

SCREENING-LEVEL HAZARD CHARACTERIZATION

p-Methylstyrene (CASRN 622-97-9)

The High Production Volume (HPV) Challenge Program¹ was conceived as a voluntary initiative aimed at developing and making publicly available screening-level health and environmental effects information on chemicals manufactured in or imported into the United States in quantities greater than one million pounds per year. In the Challenge Program, producers and importers of HPV chemicals voluntarily sponsored chemicals; sponsorship entailed the identification and initial assessment of the adequacy of existing toxicity data/information, conducting new testing if adequate data did not exist, and making both new and existing data and information available to the public. Each complete data submission contains data on 18 internationally agreed to “SIDS” (Screening Information Data Set^{1,2}) endpoints that are screening-level indicators of potential hazards (toxicity) for humans or the environment.

The Environmental Protection Agency’s Office of Pollution Prevention and Toxics (OPPT) is evaluating the data submitted in the HPV Challenge Program on approximately 1400 sponsored chemicals by developing hazard characterizations (HCs). These HCs consist of an evaluation of the quality and completeness of the data set provided in the Challenge Program submissions. They are not intended to be definitive statements regarding the possibility of unreasonable risk of injury to health or the environment.

The evaluation is performed according to established EPA guidance^{2,3} and is based primarily on hazard data provided by sponsors; however, in preparing the hazard characterization, EPA considered its own comments and public comments on the original submission as well as the sponsor’s responses to comments and revisions made to the submission. In order to determine whether any new hazard information was developed since the time of the HPV submission, a search of the following databases was made from one year prior to the date of the HPV Challenge submission to the present: (ChemID to locate available data sources including Medline/PubMed, Toxline, HSDB, IRIS, NTP, ATSDR, IARC, EXTOXNET, EPA SRS, etc.), STN/CAS online databases (Registry file for locators, ChemAbs for toxicology data, RTECS, Merck, etc.) and Science Direct. OPPT’s focus on these specific sources is based on their being of high quality, highly relevant to hazard characterization, and publicly available.

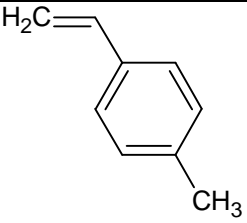
OPPT does not develop HCs for those HPV chemicals which have already been assessed internationally through the HPV program of the Organization for Economic Cooperation and Development (OECD) and for which Screening Initial Data Set (SIDS) Initial Assessment Reports (SIAR) and SIDS Initial Assessment Profiles (SIAP) are available. These documents are presented in an international forum that involves review and endorsement by governmental authorities around the world. OPPT is an active participant in these meetings and accepts these documents as reliable screening-level hazard assessments.

¹ U.S. EPA. High Production Volume (HPV) Challenge Program; <http://www.epa.gov/chemrtk/index.htm>.

² U.S. EPA. HPV Challenge Program – Information Sources; <http://www.epa.gov/chemrtk/pubs/general/guidocs.htm>.

³ U.S. EPA. Risk Assessment Guidelines; <http://cfpub.epa.gov/ncea/raf/rafguid.cfm>.

These hazard characterizations are technical documents intended to inform subsequent decisions and actions by OPPT. Accordingly, the documents are not written with the goal of informing the general public. However, they do provide a vehicle for public access to a concise assessment of the raw technical data on HPV chemicals and provide information previously not readily available to the public.

Chemical Abstract Service Registry Number (CASRN)	622-97-9
Chemical Abstract Index Name	Benzene, 1-ethenyl-4-methyl
Structural Formula	
Summary	
<p>CASRN 622-97-9 is a liquid with moderate water solubility and high vapor pressure. It is expected to have moderate mobility in soil. Volatilization of this chemical is considered high based on its Henry's Law constant. The rate of hydrolysis is considered negligible. The rate of atmospheric photooxidation is considered rapid. This chemical is expected to have moderate persistence (P2) and low bioaccumulation potential (B1).</p>	
<p>The acute toxicity of CASRN 622-97-9 is low via the oral (rats and mice), dermal (rabbits) and inhalation (rats) routes of exposure. CASRN 622-97-9 was not irritating to rabbit skin in an irritation study and was not irritating to rabbit eyes. Repeated 90-day oral exposures of rats to CASRN 622-97-9 significantly decreased survival and growth rates and caused severe irritation and lung lesions at 700 and 1500 mg/kg/day. The LOAEL and NOAEL values were 700 and 300 mg/kg/day, respectively. Dermal exposure of rabbits to CASRN 622-97-9 showed decreased body weight gain and dose-related changes in hematology and clinical chemistry parameters, indicative of an inflammatory response. The LOAEL was 500 mg/kg/day and the NOAEL was not established. Following the repeated inhalation exposure of rats to CASRN 622-97-9 for 13 weeks, the LOAEC and NOAEC values were 7.7 and 2.4 mg/L/day, respectively, based on mortality, decreased body weight and increased glutamate pyruvate transaminase and alkaline phosphatase activities. In the 2-generation study, the NOAEL for reproductive toxicity was 500 mg/kg-bw/day based on no effects at the highest dose tested. The LOAEL and NOAEL values for offspring toxicity were 500 and 200 mg/kg/day, respectively, based on pup mortality and decreased pup weights per litter. In two separate oral prenatal developmental toxicity studies in rats and rabbits, the NOAELs for maternal and developmental toxicity were 600 mg/kg-bw/day (highest dose tested) for rats and 150 mg/kg-bw/day (highest dose tested) for rabbits. CASRN 622-97-9 did not induce gene mutations <i>in vitro</i> or chromosome aberrations <i>in vivo</i>. It did not show evidence for carcinogenicity in cancer bioassays in rats and mice.</p>	
<p>For CASRN 622-97-9, the measured 96-hour LC₅₀ for fish was 2.8 mg/L, the measured 48-hour EC₅₀ for aquatic invertebrates was 1.3 mg/L and the measured 72-hour EC₅₀ was 4.3 mg/L for aquatic plants (growth rate) and 2.6 mg/L (biomass).</p>	
<p>No data gaps were identified under the HPV Challenge Program.</p>	

