Reconsideration of Six Chemicals Listed under Proposition 65 as Known to Cause Reproductive Toxicity

Chemicals Listed via the Labor Code Mechanism:

n-Butyl glycidyl ether

Diglycidyl ether

Phenyl glycidyl ether

Methyl n-butyl ketone

Methyl isopropyl ketone

α-Methyl styrene

Office of Environmental Health Hazard Assessment
California Environmental Protection Agency

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Background

Proposition 65[1] requires the State to publish a list of chemicals known to cause cancer or reproductive toxicity. This list must be updated at least once a year. Reproductive toxicity includes developmental toxicity, and female and male reproductive toxicity. Chemicals added to the list as known to cause reproductive toxicity affect one or more of these endpoints.

The chemicals covered in this document (See Table 1 below) were added to the list as known to cause reproductive toxicity because they were identified by reference as such in the California Labor Code. Proposition 65 thus required their inclusion on the list, as discussed in greater detail below. There are three additional ways for a chemical to be added to the Proposition 65 list: 1) The Developmental and Reproductive Toxicant Identification Committee (DART IC) finds that the chemical has been clearly shown to cause reproductive toxicity. 2) An organization designated as an "authoritative body" by the DART IC has identified it as causing reproductive toxicity[2]. 3) An agency of the state or federal government requires that it be labeled or identified as causing reproductive toxicity.

Reason for Reconsideration of Listing

Because of recent changes in federal regulations, the chemicals identified in Table 1 no longer meet the criteria for inclusion on the list on the basis of the Labor Code mechanism. Each of these chemicals are being presented to the DART IC for a decision as to whether they have been clearly shown through scientifically valid testing according to generally accepted principles to cause reproductive toxicity. If the Committee makes that determination, the chemical will remain on the list.

The six chemicals were added to the list on the basis of a Threshold Limit Value (TLV) developed by the American Conference of Governmental Industrial Hygienists (ACGIH) that was based on reproductive or developmental toxicity. All the chemicals in Table 1

[1] The Safe Drinking Water and Toxic Enforcement Act of 1986: Health and Safety Code section 25249.5 *et seq.,* passed by voter initiative.

[2] Title 27, California Code of Regulations, section 25306(l). The authoritative bodies are: U.S. Environmental Protection Agency, U.S. Food and Drug Administration, National Institute for Occupational Safety and Health, National Toxicology Program solely as to final reports of the National Toxicology Program's Center for Evaluation of Risks to Human Reproduction, and International Agency for Research on Cancer solely as to transplacental carcinogenicity.

were listed as known to cause reproductive toxicity based on their ACGIH TLV. The TLV provided a basis for listing via the Labor Code because:

- Proposition 65 provides that the list of chemicals known to the state to cause reproductive toxicity "shall include at a minimum those substances identified by reference in Labor Code Section 6382(b)(1) and those substances identified additionally by reference in Labor Code Section 6382(d)[3]".

- California Labor Code Section 6382(d) further provides that "…any substance within the scope of the federal Hazard Communication Standard (29 C.F.R. Section 1910.1200) is a hazardous substance subject to this chapter".

- Until 2012, the federal Hazard Communication Standard (HCS) incorporated TLVs as a definitive source for establishing that a chemical is hazardous.

In March 2012, the federal HCS was amended to remove reference to ACGIH TLVs as a mandatory basis for establishing that chemicals are hazardous. Consequently, a TLV based on reproductive or developmental toxicity no longer provides the basis for listing a chemical as known to the state to cause reproductive toxicity under Propostion 65.

Table 1. Chemicals under Reconsideration for Listing as Known to Cause Reproductive Toxicity

Chemical	CAS Number	Basis for TLV
n-Butyl glycidyl ether	2426-08-6	Male reproductive toxicity ("reproductive damage")
Diglycidyl ether	2238-07-5	Male reproductive toxicity ("male reproductive damage")
Phenyl glycidyl ether	122-60-1	Male reproductive toxicity ("testicular damage")
Methyl n-butyl ketone	591-78-6	Male reproductive toxicity ("testicular damage")
Methyl isopropyl ketone	563-80-4	Developmental toxicity ("embryo/fetal damage")
α-Methyl styrene	98-83-9	Female reproductive toxicity ("female reproductive damage")

[3] Health and Safety Code section 25249.8(a)

Reconsideration Procedure

These chemicals are being brought to the DART IC because they do not meet the criteria for inclusion on the list by any of the administrative listing mechanisms outlined above.

