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

Chemicals Listed via the Labor Code Mechanism:

tert-Amyl methyl ether (TAME)

2-Chloropropionic acid

N,N'-Dimethylacetamide (DMAC)

2-Ethylhexanoic acid

Ethyl-tert-butyl ether (ETBE)

p,p'-Oxybis(benzenesulfonyl hydrazide)

1,3,5-Triglycidyl-s-triazinetrione

4-Vinyl-cyclohexene (VCH)

Vinyl cyclohexene dioxide (VCD)

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. 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 nine 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 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:

[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

- 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 HSC 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
tert-Amyl methyl ether (TAME)	994-05-8	Developmental toxicity ("embryo/fetal damage")
2-Chloropropionic acid	598-78-7	Male reproductive toxicity ("male reproductive damage")
N,N-Dimethyl acetamide (DMAC)	127-19-5	Developmental toxicity ("embryo/fetal damage")
2-Ethylhexanoic acid	149-57-5	Developmental toxicity ("teratogenic effects")
Ethyl-tert-butyl ether (ETBE)	637-92-3	Male reproductive toxicity ("testicular damage")
p,p'-Oxybis(benzenesulfonyl hydrazide)	80-51-3	Developmental toxicity ("teratogenic effects")
1,3,5-Triglycidyl-s-triazinetrione	2451-62-9	Male reproductive toxicity ("male reproductive damage")

[3] HSC section 25249.8(a)

Table 1 continued

Chemical	CAS Number	Basis for TLV
4-Vinyl-cyclohexene (VCH)	100-40-3	Female and male reproductive toxicity ("female & male reproductive damage")
Vinyl cyclohexene dioxide (4-vinyl-1-cyclohexene diepoxide; VCD)	106-87-6	Female and male reproductive toxicity ("female & male reproductive damage")

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 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 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.

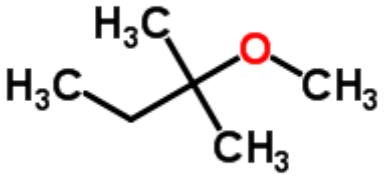
Relevant studies were identified for all but one of the chemicals. No relevant data on reproductive or developmental toxicity were identified for p,p'-oxybis(benzenesulfonyl hydrazide).

For the chemicals 4-vinyl-cyclohexene (VCH) and vinyl cyclohexene epoxide (VCD), a very large body of relevant data was identified. VCD is a metabolite of VCH. Most of the data on both of these chemicals relate to female reproductive toxicity, as these compounds are used as model compounds for this type of toxicity. Due to the volume of references on these chemicals, two recent reviews of the female reproductive toxicity of VCH and VCD published in peer-reviewed scientific journals are provided in Appendix B, rather than the data being tabulated. The relatively small number of studies relevant to developmental and male

reproductive toxicity of VCD and VCH have been summarized in tables. All of the complete study reports on both chemicals for male reproductive, female reproductive and developmental toxicity have been provided on CD to the DART IC and are available to the public upon request.

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 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.

tert-Amyl Methyl Ether (TAME)



Molecular Formula: $C_6H_{14}O$

tert-Amyl methyl ether is mostly used as an oxygenate for gasoline. It is added to increase octane enhancement and to raise the oxygen content in gasoline to help reduce emissions.

Relevant Studies

Berger, T. and C. M. Horner (2003). "In vivo exposure of female rats to toxicants may affect oocyte quality". Reprod Toxicol **17**(3): 273-81.

Tyl, R. W., C. B. Myers, M. C. Marr, P. A. Fail, J. C. Seely, B. Elswick, A. James and F. Welsch (2003). "Two-generation reproductive toxicity study of inhaled tertiary amyl methyl ether (TAME) vapor in CD rats". J Appl Toxicol **23**(6): 397-410.