The sponsor, Deltech Corporation, submitted a Test Plan and Robust Summaries to EPA for *p*-methylstyrene (CASRN 622-97-9; CA name: benzene, 1-ethenyl-4-methyl) on May 15, 2001. EPA posted the submission on the RTK HPV Challenge Web site on July 10, 2001 (<http://www.epa.gov/chemrtk/pubs/summaries/pmstyrn/c13044tc.htm>). EPA comments on the submission were posted to the website on December 3, 2001. Public comments were also received and posted to the website.

1. Chemical Identity

1.1 Identification and Purity

The HPV submission⁴ for this chemical did not include information on identification and purity in the Test Plan (2001). However, where indicated in the robust summaries, the purity of the test substance was approximately 97% or assumed 100% (2001).

1.2 Physical-Chemical Properties

The physical-chemical properties of *p*-methylstyrene are summarized in Table 1. *p*-Methylstyrene is a liquid with moderate water solubility and high vapor pressure.

Property	Value
CASRN	622-97-9
Molecular Weight	118.18
Physical State	Liquid
Melting Point	-34°C
Boiling Point	173°C
Vapor Pressure	1.81 mm Hg at 25°C (estimated); 2.82 mm Hg at 31.8°C
Water Solubility	25–40 mg/L at 25°C
Dissociation Constant (pK _a)	Not applicable
Henry's Law Constant	3.01 × 10 ⁻³ atm·m ³ /mole (estimated)
Log K _{ow}	3.35

¹Deltech Corporation. May 15, 2001. Robust Summary for *p*-Methylstyrene.
<http://www.epa.gov/chemrtk/pubs/summaries/pmstyrn/c13044tc.htm>.

⁴ Deltech Corporation, 2001. Robust Summary Styrene, *p*-methyl- CASRN 622-97-9. Accessed 12/05/08.
<http://www.epa.gov/chemrtk/pubs/summaries/pmstyrn/c13044tc.htm>.

2. General Information on Exposure

2.1 Production Volume and Use Pattern

p-Methylstyrene had an aggregated production volume in the United States of 1 million to 10 million pounds during calendar year 2005.

Non-confidential information in the IUR⁵ indicated that the industrial processing and uses of this chemical include processing as an intermediate, or as a reactant in coloring agents and pigments; adhesives and binding agent; “other”. The HPV submission⁶ for this chemical did not include information on use. The HSDB indicated that this chemical is used as a reactive monomer in the production of polymers⁷.

2.2 Environmental Exposure and Fate

No quantitative information is available on releases of this chemical to the environment.

The environmental fate properties are provided in Table 2. *p*-Methylstyrene is a liquid with moderate water solubility and high vapor pressure. It is expected to have moderate mobility in soil. Volatilization of *p*-methylstyrene is considered high based on its Henry’s Law constant. The rate of hydrolysis is considered negligible. The rate of atmospheric photooxidation is considered rapid. *p*-Methylstyrene is expected to have moderate persistence (P2) and low bioaccumulation potential (B1).

⁵ USEPA, 2008. 2006 Confidential Inventory Update Reporting Database. Version 1.02

⁶ Deltech Corporation, 2001. Robust Summary Styrene, *p*-methyl- CASRN 622-97-9. Accessed 12/05/08.
<http://www.epa.gov/chemrtk/pubs/summaries/pmstyrn/c13044tc.htm>.

⁷ HSDB, 2008. Hazardous Substances Data Bank. 4-Vinyltoluene. Accessed, 12/17/08. <http://toxnet.nlm.nih.gov/>.

