MUTATIONS)

Test Substance

Remark	p-methylstyrene Test Article ID#: MCTR-134-79 Purity: 100% assumed for dosage calculations Additives: None reported Solvent carrier: Dimethylsulfoxide (DMSO) Contaminants: None reported Chemical formula: C9H10
Chemical Category	

Method

Method/Guideline Followed	Other
Test Type	Mitotic recombination in Saccharomyces cerevisiae
System of Testing	Non-bacterial
GLP	**Yes**
Year study performed	1980
Species	Saccharomyces cerevisiae
Metabolic Activation	A 9,000 x g supernatant prepared from Sprague-Dawley adult male rat liver induced by Aroclor 1254
Concentration	0.01, 0.10, 0.50, 1.00, 2.00, and 2.50 microliters/plate
Statistical Method	None reported
Remarks for Method	The objective of this study was to evaluate the test substance for its genetic activity in Saccharomyces cerevisiae yeast strain D5 (genotype ade 2-40/+, +/ade 2-l 19) with and without the addition of metabolic activation preparations. The concentrations were 0.01, 0.10, 0.50, 1 .00, 2.00, and 2.50 microliters/plate (DMSO solvent carrier). An initial assay was conducted using 0.1 to 50 microliters/plate, but was repeated using the previous concentrations due to toxicity. Cell cultures for the study were prepared from a single isolated colony of the indicator organism. Approximately 1 0.E7 cells from a suspension stock of the indicator strain were added to a screw-capped test tube containing 0.5 ml of phosphate buffer, pH 7.4. In the activation assay, an aliquot of reaction mixture (0.5 ml containing 9,000 x g Sprague-Dawley adult male liver homogenate induced by Aroclor 1254) was added in place of the buffer. All tubes were incubated at 30 degrees C in a shaker water bath for 3 hours. Samples were removed and plated onto complete medium (5 plates per concentrations) and incubated at 30 degrees C for 4 days. The plates were screened for pigmented colonies and sectors using a dissecting microscope with variable magnification.

Admin Record Number: **ROBUST SUMMARY**

Styrene, p-methyl-

CAS# 622979 **Study Number: 5**

HUMAN HEALTH EFFECTS ELEMENTS:

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A DMSO negative control (0.05 ml) was employed for each indicator strain and performed in the absence and presence of S9 mix. Positive controls for nonactivation and activation assays were ethylmethanesulfonate (EMS, 27.8 microliters/ml) and dimethylnitrosamine (DMN, 277.8 microliters/ml), respectively.

Results are considered positive if the total recombinogenic events in a test are equal to or greater than 2.0 times the spontaneous events. Recombinational events are scored by monitoring pigment production in colonies. Heterozygous colonies are white, homozygous colonies from reciprocal exchange events are red-pink and colonies derived from non-reciprocal events are red, pink or a mottled red-white or pink and white mixture. An accompanying dose-related effect is also necessary to give confidence to the increase. Plate assays are required to demonstrate clear dose-related increases in mutant counts over at least three consecutive concentrations to be considered positive.

Results

Result Positive

Cytotoxic Concentration 2.5 microliters/plate, with and without activation

Genotoxic Effects Dose-response

Statistical results None reported

Results Remarks Total events per 10.E4 survivors (included the sum of pink/white, red/white, pink/red, red and pink colonies) for the nonactivation test was 15, 320*, 12, 9, 19, 24, 733*, and 21 for solvent control, positive control, 0.01, 0.1, 0.5, 1 .0, 2.0, and 2.5 microliters/plate, respectively. *indicates events per 10.E2 survivors. The frequency per 10.E5 survivors for the nonactivation test was 3.94, 815.08, 5.35, 3.28, 15.50, 73.17, 5699.84, and 10500 for solvent control, positive control, 0.01, 0.1, 0.5, 1 .0, 2.0, and 2.5 microliters/plate, respectively. The total events for the activation test was 14, 39, 8, 23, 27, 43, 503*, and 57 for solvent control, positive control, 0.01, 0.1, 0.5, 1 .0, 2.0, and 2.5 microliters/plate, respectively. *indicates events per 10E2 survivors. Frequency per 10.E5 survivors for the activation test was 4.74, 37.94, 3.48, 6.50, 13.50, 21.37, 1620.49, and 7125.00, respectively.