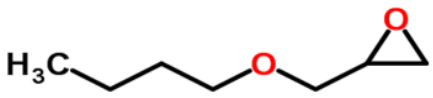
The Office of Environmental Health Hazard Assessment (OEHHA) has, through a contract with the Sheldon Margen Public Health Library at the University of California, Berkeley, conducted literature searches to identify studies that potentially provide information on the reproductive toxicity of each chemical. The searches covered the three major reproductive toxicity endpoints, namely developmental toxicity and male and female reproductive toxicity. The databases searched and parameters used in these searches are described in Appendix A.

The results of these searches were reviewed by OEHHA staff and all studies that provided data on reproductive toxicity were identified. For each chemical the design parameters and results of these studies on male reproductive, female reproductive and developmental toxicity are summarized in a table, except as specified below. The complete study reports for these chemicals have been provided to the DART IC and are available to the public upon request. Relevant studies were identified for all of the chemicals.

For completeness, the original ACGIH documents supporting development of the TLVs have also been provided to the DART IC on CD. These documents were not used in the process that resulted in the listing under Proposition 65 of the chemicals identified in Table 1. Rather, identification of a TLV based in whole or in part on a reproductive toxicity endpoint in the document “Threshold Limit Values for Chemical Substances and Physical Agents in the Environment, American Conference of Governmental Industrial Hygienists (ACGIH)” (latest edition) resulted in the listing. Relevant entries from that document also have been provided on CD to the committee.

Glycidyl ethers

n-Butyl glycidyl ether



Molecular Formula: $C_7H_{14}O_2$

Used as a reactive diluent in epoxy resins, and as an acid acceptor for stabilizing chlorinated solvents and chemical intermediates.

Relevant Studies

Anderson, H., C. Hine, R. Guzman and J. Wellington (1957). Chronic Vapor Toxicity of n-Butyl Glycidyl Ether. Prepared by the Department of Pharmacology and Experimental Therapeutics, UCSF for the Shell Development Company.

Pullin, T. G. and M. S. Legator (1977). Integrated Mutagenicity Testing Program on Several Epoxy Compounds. R&D Report Dow Chemical.

Whorton, E. B., Jr., T. G. Pullin, A. F. Frost, A. Onofre, M. S. Legator and D. S. Folse (1983). "Dominant lethal effects of n-butyl glycidyl ether in mice". Mutat Res **124**(3-4): 225-33.

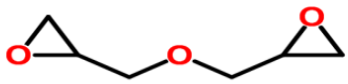
n-Butyl glycidyl ether

Reference	Experimental Parameters					Endpoints Assessed	Results (Effects/NOEL/LOEL)		Comments
	Chemical (Source/ Purity/ Preparation)	Animal Model (Species/ Strain/Sex/ Age) N	Study Design	Exposure (Route/ Period/ Frequency/ Vehicle)	Doses or Concen-trations		Systemic Toxicity	Reproductive Toxicity	
Anderson et al. 1957 (Shell)	n-Butyl glycidyl ether Source/purity not stated	Rats male pubertal (by weight) N=10/group	Subchronic Toxicity	Inhalation 7 h/day. 5 days/week, 10 weeks	0 (uncontaminated air), 0.2, 0.4, 0.8, 1.6 g/m^3 (38, 75, 150, 300 ppm)	Organ weight and pathology at end of study	↑ mortality: 10% 0.8 g/m^3; 50% 1.6 g/m^3 Reduced weight gain at 0.8 ($p<0.01$) and 1.6 ($p<0.05$) g/m^3 Increased lung and liver weights, 1.6 g/m^3 (statistically significant) Bronchopneumonia and organ pathology seen in lung, liver above 0.2 g/m^3 Animals reported to be normal in appearance and activity in 0.2 and 0.4 g/m^3 groups	Atrophic testes in 4/5 surviving animals (and 1 animal that died after 40 exposures) at 1.6 g/m^3; very small testes in 1/10 at 1.6 g/m^3; slight patchy testes atrophy in 1/10 at 0.4 g/m^3. Only 1 case with testes atrophy had no other organ pathology (0.4 g/m^3).	Testes pathology considered nonspecific by study authors
Pullin and Legator 1977 (Dow)	n-Butyl glycidyl ether Source/purity not stated	B6D2F1 male mice 8-10 weeks old N=10/group	Dominant Lethal	Dermal on depilated back, 3×/week, 8 weeks; 1 week before mating	0 (saline), 1.5 g/kg. Positive control triethylene melamine	Pregnancy rate, implantations, fetal mortality	Not reported	Lower pregnancy rate one and two weeks after exposure ($p=0.05$) Greater fetal mortality and postimplantation loss ($p=0.04$). LOEL 1.5 g/kg	