Table 2. Environmental Fate Characteristics of <i>p</i>-Methylstyrene¹	
Property	Value
Photodegradation Half-life	12.2 hours (estimated) Weakly absorbs light >290 nm
Hydrolysis Half-life	Stable
Biodegradation	>95% after 19 days (measured in acclimated, activated sludge) (primary removal by volatilization); 32% after 20 days (not readily biodegradable); 0% after 28 days (measured data for <i>m</i> - or <i>p</i> -ethylstyrene, CASRN 28106-30-1) ²
Bioconcentration	BCF = 31.6 (measured in goldfish); BCF = 4.9, 9.2, 4.0 (measured in channel catfish); BCF = 110 (measured in bluegill sunfish)
Log K _{oc}	2.6 to 3.2 (estimated)
Fugacity (Level I Model)	Air = 100% Water = 7.04×10 ⁻⁴ % Soil = 1.39×10 ⁻³ % Sediment = 3.09×10 ⁻⁵ % Suspended sediment = 9.65×10 ⁻⁵ %
Persistence ³	P2 (moderate)
Bioaccumulation ³	B1 (low)

¹Deltech Corporation. May 15, 2001. Robust Summary for *p*-Methylstyrene.

<http://www.epa.gov/chemrtk/pubs/summaries/pmstyrn/c13044tc.htm>.

²National Institute of Technology and Evaluation. 2002. Biodegradation and Bioconcentration of Existing Chemical Substances under the Chemical Substances Control Law.

http://www.safe.nite.go.jp/english/kizon/KIZON_start_hazkizon.html.

³Federal Register. 1999. Category for Persistent, Bioaccumulative, and Toxic New Chemical Substances. *Federal Register* 64, Number 213 (November 4, 1999) pp. 60194–60204.

Bold = measured data

3 Human Health Hazard

The human health hazard data are summarized in Table 3.

Acute Oral Toxicity

(1) CD-1 mice (5/sex/group, fasted) were administered *p*-methylstyrene in cottonseed oil via gavage at 215, 462, 993, 2135 or 4590 mg/kg-bw and observed for 14 days. Mortality was 0/10, 0/10, 4/10, 10/10 and 10/10, respectively. Surviving animals appeared normal by the end of the 14-day observation period.

LD₅₀ = 1072 mg/kg-bw

(2) Fischer 344 rats (10/sex/group, fasted) were administered *p*-methylstyrene in corn oil via gavage at 1260, 1780, 2510, 3550 or 5010 mg/kg-bw and observed for 14 days. The mortality was 0/20, 2/20, 7/20, 20/20 and 20/20, respectively. Surviving animals appeared normal by the end of the 14-day observation period. (The 5010 mg/kg-bw dose was not included in the LD₅₀ calculation.)

LD₅₀ = 2523 mg/kg-bw

(3) Sprague-Dawley rats (5/sex/group, fasted) were administered *p*-methylstyrene in olive oil via gavage at doses of 1480, 2000, 2700, 3650 (5 males and 4 females dosed) or 4935 mg/kg-bw and observed for 14 days. The mortality was 0/10, 0/10, 1/10, 1/9 and 5/10, respectively.

LD₅₀ = 4935 mg/kg-bw

(4) Swiss Webster mice (10/sex/group, fasted) were administered *p*-methylstyrene neat, in corn oil, or in olive oil via gavage at 600, 810, 1095, 1480 or 2000 mg/kg-bw and observed for 14 days. The mortality was 1/20, 5/20, 7/20, 18/20 and 20/20 for the neat dose groups; 1/20, 0/20, 7/20, 17/20 and 20/20 for the corn oil groups; and 2/20, 1/20, 4/20, 17/20 and 19/20 for the olive oil groups. Lower LD₅₀ values were consistently calculated for females compared with males for the neat test substance (reported below).

LD₅₀ = 1150 mg/kg-bw

Acute Inhalation Toxicity

(1) Sprague-Dawley rats (5/sex/dose) were exposed to *p*-methylstyrene vapor at a nominal concentration of 3500 ppm (approximately 16.9 mg/L) for 4 hours and were observed for 14 days. None of the rats died during the 14-day observation period.

LC₅₀ > ~16.9 mg/L

(2) Sprague-Dawley rats (5/sex/dose) were exposed to *p*-methylstyrene vapor at mean measured concentrations of 1960 ppm (first exposure, approximately 9.5 mg/L) for 4 hours and observed for 14-days followed by a second exposure at 1510 ppm (approximately 7.3 mg/L) for 4 hours and were observed for 14 days. Ocular and nasal irritation and neuromuscular impairment were seen at both concentrations; these signs were reversible within two days following cessation of exposure. One male rat died on day 1 after the first exposure. None of the rats died within the 14-day observation period.