Welsch, F., B. Elswick, R. A. James, M. C. Marr, C. B. Myers and R. W. Tyl (2003). "Developmental toxicity evaluation of inhaled tertiary amyl methyl ether in mice and rats". J Appl Toxicol **23**(6): 387-95.

tert-Amyl Methyl Ether (TAME)

Reference	Experimental Parameters					Endpoints Assessed Parents/ Offspring	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		Parents	Offspring	
Welsch et al. 2003	TAME (Chevron Research and Technology. Richmond, CA) Purity = 98.9%	CD·1 Mice. Pregnant female, 11wks old 25/group	Developmental toxicity study. Dams sacrificed on GD17 and fetuses dissected for physical examination.	Inhalation; 11 days (GD6-16); 6h/day Filtered fresh air vehicle	0 , 250, 1500, 3500 ppm	Parents: Dam toxicity: survival, organ wt, BW Offspring: Fetal survival, organ wt, developmental landmark, ossification, physical evaluation, and neurodevelopment	4 deaths (16%) at high dose; 1 on each day: GD 6,7,8,9 Increased liver wt. at 1500, 3500 ppm Reduced BW: 27% at 3500 ppm p<0.01	40% Reduced fetal BW/ litter at 3500 ppm (p<0.01); increased incidence of late fetal death at 3500 ppm; extra rib(s) on lumbar vertebra no. 1 in all groups, misaligned sternebrae at 0, 250 and 1500 ppm; reduced ossification in lumbar centrum at 1500 ppm and in thoracic centrum and pubis at 3500 ppm and floating extra rib cartilage at 1500 ppm Increased fetal external malformations: Cleft palate 18% of litters at 1500 ppm (NS) and 31.6% of litters at 3500 ppm (p<0.01) LOEL=1500 ppm; enlarged lateral ventricles of the fetal cerebrum at 3500 ppm NOEL = 250 ppm	
	As above	CD (Sprague-Dawley) rats; Female pregnant: 10 wks old 25/group	Developmental toxicity study. Dams sacrificed on GD20	Inhalation; 14 days (GD6-19); 6h/day Filtered fresh air vehicle	0, 250, 1500, 3500 ppm	Parents: Dam survival, BW Offspring: Fetal survival, organ wt., developmental landmark, ossification, physical evaluation, and neurodevelopment	Reduced BW: 7% at 1500 ppm (p<0.05) 22% at 3500 ppm (p<0.01)	No significant increase in fetal death in any treatment group. 5% reduction in fetal BW/ litter at 3500 ppm (p<0.01)	

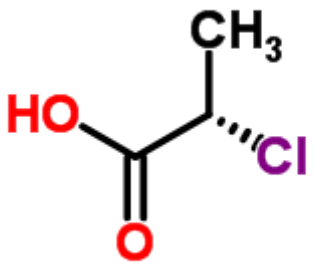
tert-Amyl Methyl Ether (continued)

Reference	Experimental Parameters					Endpoints Assessed Parents/ Offspring	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	
Tyl et al. 2003	TAME (Chevron Research and Technology. Richmond, CA) Purity= 98.8%	CD (Sprague-Dawley) rats, virgin females and virgin males; 35 days old 30/sex/ group	Two generation reproductive study. F1 and F2 offspring weaned on PND 28. Exposure of F1 and F2 started on PND29. Pregnant dams were not exposed beginning on gd 20. Dams with litters were not exposed on PND 0 through PND 4.	Inhalation for 6 h/day, 5 days/ week, during the prebreeding exposure periods (10 weeks) and the postmating holding period (males). During mating, gestation and lactation of F1 and F2 litters, exposures were 6 h/day, 7 days/ week.	0 (filtered fresh air), 250, 1500, 3000 ppm	Parents: Dam toxicity: survival, organ wt, BW and feed consumption Offspring: Fetal survival, BW, vaginal patency and preputial separation for F1. Anogenital distance at birth (PND 0) for F2. Reproductive organs of animals suspected of reduced fertility were subjected to histopathological evaluation.	Reduced BW during lactation at 3000 ppm ($p<0.05$).	F1: reduced BW throughout lactation in both sexes at 3000 ppm ($p<0.05$); in females only at 1500 ppm on PND 7 and 14, in both sexes at 1500 ppm on PND 21 and 28 ($p<0.05$). F2: deceased survival index at 3000 ppm at PND 4 and 21. Decreased BW throughout lactation in both sexes at 3000 ppm ($p<0.05$); and on PND 14 and 21 at 1500 ppm ($p<0.05$) . The NOAEL for offspring toxicity was 250 ppm.	
							Systemic Toxicity	Reproductive Toxicity	
							As above	Reduced estrous cycle length (4.22 vs 3.96 days, $p<0.05$) and increased gestational length (22 vs 22.3, $p<0.05$) at 1500 ppm. F0: Increased % abnormal sperm: 3.1% at 1500 ppm and 5.5% ($p<0.05$) at 3000 ppm, (control=2.3%)	