n-Butyl glycidyl ether

Reference	Experimental Parameters					Endpoints Assessed	Results (Effects/NOEL/LOEL)		Comments
	Chemical (Source/ Purity/ Preparation)	Animal Model (Species/ Strain/Sex/ Age) N	Study Design	Exposure (Route/ Period/ Frequency/ Vehicle)	Doses or Concen-trations		Systemic Toxicity	Reproductive Toxicity	
Whorton et al. 1983	n-Butyl glycidyl ether Shell-Petroleum, >95% pure	BDF mice 8-10 weeks of age N=36-42 males/group, 3 females/week /male	Dominant Lethal (2 replicate experiments conducted)	Dermal on depilated back, 3×/week for 8 weeks	0 (saline), 0.375, 0.75, 1.5 g/kg-d No positive control	Males: weekly body weight, testicular pathology Females: pregnancy, implants, fetal death	Body weight taken but not reported	No significant dose-related testicular changes Greater fetal death rate, 1.5 g/kg-d, 1st week of mating only in the second experiment ($p<0.05$)	Discrepancy between control fetal death rate in 2 experiments: considered unusually high in the first experiment

Diglycidyl ether



Molecular Formula: $C_6H_{10}O_3$

Diglycidyl ether is used as a diluent for epoxy resins, as a textile-treating agent and as a stabilizer for chlorinated organic compounds.

Relevant Studies

Hine, C. H., J. K. Kodama, R. J. Guzman, M. K. Dunlap, R. Lima and G. S. Loquvam (1961). "Effects of diglycidyl ether on blood of animals". Arch Environ Hlth **2**: 31-44.

Diglycidyl ether

Reference	Experimental Parameters					Endpoints Assessed	Results (Effects/NOEL/LOEL)		Comments
	Chemical (Source/ Purity/ Preparation)	Animal Model (Species/ Strain/Sex/ Age) N	Study Design	Exposure (Route/Period/ Frequency/ Vehicle)	Doses/ Concen-trations		Systemic Toxicity	Reproductive Toxicity	
Hine et al. 1961	Diglycidyl ether (DGE) Shell Oil Company Purity not provided	Young Long-Evans male rats (115 to 145 g) N=5/group	Effect of DGE on the circulating blood and blood production, using 3 animal models and various routes of administration.	Cutaneous repeated application on shaved back. DGE applied daily, 5 days per week for 4 weeks.	Applications of 0, 125, 250 or 500 mg/kg	Peripheral blood, bone, marrow, body weight, and mortality Physical observation and histology for testes, skin, lymphoid, kidney, adrenal medulla and pancreas at necropsy	Weight loss at all doses. 2 deaths each in the 125, 250 and 500 mg/kg groups. Applications of 250 and 500 mg/kg were stopped after the sixth application, because of the 2 deaths that had already occurred in each group. Blood parameters such as leukocyte count were reduced in all 3 treated groups. Necrosis of the skin, lymphoid and kidney. Hemorrage of the adrenal medulla	Focal necrosis of the testes at all doses. No specific findings for the different dose groups were provided (p values not provided)	
					Applications of 15, 30 or 60 mg/kg (no concurrent control group reported)	As above	Weight gain reported to be significantly retarded at 30 and 60 mg/kg (no data provided). No deaths; no visceral abnormalities.	No adverse effect on testes reported. No data provided.	

Diglycidyl ether (continued)

Reference	Experimental Parameters					Endpoints Assessed	Results (Effects/NOEL/LOEL)		Comments
	Chemical (Source/ Purity/ Preparation)	Animal Model (Species/ Strain/Sex/ Age) N	Study Design	Exposure (Route/Period/ Frequency/ Vehicle)	Doses/ Concen-trations		Systemic Toxicity	Reproductive Toxicity	
Hine et al. 1961 (continued)	Diglycidyl ether Shell Oil Company Purity not provided	Young Long-Evans male rats (115 to 145 g) N=30/exposed group N=10 unexposed controls	Chronic exposure study. After the final exposure the control rats and 15 exposed rats were killed for necropsy No information on testicular parameters provided on the remaining exposed rats.	Repeated inhalation; exposed for 4 h/day, 5 days/week (total of 19 exposures over 29 days).	Target concentration 3 ppm (Actual concentration varied between 1.3 and 2.5 ppm)	Same as above	5 animals died, histology on 3 of them reported: One had bronchopneumonia and necrosis of the pancreas and spleen; the second had pneumonia, and the third was apparently normal. Reduced percentage body weight gain (reported to be significant; p value not provided). Effects on total leukocyte count, percentage of polymorphonuclear (PMN) cells, number of nucleated cells in the femoral marrow.	In the 15 treated animals: one case of necrosis of the tubules of the testes. The authors reported an apparent increase in testes weight (believed to reflect the retardation in weight gain)	
		Long-Evans male rats N=10	Chronic exposure study	Inhalation. exposure for 4 h/day, 5 days/ week for 60 exposures in 90 days	0.3 ppm	Same as above	One animal had acute peribronchiolitis. Not reported if it was one of the 5 showing reproductive toxicity. No other systemic toxicity was found.	Five rats had poorly defined focal degeneration of the germinal epithelium.	