LC₅₀ > ~9.5 mg/L

Acute Dermal Toxicity

New Zealand white rabbits (2/sex/dose) were administered *p*-methylstyrene dermally on to the backs at 0.5, 1.0, 2.0, 4.0 or 5.0 mL/kg-bw (corresponding to 500, 1000, 2000, 4000 or 5000 mg/kg-bw) for 24 hours and observed for 14 days; the application sites were not occluded. No mortality occurred during the study. Mild to moderate erythema and very mild edema were noted at the application sites. A dose-related occurrence of coriaceous (leathery) skin was seen in most rabbits with mild responses in the four lowest doses and moderate response at 5.0 mL/kg-bw.

LD₅₀ > ~5000 mg/kg-bw

Repeated-Dose Toxicity

Oral

(1) Fischer 344 rats (15/sex/dose) were administered *p*-methylstyrene (in olive oil) via gavage at 0, 50, 100, 300, 700 or 1500 mg/kg-bw/day for 90 consecutive days. The following tissues from all animals were examined microscopically: lungs, liver, kidneys, testes or ovaries and prostate or uterus. Statistically significant (significance not provided) increased mortality was observed at 700 (3/30) and 1500 (19/30) mg/kg-bw/day, compared with controls (0/30). The mean body weights for the treated males were consistently lower than that of the control group throughout the study, and the growth rate was significantly lower than that of the controls through week 13 for 700 and 1500 mg/kg-bw/day. No significant treatment-related effects were noted in gross histopathology, or in hematology, blood chemistry or urinalysis parameters. Rats at 700- and 1500-mg/kg-bw/day showed severe irritation of bronchioles and bronchiolar epithelium and exacerbation of focal hyperplasia of bronchioles and bronchiolar epithelium, and multifocal chronic pneumonitis.

LOAEL = 700 mg/kg-bw/day (based on mortality, lung lesions and reduced body weight and body weight gain)

NOAEL = 300 mg/kg-bw/day

(2) Beagle dogs (2/sex/group) were administered *p*-methylstyrene via gelatin capsules at 0, 30, 100, 300 or 1000 mg/kg-bw/day for 28 days. There was no effect on body weights. Absolute and relative liver weights were increased at 1000-mg/kg-bw/day compared with controls. No exposure-related effects were observed on survival, hematology, clinical chemistry or urinalysis. Clinical observations showed that doses of 300 mg/kg-bw/day and above produced tremors; however, no corresponding histopathology was noted. Tremors were observed only sporadically at 30 and 100-mg/kg-bw/day.

NOAEL = 1000 mg/kg-bw/day (based on no significant effects at the highest dose tested)

Inhalation

Sprague-Dawley rats (15/sex/concentration) were exposed (whole body) to *p*-methylstyrene vapor at 0, 100, 500 or 1600 ppm (reduced to 1300 ppm after 7 weeks due to excess mortality) for 6 hours/day, 5 days/week for 13 weeks. Mean measured concentrations were 0, 101, 505 and 1583 ppm (1313 ppm after 7 weeks; corresponding to 0, 0.5, 2.4 and 7.7 mg/L and 6.4 mg/L after 7 weeks). At the highest concentration: (1) six rats died (1 male, 5 females) during the first 8 weeks of the study; (2) mean body weights were statistically significantly ($p < 0.01$) decreased compared with controls; (3) serum glutamate pyruvate transaminase (both sexes) and alkaline phosphatase (females) activities were increased after 5 weeks; and (4) significantly elevated absolute and relative liver and ovarian weights were observed in females. Gross and microscopic examination of tissues found no exposure-related effects. No exposure-related adverse effects were apparent at 101 or 505 ppm.

LOAEL = 7.7 mg/L (based on mortality, reduced body weight, increased serum glutamate pyruvate transaminase and alkaline phosphatase activities)

NOAEL = 2.4 mg/L

Dermal

New Zealand white rabbits (3/sex/group) were administered undiluted *p*-methylstyrene dermally on to shaved application sites at 0, 0.5 or 2.0 mL/kg-bw/day (corresponding to approximately 0, 500 or 2000 mg/kg-bw/day) for 6 hours/day for 21 consecutive days. By the final week of the study, moderate and severe skin irritation (marked coriaceous skin, fissuring and sloughing of the skin at the application site) was observed at 0.5 and 2.0-mL/kg-bw/day, respectively. Dose-related reduction in body weight gain was observed at both doses. Effects indicative of an inflammatory process associated with this skin irritation were (1) a dose-related increase in neutrophils and (2) increased activities of SGOT in both exposed groups. No exposure-related effects were noted during gross and microscopic examination of tissues, other than the skin.