The results of this test conducted in the presence and absence of metabolic activation demonstrated an increase in the frequency of both types of mitotic recombination. The total "events per 10.E5 survivors" exceeded the spontaneous conversion rate by greater than two fold at 0.5, 1 .0, 2.0, and 2.5 microliters/plate with and without activation. At a dose of 2.5 microliters/plate, low survival of less than 1% inflated the total event ratio. A dose of 2.0 microliters/plate was administered in a second study to provide an intermediate dose between 1 .O and 2.5 microliters/plate. The results of this supplemental test showed a very high incidence of recombinational events, exceeding even positive control values by two to three-fold.

Conclusions

The test compound MCTR-134-79 exhibited genetic activity with the strain D5 in the

HUMAN HEALTH EFFECTS ELEMENTS:

TOXICITY IN VITRO (GENE MUTATIONS)

activation and nonactivation assays conducted in this evaluation and is considered to induce mitotic recombinations under these test conditions. These tests indicate that the test compound does not require metabolic activation to cause genetic activity and that the parent compound can cause mitotic recombinations.

Although mitotic recombination is not a mutagenic event, it is known that most mutagenic agents stimulate mitotic segregation to frequencies exceeding the usual spontaneous frequency.

Data Qualitv

Reliability Acceptable

Data Reliability Remarks The key parameters (i.e., concentrations, use of positive and negative controls) were appropriate and described in the study.

Reference

Remarks

Mutagenicity Evaluation of MCTR-134-79 in the Mitotic Recombination Assay with the Yeast Strain D5. Litton Bionetics, Inc. Project No. 20988, April, 1980. M1340-79.
Ames, et. al. Mutation Research, 31:347-364, 1975.
Zimmerman, Mutation Research, 21:263-269, 1973.

General

Admin Record Number: **ROBUST SUMMARY**

Styrene, p-methyl-

CAS# 622979 Study **Number: 6**

HUMAN HEALTH EFFECTS ELEMENTS:

TOXICITY IN VITRO (GENE MUTATIONS)

Test Substance

Remark	p-methylstyrene Test Article ID#: MCTR-135-79 Purity:100% assumed for dosage calculations Additives: None reported Solvent carrier: Dimethylsulfoxide (DMSO, 1%) Contaminants: None reported Chemical formula: C9H10
Chemical Category	

Method

Method/Guideline Followed	Other
Test Type	Unscheduled DNA synthesis
System of Testing	Non-bacterial
GLP	**Yes**
Year study performed	**1979**
Species	Primary cultures - rat hepatocytes
Metabolic Activation	None
Concentration	0.625, 1.25, 2.5, 5.0, 10, 20, and 40 nanoliters/ml
Statistical Method	None reported
Remarks for Method	The objective of this assay was to detect DNA damage caused by the test substance or an active metabolite by measuring unscheduled DNA synthesis (UDS) in primary rat hepatocytes obtained from adult male Fischer 344 rats (150-300 g). The existence and degree of DNA damage was inferrred from an increase in nuclear grain counts compared to untreated hepatocytes. The types of detectable DNA damage are unspecified but must be recognizable by the cellular repair system and result in the incorporation of new bases (including 3-H-thymidine) into the DNA. This assay is based on the procedures described by Williams (1977). The UDS assay is initiated by replacing the media in the culture dishes with Williams' Medium E (WME) containing only 1 .O% fetal bovine serum and the test material at the following concentrations; 0.625, 1.25, 2.5, 5.0, 10, 20, and 40 nanoliters/ml (dissolved in DMSO). All concentrations and positive (2-acetyl aminofluorene, 400 ug/ml) and negative (DMSO 1%) controls were performed on six cultures. After treatment for one hour, the test material was removed and the cell monolayers were washed twice with incomplete WME. Three of the cultures for each treatment were used to monitor the toxicity of treatment. The other three cultures were labeled with 3H-thymidine (1 microCi/ml), incubated for 3 hours and then washed with complete WME containing 1 mM thymidine. The toxicity of each treatment was monitored

HUMAN HEALTH EFFECTS ELEMENTS:

TOXICITY IN VITRO (GENE MUTATIONS)

by performing viable cell counts on one culture 2 to 3 hours after treatment and on two cultures about 24 hours later. The nuclei in the labeled cells were fixed and mounted on glass slides and stained and processed according to standard methods. The cells were examined microscopically at approximately 1500x magnification under oil immersion and the field was displayed on the video screen of an automatic counter. UDS was measured by counting nuclear grains and subtracting the average number of grains in three nuclear-sized areas adjacent to each nucleus (background count). This value was referred to as the net nuclear grain count. The net nuclear grain count was detennined for 50 randomly selected cells on each coverslip. The mean net nuclear grain count was determined from the triplicate coverslips (150 total nuclei) for each treatment conditions. The assay was performed twice due to poor growth of both test and control hepatocyte cultures in the first assay.

The test material is considered active in the UDS assay at applied concentrations that cause: 1) An increase in the mean nuclear grain count to at least 6 grains/nucleus in excess of the concurrent negative control value,and/or 2) The percent of nuclei with 6 or more grains to increase above the examined population, in excess of the concurrent negative control, and/or 3) The percent of nuclei with 20 or more grains to reach or exceed 2% of the examined population. If the negative control shows an average of 6 grains/nucleus or 1% of the cells have 20 grains/nucleus, the assay will normally be considered invalid. A dose related increase in UDS for at least two consecutive concentrations is also desirable to evaluate a test material as active in this assay. The positive controls are used to demonstrate that the cell population employed was responsive and the methodology adequate for the detection of UDS.

Results

Result	Positive
Cytotoxic Concentration	40 nanoliters/ml
Genotoxic Effects	Dose-response
Statistical results	None reported
Results Remarks	The results will be presented in the following sequence: solvent control, positive control, 0.625, 1.25, 2.5, 5.0, 10, 20, and 40 nanoliters/ml, respectively. The UDS grains/nucleus were 1.54, 5.84, 2.39, 1.46, 1.35, 3.63, 4.31, 2.59, and 1.61. The average % nuclei with > or equal to 6 grains was 8.0, 42.7, 11.3, 7.3, 6.7, 19.3, 26.0, 12.0, and 8.0. The average % nuclei with > or equal to 20 grains was 0, 0.67, 0.67, 0, 0.67, 1.3, 2.0, 1.3, and 0. The % survival at 3 hours was 100, 57.2, Not counted, 102.3, 110.4, 94.7, 73.9, 70.5, and 45.5. The % survival at 24 hours was 100, 66.5, Not counted, 71 .1, 73.8, 73.6, 76, 66.8, and 47.6. The test material was immiscible with water at 50 microliters/ml but dissolved easily into DMSO at 500 microliters/ml. The test material caused about a 55% reduction in viability during the labeling period after exposure to 40 nl/ml. The toxicity decreased with decreasing dose until no apparent effect was observed at 2.5 nl/ml. By 24 hours after

HUMAN HEALTH EFFECTS ELEMENTS:

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treatment, some further reduction in viability occurred over the 1.25 to 5 nl/ml dose range, but most of the toxic action occurred early after the exposure period. These results showed that an appropriate concentration range was assayed to search for UDS activity.

Treatments with 0.625 nl/ml to 2.5 nl/ml did not result in nuclear grain distributions significantly different from the control solvent. However, increases in all three UDS parameters were observed for the 5 and 10 nl/ml treatments. Thus, the mean grain count approached the positive control value, more than 18% of the nuclei had 6 or more grains above background, and the percentage of nuclei with 20 or more grains exceeded the positive control. Greater activity was observed for the 10 nl/ml treatment than for 5 nl/ml, but the response faded for the 20 nl/ml treatment, where the primary evidence for UDS was the increase in nuclei with 20 or more grains. At 40 nl/ml, the labeling pattern returned to the solvent control values. High toxicity was evident at the high dose in that cells were rounded and difficult to analyze, nuclei were extruded from the cytoplasm, and not many of the cells contained any grains. The interpretation of these results is that UDS activity became detectable at 5 nl/ml and increased with dose until DNA repair was inhibited by the disruption of cellular processes at test concentrations exceeding 10 nl/ml.