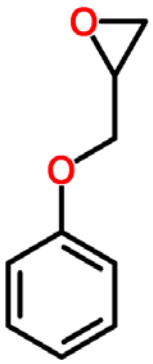
Diglycidyl ether (continued)

Reference	Experimental Parameters					Endpoints Assessed	Results (Effects/NOEL/LOEL)		Comments
	Chemical (Source/ Purity/ Preparation)	Animal Model (Species/ Strain/Sex/ Age) N	Study Design	Exposure (Route/Period/ Frequency/ Vehicle)	Doses/ Concen-trations		Systemic Toxicity	Reproductive Toxicity	
Hine et al. 1961 (continued)	Diglycidyl ether Shell Oil Company Purity not provided	Young male New Zealand rabbits (1.9 to 4.2 kg) N=3/treatment groupgroup	Effect of DGE on the circulating blood and blood production.	Single Inhalation exposure (24h duration)	Groups of 3 rabbits were exposed for 24 hours to graded (3, 6, 12 or 24 ppm) concentrations of DGE vapor (no control group reported)	Peripheral blood, bone, marrow, and Weight Gain. Physical observation and histology for testes, skin, lymphoid, kidney, adrenal medulla and pancreas at necropsy	Two rabbits in the 24 ppm group died on the evening of the fifth day since first exposure, having suffered 30% and 35% weight loss. One had confluent bronchopneumonia and serous hepatitis and the other had focal atelectasis, eribronchiolitis, and focal hemorrhage in the kidneys and lungs. The third rabbit died 2 days later, with 35'% weight loss, and was not necropsied. Rabbits exposed to lower levels showed no gross changes at necropsy and were not studied histologically	The first two animals that died had greatly atrophied testes. No additional testicular effects were reported	

Diglycidyl ether (continued)

Reference	Experimental Parameters					Endpoints Assessed	Results (Effects/NOEL/LOEL)		Comments
	Chemical (Source/ Purity/ Preparation)	Animal Model (Species/ Strain/Sex/ Age) N	Study Design	Exposure (Route/Period/ Frequency/ Vehicle)	Doses/ Concen-trations		Systemic Toxicity	Reproductive Toxicity	
Hine et al. 1961 (continued)	Diglycidyl ether Shell Oil Company Purity not provided	Male mongrel dogs N=3	Effect of DGE on the circulating blood and blood production	Intravenously with the aid of sodium pentobarbital. 1 injection/ week	25 mg/kg	Peripheral Blood, Bone, Marrow, and Weight Gain. Physical observation and histology for testes, skin, lymphoid, kidney, adrenal medulla and pancreas at necropsy	The leukocyte count was low in this group of 3 dogs ($p < 0.01$). 2 dogs died: one 6 days after the third weekly injection. The other died 7 days after the second injection. Deaths occured apparently of pneumonia. The pathologist reported infarction of all lung tissue submitted.	The animal that died 7 days after the second injection, presented hyaline degeneration of the testicular tubules	

Phenyl glycidyl ether



Molecular Formula: $C_9H_{10}O_2$

Component of various curing agents and epoxy resins.

Relevant Studies

Terrill, J. B. and K. P. Lee (1977). "The inhalation toxicity of phenylglycidyl ether. I. 90-day inhalation study". Toxicol Appl Pharmacol **42**(2): 263-9.

Terrill, J. B., K. P. Lee, R. Culik and G. L. Kennedy, Jr. (1982). "The inhalation toxicity of phenylglycidyl ether: reproduction, mutagenic, teratogenic, and cytogenic studies". Toxicology and applied pharmacology **64**(2): 204-12.