LOAEL = 500 mg/kg-bw/day (based on reduced body weight gain and changes in hematology and clinical chemistry parameters indicative of an inflammatory response)

NOAEL = not established

Reproductive Toxicity

In a two-generation reproductive toxicity study, Sprague-Dawley rats (25-30/sex/dose) were administered *p*-methylstyrene (in olive oil) via gavage at 0, 25, 200 or 500 mg/kg-bw/day for 14 weeks prior to mating. Another group received 600 mg/kg-bw/day for one generation—excess mortality precluded evaluation of reproductive performance in the F1 generation at this dose. Treatment continued through mating, gestation, delivery, and lactation. F1 weanling rats were selected for continued treatment and evaluation (40/sex/dose; 20/sex/control group) and were treated for 17 weeks before mating, and during mating, gestation, delivery, and lactation. Necropsy was performed on F0 and F1 adults (10 males and 25 females/generation/dose) at the end of respective lactation periods. Histopathological evaluation of tissues was conducted from sacrificed adults from the control and 500-mg/kg-bw/day groups. F0 results: Mortality was 2/54, 0/25, 2/25, 10/30 and 12/25 (males) and 2/55, 1/25, 3/27, 7/30 and 14/25 (females) in the 0, 25, 200, 500 and 600 mg/kg-bw/day groups, respectively. Dose-related decrease in body-weight gain was observed at doses ≥ 200 mg/kg-bw/day (no magnitude or statistical significance was provided). No statistically significant differences between the treated and control groups were found in the following reproductive function parameters: fertility index, duration of gestation, gestation index and mean number of implantations per litter. At the two highest doses, small increases in pup mortality and decreases in pup body weight per litter were observed. Gross necropsy and microscopic examination of tissues revealed no treatment-related effects, except for tracheo-bronchial lesions which were regarded as effects from aspiration of gavaged test material. F1 results: Mortality was 0/40, 0/40, 1/40 and 19/40 (males) and 1/40, 2/40, 3/40 and 8/40 (females) at 0, 25, 200, 500 and 600 mg/kg-bw-day, respectively. Treatment-related clinical signs were similar to those described for the F0 generation. Dose-related decrease in body-weight gains were observed at doses ≥ 25 and at 500 mg/kg-bw/day in males and females, respectively, compared with controls (no magnitude or statistical significance was provided). No statistically significant differences were seen between the treatment and control groups in the following reproductive function parameters: percentage of rats which mated, mean number of days required to mate, fertility index, duration of gestation, gestation index, pup mortality per litter, pup sex ratio or pup body weight per litter. A small increase in the incidence of stillborn pups was observed at 500 mg/kg-bw-day, but this was not statistically significantly different from controls. Gross necropsy and microscopic examination of tissues revealed no treatment-

related effects, except for tracheo bronchial lesions which were regarded as effects from aspiration of gavaged test material.

NOAEL (reproductive toxicity) = 500 mg/kg-bw/day (based on no effects at the highest dose tested)

LOAEL (offspring toxicity) = 500 mg/kg-bw/day (based on increase in F1 and F2 pup mortality and decreased pup weight per litter)

NOAEL (offspring toxicity) = 200 mg/kg-bw/day

Developmental Toxicity

(1) Pregnant Sprague-Dawley rats (20/dose) were administered single doses of *p*-methylstyrene (in corn oil) via gavage at 0, 60, 190 or 600 mg/kg-bw/day during gestation days 6 through 15. There were no significant differences between any treatment group and the control group in maternal body weight, pregnancy, implantation, numbers of live or dead fetuses or number of resorptions per dam. Elevated incidences of litters or fetuses with skeletal or soft tissue anomalies or malformations were not consistently observed in treated groups. At 60-mg/kg-bw/day, increased litters with fetuses with rudimentary ribs or delayed ossification of vertebrae were observed. At 190-mg/kg-bw/day, increased litters with fetuses with extra ribs or delayed ossification of vertebrae were observed. However, increased incidences of these skeletal variations were not seen at 600 mg/kg-bw/day.

NOAEL (maternal/developmental toxicity) = 600 mg/kg-bw/day (based on no effects at the highest dose tested)

(2) Pregnant CD-1 rats (25 /dose) were administered *p*-methylstyrene (in olive oil) via gavage at 0, 50, 300 or 600 mg/kg-bw/day during gestation days 6 through 19. A reduction in mean maternal body weight gain (not statistically significant) was noted in the treated groups compared with the control group. There were no significant differences in mean number of viable fetuses, early or late resorptions, post-implantation loss, total implantations or fetal sex distribution between any treatment group and the control group. Malformations were restricted to a meningocele (protrusion of the spinal membrane) observed in one fetus in one litter of the 600-mg/kg-bw/day group. The incidences of fetuses or litters with skeletal or visceral variations in treated groups were comparable to the control group.

NOAEL (maternal/developmental toxicity) = 600 mg/kg-bw/day (based on no effects at the highest dose tested)

(3) Pregnant Dutch Belted rabbits (16/dose) were administered undiluted *p*-methylstyrene via gavage at 0, 50, 100 or 150 mg/kg-bw/day during gestation days 6 through 27. No treatment-related differences were noted for maternal body weight gain or survival, mean numbers of corpora lutea, total implantations, early or late resorptions, post-implantation loss, viable fetuses, fetal sex distributions or mean fetal weight. No fetuses with malformations were noted in treated or control groups. Incidences of fetuses or litters with variations in skeletal or soft tissues were comparable to the control group.