The 2-AAF positive control did not induce the usual UDS response in this assay. Typically, the average grain count is at least 10 above the solvent control and the percentage of heavy labeling (20 or more grains) is 10% or greater. The reason for the low response is unknown, but questions about the metabolic state of the hepatocytes might by raised. Because of the low 2-AAF activity, the evaluation of the test results relied more on a comparison with the 2-AAF-induced response than on the predetenined UDS criteria. It is reasonably clear, however, that UDS activity did occur for the 5 and 10 nl/ml treatments. The number of heavily-labeled nuclei (blackened with numerous grains) was low in this assay. On each coverslip 500 cells were screened for heavy labeling. Among 13,500 cells thus screened in the entire assay, an average of 0.11% (15 cells) were heavily labeled. Thus, very few hepatocytes were undergoing DNA replication and this normal process did not interfere with the measurement of UDS.

Conclusions

The test material, MCTR-135-79, induced unscheduled DNA synthesis (UDS) in primary rat hepatocytes over the concentration range of 5 nl/ml to 20 nl/ml. Treatment with 40 nl/ml was very toxic and no UDS was observable. The test material is therefore considered to be active in the Primary Rat Hepatocyte UDS Assay.

Data Qualitv

Reliability Acceptable

Data Reliability Remarks The key parameters (i.e., number of concentrations, use of positive and negative controls) were appropriate and described in the study. Even though there were questions concerning the metabolic state of the hepatocytes based on the low positive control response, it was clear that UDS activity did occur in the 5 and 10 nl/ml treatments.

Reference

Admin Record Number:

ROBUST SUMMARY

Styrene, p-methyl-

CAS# 622979

Study Number: 6

HUMAN HEALTH EFFECTS ELEMENTS:

TOXICITY IN VITRO (GENE MUTATIONS)

Remarks

Evaluation of MCTR-13579 in the Primary Rat Hepatocyte Unscheduled DNA Synthesis Assay, Litton Bionetics, Inc. Project No. 20991. April 1980, M1350-79.
Williams, Cancer Research, 37:1845-1851, 1977.

General

Admin Record Number: **ROBUST SUMMARY**

Styrene, p-methyl-

CAS# 622979 Study **Number: 1**

HUMAN HEALTH EFFECTS ELEMENTS:

TOXICITY IN VIVO (CHROMOSOMAL ABERRATIONS)

Test Substance

Remarks	p-methylstyrene Test Article ID#: MCTR-138-79 (T1563) Purity: 100% assumed for dosage calculations Additives: None reported Solvent carrier: Olive oil Contaminants: None reported Chemical formula: C9H10
Chemical Category	

Method

Method/Guideline Followed	Other
Test Type	Cytogenetic assay
GLP	Yes
Year Study Performed	1979
Species	rat
Strain	Sprague-Dawley
Sex	M
Number of Males per Dose	5
Number of Females per Dose	**0**
Route of Administration	Oral
Doses	0.15, , 0.5, and 1.5 ml/kg/day
Exposure Period	daily for 5 days
Statistical Method	One-sided t-test, chi-square analysis
Remarks for Method	The purpose of this study was to use the in vivo cytogenetics assay in rodents to investigate the mutagenic potential of the test substance. Male Sprague-Dawley rats (4-6 weeks old, 125-l 75 grams) were quarantined for 11 days prior to initiation of the assay, during which time they were found to be free of parasites, pathogenic bacteria, and appropriate murine viruses. The test substance was suspended in olive oil and dosed orally by gavage with 0.15, 0.5, and 1.5 ml/kg/day for five consecutive days (five animals/dose). The test article/olive oil was given at a volume of 4 ml/kg body weight/day. Olive oil (negative control) was administered by gavage at a dose level of 4.0 ml/kg/day for five days. Triethylenemelamine (TEM, positive control) was given intraperitoneally (IP) as a single dose of 0.5 mg/kg one day prior to sacrifice. All rats

HUMAN HEALTH EFFECTS ELEMENTS:

TOXICITY IN VIVO (CHROMOSOMAL ABERRATIONS)

were given colchicine at 4 mg/kg IP 4 hours prior to sacrifice. The rats were sacrificed by carbon monoxide asphyxiation; the femurs were removed and the marrow was flushed into HBSS. After centrifugation at 800-l 000 rpm for 8-l 0 minutes, the cells were treated with 0.075 M KCI for 20-30 minutes at 37 degrees C. The cells were again centrifuged and washed twice with 5 ml Carnoy's fixative. The cells were resuspended in 5 ml Carnoy's fixative and treated overnight (16-20 hr) at 4 degrees C. The cells were centrifuged at 800-l 000 rpm for 8-10 minutes and the cell pellet was resuspended to opalescence in fresh Carnoy's fixative. Two to five slides were prepared from each animal. Slides were stained with Giemsa and permanently mounted. A minimum of 50 metaphase spreads from each animal was examined and scored for chromatid and chromosomal gaps and breaks, fragmentation, structural rearrangements, and ploidy. The mitotic index was recorded for each rat as the number of cells in mitosis/l 00 cells observed.

The criteria for determination of a valid test is as follows: The percentage of cells in the negative control group demonstrating chromosomes and chromatid breaks and gaps must be less than or equal to 3% of the total **cells analyzed.** The percentage of cells demonstrating aberrations of any type must not exceed 10% in the negative control group. The number of cells with aberrations must be at least 45% of the total cells analyzed in the positive control group.

Chi-square analysis using a 2 x 2 contingency table was used to ascertain significant relationships between the number of cells with aberrations in the treatment group relative to the negative control. The t-test was used to compare pairwise the number of aberrations per cell of the treatment group with that of the negative control.

Results

Effects on Mitosis None

Genotoxic Effects Negative

Statistical Results No treatment significant from negative control at $p<0.05$

Results Remarks No treatment-related mortality was observed. The mitotic index (5 replicates) is as follows: negative control (8, 10, 8, 3, 4), positive control (<1, 2, 1, <1, <1), 0.15 ml/kg/day (13, 3, 5, 5, 4), 0.05 ml/kg/day (6, 4, 10, 12, 12), and 1.5 ml/kg/day (12, 11, 12, 3, 12). The percentage of total cells analyzed with aberrations were 9.6, 53.7, 9.6, 10.0, and 12.0 for negative control, positive control, 0.15, 0.50, and 1.5 ml/kg/day, respectively. The aberrations per cell were 0.10, 2.11, 0.12, 0.11, and 0.13 for negative control, positive control, 0.15, 0.50, and 1.5 ml/kg/day.

The test substance was not a mitotic inhibitor when compared to the negative controls. The positive control TEM was a moderate inhibitor. The percentage of cells with aberrations for all treatment groups was not statistically increased from the negative control group ($p<0.05$). The group receiving TEM demonstrated severe damage, with approximately 54% of all cells **analyzed containing one or more aberrations.** The

HUMAN HEALTH EFFECTS ELEMENTS:

TOXICITY IN VIVO (CHROMOSOMAL ABERRATIONS)

severity of damage within the cells for all treatment groups were not significantly increased from negative controls ($p<0.05$). The group receiving TEM positive control demonstrated severe damage.

Conclusions

The positive and negative controls fulfilled the requirements for the determination of a valid test.

The test article appeared to exhibit little **or** no clastogenic activity. Under the conditions employed in the assay, the data suggest that the test article exhibits little or no mutagenic activity in the in vivo cytogenetics assay. It did not alter the structural or numerical configuration of chromosomes from treated animals beyond that observed in negative controls. However, the negative control animals had an abnormally high number of aberrant cells compared to published spontaneous chromosome breakage values. However, consistant criteria for identifying abnormalities in negative control and treated groups allow acceptance of the validity of the results.

Data Quality

Reliability Acceptable

Data Reliability Remarks The key parameters (number of animals, concentrations, positive and negative controls, observations, etc.) was appropriate and described in the study.

Reference

Remarks

Activity of T1563 in the In Vivo Cytogenetics Assay in Rodents, Microbiological Associates Study No. Al 380. Ml 380-79.
Kilian, et al. Handbook of Mutagenicity Test Procedures, Elsevier Scientific Publishing Company, New York, pp. 243-260, 1977.