tert-Amyl Methyl 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	
Berger and Horner 2003	TAME (Aldrich Chemical Co). (purity not specified)	Sprague-Dawley rats, males, 100 days old, provided semen (collected from dissected epididy-mides) Females, 28–45 days old , provided ovocites.	*In vivo* treatment of females/*In vitro* fertilization study Exposed females were induced to ovulate and the ovocites collected and incubated with diluted sperm for 20 h.	Drinking water Females were exposed for 2 weeks prior to oocyte harvest.	0 or 0.3% in drinking water	Oocyte fertilization	N/A	Reduced percentage of oocytes fertilized following exposure of females to 0.3% TAME (65% versus 84% in controls; $p<0.05$); and decreased penetrated sperm/oocyte (1.53 versus 1.84) for TAME-exposed females, S.E.M. = 0.20; $p < 0.10$	

2-Chloropropionic Acid



Molecular Formula: $C_3H_5ClO_2$

2-Chloropropionic acid is a chemical intermediate used in the manufacture of pharmaceuticals and pesticides.

In the two studies by Yount et al. described in the table below, 2-chloropropionic acid as a neutral sodium salt, known as sodium 2-chloropropionate, was administered to rat testicular cells and rats. 2-Chloropropionic acid is a weak acid, so there will be an equilibrium between the acid and its anion (2-chloropropionate) in solution. Therfore, administration of 2-chloropropionate (in salt form) exposes an animal to 2-chloropropionic acid.

Relevant Studies

Yount, E. A. and R. A. Harris (1982a). "Ketone-Body and Acetate Formation from Oleate by Isolated Rat Testicular Cells". Arch of Biochem Biophysics **217**(2): 503-11.

Yount, E. A., S. Y. Felten, B. L. Oconnor, R. G. Peterson, R. S. Powell, M. N. Yum and R. A. Harris (1982b). "Comparison of the metabolic and toxic effects of 2-chloropropionate and dichloroacetate." J Pharmacol Exper Ther 222(2): 501-508.

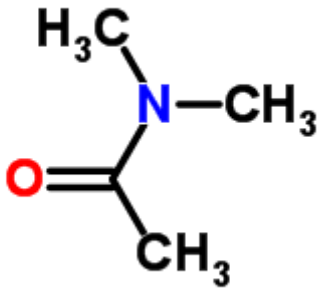
2-Chloropropionic Acid

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	
Yount et al. 1982a	DL-2-chloro-proprionic acid (used as a neutral solution of its sodium salt) Aldrich Chemical Company	Isolated testicular cells from Wistar rats from Harland Laboratories (Indianapolis, IN) Testes from one adult rat, or pooled testes from 8 or more 24- to 27-day old rats, or 40 14-day old rats	In vitro cell culture Cells were 97%+ viable as estimated by trypan blue exclusion The study examined metabolic effects of 2-chloropro-prionic acid oxidation, as well as some general features of energy metabolism.	Incubation of isolated cells 60 minutes in culture	Unspecified	Metabolism in testicular cells	N/A	2-chloropropionate did not activate the pyruvate dehydro-genase complex in the testes which did not increase the production of $^{14}CO_2$ from [U-^{14}C]glucose and diminish lactate and pyruvate accumulation.	Since all results obtained with 2-chloropropionic acid were negative, the focus became the capacity of isolated testicular cells to produce ketone bodies. Cellular composition of the isolated cell preparations was not defined.