NOAEL (maternal/developmental toxicity) = 150 mg/kg-bw/day (based on no effects at the highest dose tested)

Genetic Toxicity – Gene Mutation

In vitro

(1) *Salmonella typhimurium* strains TA98, TA100, TA 1535, TA1537 and TA1538, were exposed to *p*-methylstyrene at 0.001, 0.01, 0.1 or 5.0 $\mu\text{L}/\text{plate}$, with and without metabolic activation. Both positive and negative controls were included, and positive controls produced an appropriate response. Cytotoxicity was not evident at any of the concentrations tested, except at 5 $\mu\text{L}/\text{plate}$ in strain TA1537.

***p*-Methylstyrene was not mutagenic in this assay.**

(2) Mouse lymphoma L5178Y cells were exposed to *p*-methylstyrene at 0.02, 0.03, 0.04, 0.06 or 0.08 $\mu\text{L}/\text{mL}$, with and without metabolic activation. Both positive and negative controls were included, and positive controls produced an appropriate response. Cytotoxicity was not evident at any of the concentrations tested.

***p*-Methylstyrene was not mutagenic in this assay.**

Genetic Toxicity – Chromosomal Aberrations

In vivo

(1) Sprague-Dawley rats (5 males/dose) were administered *p*-methylstyrene (in olive oil) via gavage at 0, 0.15, 0.5 or 1.5 $\text{mL}/\text{kg-bw}/\text{day}$ (corresponding to 0, ~150, 500 or 1500 $\text{mg}/\text{kg-bw}/\text{day}$) for 5 consecutive days. Triethylenemelamine was used as a positive control (single intraperitoneal injection one day prior to sacrifice). Animals were sacrificed 24 hours after the dose administration, and the percentages of bone marrow cells showing chromosomal aberrations and the number of aberrations per cell were assessed. The positive control increased the frequency of cells with chromosomal aberrations by about 4-fold, compared with the negative control group. The percentages of cells with chromosomal aberrations in the *p*-methylstyrene exposed groups were comparable to the negative control group.

***p*-Methylstyrene did not induce chromosomal aberrations in this assay.**

(2) Sprague-Dawley rats (6 males/dose) were administered *p*-methylstyrene (n methocel K4M) via gavage at 0, 134, 450 or 1340 $\text{mg}/\text{kg-bw}/\text{day}$ for 5 consecutive days. Cyclophosphamide was used as a positive control. Animals were sacrificed 24 hours after the dose administration and the percentages of bone marrow cells showing chromosomal aberrations and the number of aberrations per cell were assessed. The positive control increased the frequency of cells with chromosomal aberrations by about 80-fold, compared with the negative control group. The percentages of cells with chromosomal aberrations in the *p*-methylstyrene exposed groups were comparable to the negative control group.

***p*-Methylstyrene did not induce chromosomal aberrations in this assay.**

Genetic Toxicity – Other Endpoints

In vitro

(1) *Saccharomyces cerevisiae* strain D5 was exposed to *p*-methylstyrene at 0.01, 0.10, 0.50, 1.0, 2.0 or 2.50 $\mu\text{L}/\text{plate}$, with and without metabolic activation. Both positive and negative controls were used in each study. The highest concentration was cytotoxic. *p*-Methylstyrene induced mitotic recombination events to high frequencies (with or without metabolic activation), exceeding positive control values by two- to three-fold.

***p*-Methylstyrene induced mitotic recombination events in this assay.**

(2) In an Unscheduled DNA synthesis (UDS) assay, primary rat hepatocytes were exposed to *p*-methylstyrene at 0.625, 1.25, 2.5, 5.0, 10, 20 or 40 nL/mL. Both positive and negative controls were used in the study. Cytotoxicity was seen at 40 nL/mL. Increased UDS activity was detected at 5, 10 and 20 nL/mL.

***p*-Methylstyrene induced unscheduled DNA synthesis in this assay.**

(3) In the Sister Chromatid Exchange (SCE) assay, cultured human lymphocytes were exposed to *p*-methylstyrene at concentrations ranging from 1 to 100 nL/mL. Negative and positive controls were included, and the positive control produced an appropriate response. Several concentrations between 50 and 100 nL/mL induced higher SCE frequencies than controls in three separate trials, but the maximum magnitude was about 1.84-fold greater than control frequencies (i.e., none were greater than 2-fold higher than negative control frequencies). Dose-related cell toxicity was evident at all test concentrations, based on a reduction in the frequency of dividing cells (mitotic index) and cell cycle delay. The positive control produced an appropriate response. The robust summary concluded that a “weak positive” response was observed, given that small, but reproducible increases in SCE frequencies were induced by the test material.