General

Admin Record Number:

ROBUST SUMMARY

Styrene, p-methyl-

CAS# 622979 **Study Number: 2**

HUMAN HEALTH EFFECTS ELEMENTS:

TOXICITY IN VIVO (CHROMOSOMAL ABERRATIONS)

Test Substance

Remarks

p-methylstyrene
Test Article ID#: MCTR-137-79
Purity: 100% assumed for dosage calculations
Additives: None reported
Solvent carrier: None
Contaminants: None reported
Chemical formula: C9H10

Chemical Category

Method

Method/Guideline Followed Other

Test Type Drosophila SLRL test

GLP Yes

Year Study Performed 1980

Species Drosophila melanogaster

Strain

Sex Both

Number of Males per Dose 0

Number of Females per Dose 0

Route of Administration Inhalation

Doses 0.07 ml delivered as an aerosol

Exposure Period immediate removal

Statistical Method Fiducial limits are computed according to Stevens

Remarks for Method

The test substance was tested in a battery of Drosophila melanogaster Mutagenesis Assays. Tests for point mutations included induction of sex-linked lethals, and somatic reversion of the white-ivory eye color mutant demonstrated by an increase in the frequency of male flies with red mosaic spots in their eyes. Chromosome aberrations and loss were measured by induction of dominant lethal mutations, Y chromosome loss and the bithorax test of Lewis in which chromosome rearrangements stimulate the development of a band of hairy tissue between thorax and abdomen, the metanotum.

Adult flies were exposed to 0.07 ml of the test substance delivered as an aerosol with immediate removal. Larvae, determined to be less sensitive to the toxic action of the test

HUMAN HEALTH EFFECTS ELEMENTS:

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substance, were exposed for 5 minutes. The number of flies exposed for each assay was not reported.

Results

Effects on Mitosis None

Genotoxic Effects Negative

Statistical Results No significant increases were observed in the frequency of "exceptions over control values" in any of the assays performed.

Results Remarks Exposure of flies to 0.07 ml carried as an aerosol into a 25 ml flask with immediate removal resulted in highly aberrant patterns of activity approaching anesthetization. The treatment resulted in about 20% mortality with some 30% of the survivors being sterile. Longer exposures resulted in almost total mortality.

The treatment with the test substance did not increase the frequencies af exceptions over those of controls in any of the assays.

Conclusions

The test substance does not induce significant genetic damage in Drosophila melanogaster and, therefore, is not a mutagen in this test system.

Data Qualitv

Reliability Acceptable

Data Reliability Remarks The methods of the generation of the aerosol and the number of flies exposed were not reported. Other methodology was adequately described in the study.

Reference

Remarks

Drosophila Mutagenicity Assay of Mobil Chemical Company Compound MCTR-137-79, Utah State University Study No. 009-647-352-9, Ml 370-79.
Lewis, Amer. Nat. 88: 225239, 1954.
Stevens, J. Genetics 43: 301-307, 1942.

General

Admin Record Number: **ROBUST SUMMARY**

Styrene, p-methyl-

CAS# 622979 **Study Number: 3**

HUMAN HEALTH EFFECTS ELEMENTS:

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Test Substance

Remarks	p-methylstyrene Test Article ID#: MCTR-138-79 Purity: 100% assumed for dosage calculations Additives: None reported Solvent carrier: 0.75% Methocel K4M Premium Contaminants: None reported Chemical formula: C9H10
Chemical Category	

Method

Method/Guideline Followed	Other
Test Type	Cytogenetic assay
GLP	Yes
Year Study Performed	1980
Species	rat
Strain	Sprague-Dawley
Sex	M
Number of Males per Dose	6
Number of Females per Dose	0
Route of Administration	Oral
Doses	0.134, 0.45, and 1.34 g/kg
Exposure Period	once daily for 5 consecutive days
Statistical Method	Chi-square analysis
Remarks for Method	The purpose of this study was to investigate whether the test substance was capable of causing a significant increase in clastogenic events in the bone marrow of rats in vivo. The test substance was prepared in Methocel K4M Premium (10 ml/kg) and given orally once daily for 5 days to male Sprague-Dawley rats (188 +- 27 g) . The concentrations of the test substance were 0.134, 0.45, and 1.34 g/kg, respectively. There were six animals per test group, five for chromosome analysis and one for absorption verification. The animals were observed for pharmacological effects 1, 2, and 6 hours after dosing. One animal from each treatment group and negative control was bled via cardiac puncture two hours after the last dose; blood **was** frozen and later analyzed for the presence of p-methylstyrene using capillary column gas chromatography. Colchicine was given

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intraperitoneally (IP; 4 mg/kg) for four hours after the last treatment and two hours before sacrifice. The positive control (cyclophosphamide, 60 mg/kg) was given 24 hours before sacrifice.