2-Chloropropionic Acid (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	
Yount et al., 1982b	2-chloropropionate acid Aldrich Chemical Company (Converted by Yount et al. to the neutral sodium salt of 2-chloropropionic acid)	Male Wistar rats from Harlan Industries or Cox Laboratory Supply Company 6 weanling rats/group	Prolonged toxicity – 12 weeks	Oral via feed for 12 weeks Food consumption measured daily, body weights assessed weekly	0.04 mol/kg concentra-tion in feed Approximate dosage: varied from 4 mmol/kg/d at the beginning of the study to about 2.5 mmol/kg/d at the end of the study	Testes and epididymal weight Testicular germ cells	N/A	Weight of testes plus epididymis were significantly less than control values. Ratio of weight of testes plus epididymis to the whole body weight was significantly smaller in the treated group compared with controls. All 6 treated rats showed evidence of testicular maturation arrest and degeneration of germ cells, some of which contained enlarged or multiple nuclei.	Multiple studies were reported in this reference. Prolonged toxicity study had data relevant to male reproductive toxicity.

N,N-Dimethylacetamide (DMAC)



Molecular Formula: C_4H_9NO

N,N-Dimethylacetamide is used as a solvent, a chemical intermediate, a carrier ingredient in pharmaceuticals, and in the production of synthetic polymers used in clothing and textiles.

Relevant Studies

Anderson, I. and L. M. Morse (1966). "The influence of solvent on the teratogenic effect of folic acid antagonist in the rat". Exp Mol Pathol **5**(2): 134-45.

Du Pont Haskell Laboratories (1997). Dimethylacetamide (DMAC): Developmental toxicity study in Sprague-Dawley rats. HL-1997-00203.

Ferenz, R. L. and G. L. Kennedy, Jr. (1986). "Reproduction study of dimethylacetamide following inhalation in the rat". Fundam Appl Toxicol **7**(1): 132-7.

Johannsen, F. R., G. J. Levinskas and J. L. Schardein (1987). "Teratogenic response of dimethylacetamide in rats". Fundam Appl Toxicol **9**(3): 550-6.

Kennedy, G. L. (2012). "Toxicology of dimethyl and monomethyl derivatives of acetamide and formamide: a second update". Crit Rev Toxicol **42**(10): 793-826.

DuPont Company. (1983). Inhalation study in rats. Unpublished results.

Kennedy, G. L., Jr. (1986). "Biological effects of acetamide, formamide, and their monomethyl and dimethyl derivatives". Crit Rev Toxicol **17**(2): 129-82.

Monsanto (1973a). Unpublished data reviewed in Kennedy (1986): ref 219c, p156

Monsanto (1973b). Unpublished data reviewed in Kennedy (1986): ref 219d, p.156

Monsanto (1973c). Unpublished data reviewed in Kennedy (1986): ref 219b, p.156Klimisch, H. J. and J. Hellwig (2000). "Developmental toxicity of dimethylacetamide in rabbits following inhalation exposure". Hum Exp Toxicol **19**(12): 676-83.

McGregor, D. B., (1981). “Tier II Mutagenic Screening of 13 NIOSH Priority Compounds”, NIOSH contract, Inveresk Research International, Musselburgh, Scotland, NTIS.

Merkle, J. and H. Zeller (1980). "[Studies on acetamides and formamides for embryotoxic and teratogenic activities in the rabbit (author's transl)]". Arzneimittelforschung **30**(9): 1557-62.

Miller, W. L., D. W. Frank and M. J. Sutton (1981). "Antifertility activity of DMA in hamsters: protection with a luteotropic complex". Proc Soc Exp Biol Med **166**(2): 199-204.

Organization for Economic Cooperation and Development (OECD) (2001). N,N-dimethylacetamide (DMAC) CAS No:127-19-5. SIDS Initial Assessment Report for 13 SIAM.