***p*-Methylstyrene was weakly positive in the induction of SCE in this assay.**

In vivo

In the dominant lethal assay, Sprague-Dawley rats (10 males/group) were administered *p*-methylstyrene (in olive oil) via gavage at 0, 0.15, 0.5 or 1.5 mL/kg-bw/day for 5 consecutive days. Triethylenemelamine was used as a positive control (single intraperitoneal injection on day 4). Three days after treatment, exposed and control males were mated with non-exposed females. The mating process was repeated through 7 weeks of mating. Mated females were sacrificed fourteen days from the mid-point of each mating period and fetal deaths and total implantations were assessed from each uterine horn. Although a few statistically significant events were sporadically recorded for some of the *p*-methylstyrene-treated groups, *p*-methylstyrene failed to induce an increase in dead implants along with a reduction in live implants compared with the negative control group. The positive control responded appropriately.

***p*-Methylstyrene did not induce dominant lethal mutations in this assay.**

Additional Information

Skin Irritation

(1) New Zealand white rabbits (6 males) were administered *p*-methylstyrene (0.5 mL) dermally on to clipped and abraded areas of skin and observed at 24 and 72 hours after application for erythema, eschar formation and edema. The application site was not occluded (because the test substance reacted with the dental dam), but the animals were harnessed to prevent oral treatment. At 24 and 72 hours, very mild erythema and edema were noted.

***p*-Methylstyrene was not irritating to rabbit skin.**

Eye Irritation

Rabbits (males and females, number and strain not stated) were instilled 0.1 mL of *p*-methylstyrene into the eye. The eyes were not washed. The observations were recorded for up to seven days. The Draize scores were 4.7, 3.7, 2.0, 0.7, 0.7 and 0.3 for 1 hour, 1, 2, 3, 4 and 7 days, respectively.

***p*-Methylstyrene was not irritating to rabbit eyes.**

Carcinogenicity

(1) Swiss mice (60/sex/dose) were administered *p*-methylstyrene (in olive oil) via gavage at 0, 10, 50 or 250 mg/kg-bw/day, 5 days/week for 83 weeks. There were no differences between the exposed and control groups (in either sex) in the incidence of animals with any specific tumor type.

***p*-Methylstyrene was not carcinogenic in this study.**

(2) Sprague-Dawley rats (60/sex/dose except for the highest dose group, which had 90/sex/group) were administered *p*-methylstyrene (in olive oil) via gavage at 0, 10, 50, 250 or 500 mg/kg-bw/day for 108 weeks. Rats were allowed to live for up to 122 weeks. A complete necropsy was performed on each rat, and microscopic examination of preserved tissues was performed for rats in the control and 500-mg/kg-bw/day group. Reduced survival was observed in males at 250 and 500 mg/kg-bw/day, beginning at about six months. Survival in these groups in the second year was about 20% lower than survival in the control group. No significant treatment-related effects were seen between treated and control groups in SGOT, SGPT, or alkaline phosphatase activities (assays were performed at 26, 54, 78, and 107 weeks). No specific or unique neoplastic lesions were observed in the treatment groups, and no significant differences were found between treated and control groups in the incidences of tumors of any type.

***p*-Methylstyrene was not carcinogenic in this study.**

Conclusion: The acute toxicity of CASRN 622-97-9 is low via the oral (rats and mice), dermal (rabbits) and inhalation (rats) routes of exposure. CASRN 622-97-9 was not irritating to rabbit skin in an irritation study and was not irritating to rabbit eyes. Repeated 90-day oral exposures of rats to CASRN 622-97-9 significantly decreased survival and growth rates and caused severe irritation and lung lesions at 700 and 1500 mg/kg/day. The LOAEL and NOAEL values were 700 and 300 mg/kg/day, respectively. Dermal exposure of rabbits to CASRN 622-97-9 showed decreased body weight gain and dose-related changes in hematology and clinical chemistry parameters, indicative of an inflammatory response. The LOAEL was 500 mg/kg/day and the NOAEL was not established. Following the repeated inhalation exposure of rats to CASRN 622-97-9 for 13 weeks, the LOAEC and NOAEC values were 7.7 and 2.4 mg/L/day, respectively, based on mortality, decreased body weight and increased glutamate pyruvate transaminase and alkaline phosphatase activities. In the 2-generation study, the NOAEL for reproductive toxicity was 500 mg/kg-bw/day based on no effects at the highest dose tested. The LOAEL and NOAEL values for offspring toxicity were 500 and 200 mg/kg/day, respectively, based on pup mortality and decreased pup weights per litter. In two separate oral prenatal developmental toxicity studies in rats and rabbits, the NOAELs for maternal and developmental toxicity were 600 mg/kg-bw/day (highest dose tested) for rats and 150 mg/kg-bw/day (highest dose tested) for rabbits. CASRN 622-97-9 did not induce gene mutations *in vitro* or chromosome aberrations *in vivo*. It did not show evidence for carcinogenicity in cancer bioassays in rats and mice.

4. Hazards to the Environment

The environmental hazard data are summarized in Table 3.