Phenyl glycidyl ether

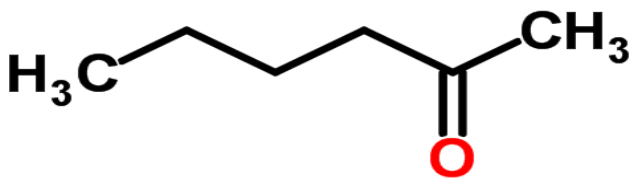
Reference	Experimental Parameters					Endpoints Assessed	Results (Effects/NOEL/LOEL)		Comments
	Chemical (Source/ Purity/ Preparation)	Animal Model (Species/ Strain/Sex/Age) N	Study Design	Exposure (Route/Period/ Frequency/ Vehicle)	Doses/ Concen-trations		Systemic Toxicity	Reproductive Toxicity	
Terrill and Lee 1977	Phenyl glycidyl ether Source not stated 99.6% pure	Sprague-Dawley (SD) male rats (250 to 260 g) N=6/group	2-week inhalation study	Inhalation 4 h/day, 5 days/week, for 2 weeks (10 exposures).	0 (control), 29 ppm (v/v)	Weighed and physically examined daily. At the end of testing, half the rats were sacrificed for histopathology. The rest of the animals were sacrificed and examined histologically after 2 weeks.	Depressed weight gain (p value not provided)	"Atrophic changes" in various organs including testes (p value not provided)	
		SD male and female rats Body weights were 250-260 g for males and 180-200 g for females N=32/sex/group	90-day study	Inhalation: 6h/day, 5 days/week (63 exposures)	0 (air),1, 5, 12 ppm	Daily physical inspection, weighted twice a week Sections of testis, prostate, ovary, uterus, mammary gland (among other tissues) were fixed	No change in body weight	No significant changes in histological examinations of relevant tissues	
		Beagle dogs (10 to 12 kg) N=6/group	As in the 90-day rat study	As in the 90-day rat study	As in the 90-day rat study	As in the 90-day rat study	No change in body weight	No significant changes in histological examinations of relevant tissues	

Phenyl glycidyl ether (continued)

Reference	Experimental Parameters					Endpoints Assessed	Results (Effects/NOEL/LOEL)		Comments
	Chemical (Source/ Purity/ Preparation)	Animal Model (Species/ Strain/Sex/Age) N	Study Design	Exposure (Route/Period/ Frequency/ Vehicle)	Doses/ Concen-trations		Parents	Offspring	
Terrill et al. 1982	Phenyl glycidyl ether Source not stated 99.6% pure	SD rats N=8 males/ group, 70 days old. N=3 females/ male/dose for mating	Two-generation rat reproduction and dominant lethal study* Untreated females were mated at the beginning of the week for 6 weeks Autopsy on GD 18 or at parturition	Inhalation: 6h/day for 19 consecutive days (males)	0 (air), 2, 6, 11 ppm	Fetility parameters. On GD 18: gross examination of uterine content and fetuses in some (1/3-1/2) of the pregnant rats. Corpora lutea (CL), implantation and resorptions. Gross pathology on rest of females at GD23 and F1 males and females at 12 weeks post weaning. Histopathology on testes of the F0 males	No increase mortality in F0	No increase in resorptions Higher number of CL and implantations on week 5 ($p<0.05$) at 2 and 11 ppm. No difference in number and survival of pups Higher number of viable fetuses on week 5 ($p<0.05$) at 2 and 11 ppm.	* This component of the study was considered invalid by U.S. EPA in its document "Glycidol and Its Derivatives Category Proposed Test Rule Action Test Rule (draft) (1986) because it, "was conducted by Industrial Bio-Test Laboratories, Northbrook, IL, a former testing facility known to have falsified data".
							Systemic Toxicity	Reproductive Toxicity	
							As above	Normal fertility: Lower number of pregnant females in week 1 at 11 ppm ($p<0.05$)	
		Pregnant female SD rats initial mean weight of approximately 200 g N=25/group	Teratogenicity Study. Dams sacrificed on GD20	Inhalation 6 h/day From GD 4 to GD 15	0 (air), 1 ,5, 12 ppm	Fetal body weight and length; number of implantations, live fetuses and resorptions. Fetuses were fixed for skeletal and soft tissue abnormalities	Parents	Offspring	
							No changes in body weight	Number of implantations, fetuses, and resorptions were similar in all groups. Fetuses had similar length and weight, and all appeared normal upon gross exam	

Ketones

Methyl n-butyl ketone



Molecular Formula: $C_6H_{12}O$

Solvent used in a wide variety of materials including paints, lacquers, ink thinners, nitrocellulose, glues, resins, oils, fats and waxes, and in printing of plasticized fabrics.