At the time of sacrifice, one femur was taken from each animal and processed for metaphase analysis according to standard procedures. A minimum of five slides were made for each animal; fifty cells were examined from each animal for clastogenic effect except two animals in the positive control which had an extremely low mitotic index.

Chi square analysis was performed to compare test values to negative control; a significant increase above control in aberrant cells is an indication of clastogenic activity by the test substance.

Results

Effects on Mitosis None

Genotoxic Effects Negative

Statistical Results No statistically significant increases in clastogenicity were observed with the test substance.

Results Remarks No mortality was observed with the test substance. A slight oral discharge and decreased activity were seen in several animals of the high dose group (1.34 g/kg), slight nasal discharge was observed in one animal of the mid dose (0.45 g/kg) and decreased activity was seen in one animal of the low dose group (0.134 g/kg). A relative concentration dose response **was** observed in the blood of animals treated with the test substance by capillary column gas chromatography. Blood concentrations were 1.5, 14, and 26.5 micrograms/ml for 0.134, 0.45, and 1.34 g test substance/kg. The number of aberrant cells/total observed were 2/250, 1491193, 2/250, 0/250, and 1/250 for the negative control (Methocel), the positive control (cyclophosphamide), 0.134, 0.45, and 1.34 g/kg, respectively.

Conclusions

The test substance, p-methylstyrene, did not significantly increase clastogenic events in the bone marrow cells above the negative control.

Data Qualitv

Reliability Acceptable

Data Reliability Remarks All key parameters (i.e., number of animals, concentrations, positive and negative controls, etc.) were appropriate and described in the study.

Reference

Remarks Metaphase Analysis of Rat Bone Marrow Cells Treated In Vivo with Para-Methylstyrene, Mobil Environmental and Health Science Laboratory Study No. 221 l-80.

Admin Record Number:

ROBUST SUMMARY

Styrene, p-methyl-

CAS# 622979

Study Number: 3

HUMAN HEALTH EFFECTS ELEMENTS:

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General

Admin Record Number: ROBUST SUMMARY

Styrene, p-methyl-

CAS# 622979 **Study Number: 4**

HUMAN HEALTH EFFECTS ELEMENTS:

TOXICITY IN VIVO (CHROMOSOMAL ABERRATIONS)

Test Substance

Remarks	p-methylstyrene Test Article ID#: MCTR-139-79 (T1601) Purity: 100% assumed for dosage calculations Additives: None reported Solvent carrier: Olive oil (1 ml/kg) Contaminants: None reported Chemical formula: C9H10
Chemical Category	

Method

Method/Guideline Followed	Other
Test Type	Dominant lethal assay
GLP	Yes
Year Study Performed	1980
Species	rat
Strain	Sprague-Dawley
Sex	M
Number of Males per Dose	10
Number of Females per Dose	0
Route of Administration	Oral
Doses	0.15, 0.5, and 1.5 ml/kg
Exposure Period	once daily for five consecutive days
Statistical Method	t-test, chi-square, analysis of regression
Remarks for Method	The purpose of the study was to determine the mutagenic potential of the test substance based on its ability to induce fetal wastage in rats. The test substance was prepared in olive oil (1 .O ml/kg volume) and given orally to male Sprague-Dawley rats (8-10 weeks old, 265-370 g body weight) once daily for five consecutive days. The concentrations of the test substance was 0.15, 0.5, and 1.5 ml/kg/day . There were 10 rats per treatment group. A negative control (olive oil, 1 .O ml/kg) and positive control (triethylenemelamine, TEM, single intraperitoneal injection of 0.5 mg/kg on day 4) were employed. Three days following the last treatment, each male was mated with two virgin females over a five day period. The male was then allowed to rest for two days, afterwhich the mating process was repeated with new virgin females until the males had been mated for seven weeks

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with two females per week. Fourteen days from the mid-point of the mating period, the females were sacrificed and the abdominal cavity exposed. The membrane was removed from each ovary and the corpora lutea for each ovary were counted and recorded separately. In addition, both uterine horns were examined and fetal deaths and total implantations were determined and recorded separately for each horn.