Acute Toxicity to Fish

(1) Bluegill sunfish (*Lepomis macrochirus*) were exposed to *p*-methylstyrene at nominal concentration of 0, 0.76, 1.5, 3.0, 6.1 or 12 mg/L under flow-through conditions for up to 7 days. The respective mean measured concentrations were 0, 0.41, 0.66, 1.2, 2.3 or 5.4 mg/L. Mortality occurred at 2.3 mg/L and above.

96-h LC₅₀ = 2.8 mg/L

(2) Fathead minnow (*Pimephales promelas*) were exposed to *p*-methylstyrene at nominal concentrations of 0, 1.6, 2.5, 4.0, 6.4 or 10 mg/L under semi-static conditions for 96 hours. The respective measured concentrations were 0, 0.82, 1.3, 2.6, 4.2 or 6.8 mg/L. Mortality was seen at 4.2 mg/L and above. (Due to the high volatility of the test substance, the test was conducted in sealed containers with little or no head space.)

96-h LC₅₀ = 5.2 mg/L

Acute Toxicity to Aquatic Invertebrates

Waterflea (*Daphnia magna*) were exposed to *p*-methylstyrene at nominal concentrations of 0, 0.95, 1.5, 2.4, 3.8 or 6.0 mg/L under static conditions for 48 hours. The respective measured concentrations were 0, 0.51, 0.81, 1.5, 2.3 or 3.8 mg/L. The study was conducted in a closed system to limit evaporation of *p*-methylstyrene.

48-h EC₅₀ = 1.3 mg/L

Toxicity to Aquatic Plants

Green algae (*Pseudokirchneriella subcapitata*) were exposed to *p*-methylstyrene at nominal concentrations of 0, 1.0, 2.0 4.0, 8.0 or 16 mg/L under static conditions (sealed vessels with no head space) for 72 hours.

72-h EC₅₀ (growth) = 4.3 mg/L

72-h EC₅₀ (biomass) = 2.6 mg/L

Conclusion: For CASRN 622-97-9, the measured 96-hour LC₅₀ for fish was 2.8 mg/L, the measured 48-hour EC₅₀ for aquatic invertebrates was 1.3 mg/L and the measured 72-hour EC₅₀ was 4.3 mg/L for aquatic plants (growth rate) and 2.6 mg/L (biomass).

Table 3: Summary Table of the Screening Information Data Set as Submitted under the U.S. HPV Challenge Program	
Endpoints	SPONSORED CHEMICAL <i>p</i>-Methylstyrene (622-97-9)
Summary of Human Health Data	
Acute Oral Toxicity LD₅₀ (mg/kg-bw)	1100
Acute Inhalation Toxicity LC₅₀ (mg/L)	> ~16.9
Acute Dermal Toxicity LD₅₀ (mg/kg-bw)	> ~5000
Repeated-Dose Toxicity NOAEL/LOAEL Oral (mg/kg-bw/day)	(rat) NOAEL = 300 LOAEL = 700 (dog, 28-d) NOAEL = 1000 (hdt)
Repeated-Dose Toxicity NOAEL/LOAEL Dermal (mg/kg-bw/day)	(Rabbit) NOAEL = not established LOAEL = 500
Repeated-Dose Toxicity NOAEL/LOAEL Inhalation (mg/L/day)	NOAEL = 2.4 LOAEL = 7.7
Reproductive Toxicity NOAEL/LOAEL Oral (mg/kg-bw/day) Reproductive Toxicity Offspring Toxicity	NOAEL = 500 (hdt) NOAEL = 200 LOAEL = 500
Developmental Toxicity NOAEL/LOAEL Oral (mg/kg-bw/day)	(rat) NOAEL = 600 (hdt) (rabbit) NOAEL = 150 (hdt)
Genetic Toxicity – Gene Mutation <i>In vitro</i>	Negative
Genetic Toxicity – Chromosomal Aberrations <i>In vivo</i>	Negative

Table 3: Summary Table of the Screening Information Data Set as Submitted under the U.S. HPV Challenge Program	
Endpoints	SPONSORED CHEMICAL <i>p</i> -Methylstyrene (622-97-9)
Genetic Toxicity – DNA Effects <i>In vitro</i> Sister Chromatid Exchange Mitotic Recombination assay Unscheduled DNA Synthesis <i>In vivo</i> Dominant Lethal Assay	Weakly positive Positive Positive Negative
Additional Information Skin Irritation Eye Irritation Carcinogenicity	Not irritating Not irritating Not carcinogenic
Summary of Environmental Effects – Aquatic Toxicity Data	
Fish 96-h LC ₅₀ (mg/L)	2.8
Aquatic Invertebrates 48-h EC ₅₀ (mg/L)	1.3
Aquatic Plants 72-h EC ₅₀ (mg/L)	
(growth)	4.3
(biomass)	2.6

hdt = highest dose tested; **Bold = Measured data**