Relevant Studies

Katz, G. V., J. L. O'Donoghue, G. D. DiVincenzo and C. J. Terhaar (1980). "Comparative neurotoxicity and metabolism of ethyl n-butyl ketone and methyl n-butyl ketone in rats". Toxicol Appl Pharmacol **52**(1): 153-8.

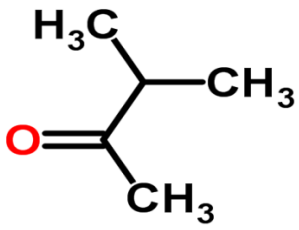
Krasavage, W. J., J. L. O'Donoghue, G. D. DiVincenzo and C. J. Terhaar (1980). "The relative neurotoxicity of methyl-n-butyl ketone, n-hexane and their metabolites". Toxicol Appl Pharmacol **52**(3): 433-41.

Peters, M. A., P. M. Hudson and R. L. Dixon (1981). "The effect totigestational exposure to methyl n-butyl ketone has on postnatal development and behavior". Ecotoxicol Environ Safety **5**(3): 291-306.

Methyl n-butyl ketone

Reference	Experimental Parameters					Endpoints Assessed	Results (Effects/NOEL/LOEL)		Comments
	Chemical (Source/ Purity/ Preparation)	Animal Model (Species/ Strain/Sex/ Age) N	Study Design	Exposure (Route/Period/ Frequency/ Vehicle)	Doses/ Concen-trations		Parents	Offspring	
Peters et al. 1981	Methyl n-butyl ketone Source/purity not stated	Fischer 344 rats 150 g N=25/group	Developmental neurotoxicity study; NIEHS contract; weaning PND 28; 500 ppm group terminated at 3 weeks of age due to technical problem There were 3 separate control groups, one for each dose group, plus a pair-fed control group for the high dose group	Inhalation GD0-GD20 6 h/day	0, 500, 1000, 2000 ppm.	Daily maternal weights; pregnancy outcome at birth; PND2 behavior observation; postnatal developmental indices; week 4, 8, 12 and month 18-20 gross and histopathology and behavioral test battery; Not all tests at all ages.	↓ maternal weight gain 1000 ppm (10%), 2000 ppm (14%); clinical signs 2000 ppm hair loss, incoordination; statistics not given; NOEL 500 ppm	↓litter size, birthweight (2000 ppm); ↓ postnatal and adult weights (males, 1000, 2000 ppm). Grip strength, maze latency, activity (1000, 2000 ppm male &/or female, at least one age). Pentobarbital increased sleeping time (2000 ppm, adult males). Decreased testes weights in weanlings; ovarian cysts 18 months, no statistics	Methods well reported; data not all reported
Katz et al. 1980	Methyl n-butyl ketone Source not stated 96.1% pure	CD rats Male N=5/group	Adult neurotoxicity study with terminal necropsy	Inhalation 72 h of exposures per week for 81 days (2 20-hour and 2 16-hour exposure periods/week	0, 700 ppm	Body weights, clinical chemistry, gross and histopathology, neurotoxicity	Systemic Toxicity Markedly reduced weight gain; decreased WBCs	Reproductive Toxicity Decreased relative testes weights; atrophy of testicular germinal epithelium described, no data presented	2,5-hexadione in serum; no statistics on data for testes
Krasavage et al. 1980	Methyl n-butyl ketone Source not stated 96.1% pure	CD rats Male N=5/group	Adult neurotoxicity study with terminal necropsy	Gavage 5 days/week for 90 days	0, 660 mg/kg	Body weights, gross and histopathology, neurotoxicity	Systemic Toxicity Reduced body weight gain	Reproductive Toxicity Atrophy of the testicular germinal epithelium described, no data presented	No statistics or data for testes

Methyl isopropyl ketone



Molecular Formula: $C_5H_{10}O$

Methyl isopropyl ketone is a pollutant from the hot gas welding of polyvinyl chloride (PVC).

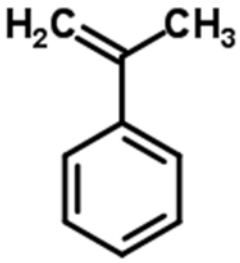
Relevant Studies

Bernard, L. G. (2001). Reproduction/Developmental Toxicity Screening Test in the Rat. Rochester New York, Toxicological Sciences Laboratory, Health and Environment Laboratories, Eastman Kodak Company.