Nine parameters were analyzed in each study; fertility index, average number of implantations per pregnant female, corpora lutea per pregnant female, preimplantation losses per pregnant female, dead implants per pregnant female, proportion of pregnant females with one or more dead implants, proportion of pregnant females with two or more dead implants, dead implants per total implants, and live implants per pregnant female.

The statistical methods used to analyze the data (if appropriate) include the following; t-test, chi-square analysis, analysis of regression, analysis of linear trend, analysis of variance, and probit analysis.

The criteria of determination of a valid test is as follows: Females mated with negative control males must show a total of 8-15 implantations and females mated to positive control males must exhibit severe fetal damage. There must be a statistically significant reduction in implantations relative to the negative controls and there must be a statistically significant increase in females with 2 or more dead implants relative to the negative controls. This damage must be seen between weeks 2 and 7 of the spermatogenic cycle.

Results

Effects on Mitosis None

Genotoxic Effects Negative

Statistical Results Random increase in preimplantation loss on week 7 at 0.5 ml/kg/day and an increase in dead implants on week 5 at 1.5 and 0.5 ml/kg/day were statistically ($p<0.05$), but not clinically significant.

Results Remarks No adult mortality was observed. Body weight loss and decreased weight gain was observed in males treated with 0.5 and 1.5 ml/kg/day. No difference was found in the fertility index between the negative control and test substance. TEM had no adverse effect on the fertility index of the positive control group. On week 7, 0.5 ml/kg/day significantly increased ($p<0.05$) the implantations per pregnant female. TEM markedly reduced implantations on weeks l-4. The variability in the numbers of corpora lutea per pregnant female rats reflected the individual variation in the female rats rather than biological activity of the doses tested. On week 7, 0.5 ml/kg/day significantly increased ($p<0.01$) the preimplantation losses. TEM markedly increased preimplantation losses on weeks l-5. On week 5, 1.5 and 0.5 ml/kg/day significantly increased ($p<0.05$) the number of dead implants per pregnancy relative to the negative control. However, there was no statistical variation ($p<0.05$) from the historic control for both dose levels. The

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linear regression analysis failed to demonstrate a dose response when the arithmetic and logarithmic doses were compared to the negative control. TEM markedly increased the number of dead implants per pregnancy on weeks 1-5. The proportion of pregnant females with one or more dead implants was unaffected by the test substance. TEM markedly increased the proportion with one or more dead implants on weeks 1-5. The proportion of pregnant females with two or more dead implants was unaffected by the test substance. TEM markedly increased the proportion with two or more dead implants on weeks l-3 and 5. The average number of dead implants per total implants was significantly increased ($p<0.05$) by 1.5 ml/kg/day on week 5. TEM markedly increased the number of dead implants per total implants on weeks l-5. The average number of live implants per pregnant female was significantly reduced at 0.5 ml/kg/day on week 7. The linear regression analysis failed to demonstrate a dose response when the arithmetic and logarithmic doses were compared to the negative control. TEM markedly reduced the number of live implants per pregnant female on weeks l-5.

Conclusions

The positive and negative controls fulfilled the requirements of a valid test.

The increase at a single dose level in preimplantation losses on week 7 and the increase in dead implants on week 5 appear to occur as random yet statistically significant events. The reduction of total implants on week 7 is presumably due to the preimplantation embryonic loss. At all doses tested, the test substance failed to induce an increase in dead implants accompanied by a reduction in live implants over any single week or weeks corresponding to stages of spermatogenesis.

MCTR-139-79 is not a mutagen in this test system.

Data Quality

Reliability Acceptable

Data Reliability Remarks All key parameters (i.e., number of animals, concentrations, negative and positive controls, etc.) were appropriate and described in the study.

Reference

Remarks Activity of T1601 in the Dominant Lethal Assay in Rodents for Mutagenicity, Microbiological Associates study No. MCTR-139-79, December 31, 1980. MI 390-79.

General