Methyl isopropyl ketone

Reference	Experimental Parameters					Endpoints Assessed	Results (Effects/NOEL/LOEL)		Comments
	Chemical (Source/ Purity/ Preparation)	Animal Model (Species/ Strain/Sex/ Age) N	Study Design	Exposure (Route/Period/ Frequency/ Vehicle)	Doses/ Concen-trations		Systemic Toxicity	Reproductive Toxicity	
Bernard 2001 Eastman Kodak	Methyl isopropyl ketone (identified by the synonym 3-methyl-2-butanone) Eastman Chemical Company >99%pure	SD rats, male and female 61 days old N=12/sex/ group	Reproductive and developmental toxicity screening study OECD 421 OPPTS 870.3550	Inhalation 6 h/day, 7days/week, 2 weeks premating to GD19	0, 1, 2.5, 5 mg/L	Fertility, sperm parameters (epididymal number, morphology, motility), pregnancy outcome, postnatal growth and mortality PND 0-4	↓Paternal food intake and body weight, 1st 2 weeks of dosing, p≤0.05, LOEL 1 mg/L ↓maternal food intake premating and 1st week of gestation, p≤0.05, LOEL 1 mg/L; ↓ maternal body weight 2nd premating week and last week of pregnancy, p≤0.05, LOEL 2.5 mg/L; maternal clinical signs during exposure, p≤0.05, NOEL 1 mg/L	None reported	Full study reviewed; postnatal mortality minimal; 100% survival in controls
							Parents	Offspring	
							As above	↓No. live pups, PND 0, 5 mg/L, p≤0.05 ↑ No. dead pups, PND 0 2.5 mg/L, p≤0.05 ↑ Pups dying PND 0 to 4, 5 mg/L, p≤0.05 NOEL 1 mg/L	

α-Methyl styrene



Molecular Formula: C_9H_{10}

Used as a polymerization monomer in the manufacture of polyester resins, acrylonitrile-butadiene-styrene, rubber-thermoplastic resin and α-methyl styrene-butadiene elastomer.

Relevant Studies

Hardin, B. D., G. P. Bond, M. R. Sikov, F. D. Andrew, R. P. Beliles and R. W. Niemeier (1981). "Testing of selected workplace chemicals for teratogenic potential". Scand J Work Environ Health **7 Suppl 4**: 66-75.

NTP (2007). "Technical Report on the Toxicology and Carcinogenesis studies of α-Metylstyrene (CAS No 98-83-9) in F344/N Rats and B6C3F1 Mice (Inhalation Studies)". National Toxicology Program. NTP TR 543. NIH Publication No. 08-4474

α-Methyl styrene

Reference	Experimental Parameters					Endpoints Assessed	Results (Effects/NOEL/LOEL)		Comments
	Chemical (Source/ Purity/ Preparation)	Animal Model (Species/ Strain/Sex/ Age) N	Study Design	Exposure (Route/ Period/ Frequency/ Vehicle)	Doses/ Concen-trations		Parents	Offspring	
Hardin et al. 1981	α-Methyl styrene Source/purity not provided	Female Sprague-Dawley rats: 250-300g N= 10-15 inseminated females/group	Developmental toxicity study Necropsy on GD 21	Intraperitoneal injection on GD1 to GD15 Vehicle: corn oil	0 (corn oil), 250 mg/kg	Parents: Gross examination of internal organs of maternal rats; brain, heart, lungs, liver, spleen, kidneys, adrenals and ovaries weighed and preserved for histopathological examination. Offspring: Fetuses weighed, measured for crown-rump length, sexed, and examined for externally visible malformations. One half to two-thirds of each litter used for internal examination. The rest of each litter preserved in ethanol for skeletal staining.	No treatment-related weight or histopathological changes were observed in maternal tissues.	Significantly increased incidence of fetal resorptions ($p < 0.05$). Altered fetal sex ratio ($p < 0.05$) with a deficit of female fetuses.	

α-Methyl styrene (continued)

Reference	Experimental Parameters					Endpoints Assessed	Results (Effects/NOEL/LOEL)		Comments
	Chemical (Source/ Purity/ Preparation)	Animal Model (Species/ Strain/Sex/ Age) N	Study Design	Exposure (Route/ Period/ Frequency/ Vehicle)	Doses/ Concen-trations		Systemic Toxicity	Reproductive toxicity	
NTP 2007	α-Methyl styrene Acros Organics, Fair Lawn, NJ Purity >99%	Male and female B6C3F1 mice 6 weeks old N=10/sex/group	3-month inhalation exposure studies. At the end of the 3-month studies, samples were collected for sperm count and motility and vaginal cytology evaluations on animals exposed to 0, 300, 600, and 1000 ppm	Whole-body inhalation: 6 h/day, 5 days/ week for 14 weeks	0, 75, 150, 300, 600, 1,000 ppm	Animals weighed initially, weekly, and at the end of the studies. Epididymal sperm concentration and motility; spermatid heads/testis; and left cauda epididymis and testis weights were evaluated in male mice at sacrifice. For 12 consecutive days prior to sacrifice of females, vaginal cytology slides were prepared to determine estrous cycle stage (i.e., diestrus, proestrus, estrus, and metestrus).	5 to 17% decrease in BW ($p<0.05$) for both males and females at 300, 600 and 1000 ppm	In male mice, decreased cauda epididymal weights were observed at 600 and 1000 ppm ($p<0.05$) No effect on other reproductive endpoints. The estrous cycle lengths of 600 and 1,000 ppm female mice were significantly longer: 3.9 days (control) vs. 4.8 and 5.2, respectively ($p<0.05$ for 600 ppm and $p<0.01$ for 1000 ppm)	
	As above	Male and female F344/N rats 6 weeks old N=10/sex/group	As above	As above	As above	As above	No difference in BW at any dose in treated rats, compared to controls.	No observable adverse reproductive effects reported in treated rats.	

Appendix A: Parameters for Literature Searches on the Reproductive Toxicity of Chemicals

Searches of the literature on the reproductive and developmental toxicity of the chemicals in Table 1 were conducted under contract by the University of California at Berkeley (Charleen Kubota, M.L.I.S.). The goal was to identify peer-reviewed open source and proprietary journal articles, print and digital books, reports and gray literature that potentially reported relevant toxicological and epidemiological information on the reproductive toxicity of the chemicals. The search sought to specifically identify all literature relevant to the assessment of evidence on male reproductive, female reproductive and developmental toxicity.

Databases

The literature search utilized the following search platforms/database vendors:

ChemSpider (Royal Society of Chemistry)
MeSH (Medical Subject Headings) (National Library of Medicine)
Developmental and Reproductive Toxicology Database (DART/ETIC) (National Library of Medicine)
EMBASE® (Elsevier)
Environmental Sciences and Pollution Management (Proquest)
PubMed (National Library of Medicine)
National Technical Research Library (NTRL v3.0) (National Technical Information Service)
ReproRisk® System: **REPROTEXT®** Reproductive Hazard Reference**, REPROTOX®** Reproductive Hazard Information, **Shepard's Catalog of Teratogenic Agents**, **TERIS Teratogen Information System (RightAnswer® Knowledge Solutions OnSite™ Applications)**
Scifinder®: CAS (Chemical Abstracts Service)
TOXLINE (National Library of Medicine)
Web of Knowledge: BIOSIS Previews®, Web of Science® (Thomson-Reuters, Inc.)

Search Process

ChemSpider was searched first to gather chemical names, synonyms, CAS registry numbers, MeSH and Chemical Abstracts Service headings for each substance before searching bibliographic databases. The MeSH database was used to identify relevant subject headings for reproductive and developmental toxicology endpoints. Relevant subject terms were entered into the PubMed Search Builder to execute a PubMed search.

The following is a typical DART chemical search strategy used to search PubMed:

("*chemical name*" [MeSh] OR CAS *registry number*[RN]) AND ("Congenital Abnormalities"[MeSh] OR "Pregnancy Complications"[MeSh] OR "Reproductive Physiological Phenomena"[MeSh] OR "Embryonic and Fetal Development"[MeSH])
In PubMed, MeSH (Medical Subject Headings) terms at the top of hierarchical lists of subject headings are automatically "exploded" in a search to retrieve citations with more specific MeSH terms. For example, the heading "Congenital Abnormalities" includes numerous specific conditions such as spina bifida and congenital heart defects. The broad subject heading "Pregnancy Complications" encompasses multiple conditions or pathological processes associated with pregnancy. Spontaneous abortion and many fetal diseases are listed under this term.

Additional databases listed above were then searched for each chemical. The search strategies were tailored according to the search features unique to each database. Web of Science, for example, was searched by entering chemical terms and refining the search by applying Web of Science categories Developmental Biology, Toxicology and/or Public, Environmental and Occupational Health. Sometimes other databases not listed here were searched as needed. For example, if there is a known behavioral endpoint linked to chemical exposure, a social science database such as PsycINFO® would be searched.