Monsanto (1973c). Unpublished data reviewed in OECD (2001): ref 55, p. 78.

Okuda, H., T. Takeuchi, H. Senoh, H. Arito, K. Nagano, S. Yamamoto and T. Matsushima (2006). "Developmental toxicity induced by inhalation exposure of pregnant rats to N,N-dimethylacetamide". J Occup Health **48**(3): 154-60.

Solomon, H. M., R. L. Ferenz, G. L. Kennedy, Jr. and R. E. Staples (1991). "Developmental toxicity of dimethylacetamide by inhalation in the rat". Fundam Appl Toxicol **16**(3): 414-22.

Stula, E. F. and W. C. Krauss (1977). "Embryotoxicity in rats and rabbits from cutaneous application of amide-type solvents and substituted ureas". Toxicol Appl Pharmacol **41**(1): 35-55.

Thiersch, J. B. (1962). "Effects of acetamindes and formamides on the rat litter in vitro". J Reprod Fertil **4**: 219.

Valentine, R., M. Hurtt, S. Frame and G. L. Kennedy, Jr. (1997). "Inhalation toxicology of dimethylacetamide (DMAC) in mice and rats: age-related effects on lethality and testicular injury". Inh Toxicol **9**: 141-56.

Von Kreybig, T., R. Preussmann and I. Kreybig (1969). "Chemische konstiution und teratogene Workung bei der ratte. II. N-alkylharnstoffe, N-alkylsulfonamide, N,N-dialkylacetamide, N-methylthioacetamide, cloracetamide.". Arzneim. Forsch **19**: 1073-6.

Wang, G. M., L. D. Kier and G. W. Pounds (1989). "Male fertility study on N,N-dimethylacetamide administered by the inhalation route to Sprague-Dawley rats". J Toxicol Environ Health **27**(3): 297-305.

N,N-Dimethylacetamide (DMAC)

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	
Thiersch 1962	DMAC; source/purity not stated	Female rats No information on strain or group size provided	Preimplantation embryotoxicity study	i.p, one injection one dose between GD 4 and14 with pregnancy outcome; one dose GD 2, 3, 4 or 5 with embryo exam	2 g/kg	Embryos flushed from uterus and examined GD 2,3,4,5	No information provided	Loss of litter, one injection between GD4 and 14. Abnormal embryo development, one injection between GD2 and 5. .	Brief report with no data or statistics
Anderson and Morse 1966	DMAC; Source/purity not stated	Holtzman rats 3-5/group	DMAC used as a solvent in a study of pyrimethamine	s.c. injection GD 10, or 10,11 or 10,11,12; No control injection	DMAC 6, 12, 18%; high dose about 500 mg/kg	Embryos examined within 3 days of dosing	No information provided	Absorbed and necrotic fetuses, 12 & 18% dose, GD10,11 and 12; 2% resorptions at 6% dose	Data table, no statistics
Von Kreybig et al. 1969	DMAC; chemicals purchased or synthesized/ purity not stated	CD rats 2-5/group	A group of acetamides was studied. Fetal exam 24 or 48 h after treatment; gross fetal exam only	injection GD 13 GD14,15 (1000 mg/kg only)	600, 800, 1000 mg/kg	No information provided	Not stated; LD50 ~3000 mg/kg	Dose related increase in malformations on GD13,14; no malformations GD15; digit and tail malformations; Dose related increase in resorptions and fetal deaths	Data table; no statistics Article in German; some data in later review; described as i.p. by Stula & Krauss

N,N-Dimethylacetamide (DMAC) (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/ Concentrations		Parents	Offspring	
Monsanto unpublished 1973a	DMAC Source and purity not described	Rats No information on strain or group size provided	Reproductive toxicity study	Dermal	120, 250, 500, 1000 mg/kg/d Control not stated	No information provided	No information provided	Reduced fertility, smaller litters, decreased fetal weight, decreased sternal ossification, 1000 mg/kg/d	Original report not available. Described in Kennedy 1986 (ref 219c, p156) Text description only; no data; no statistics
Monsanto unpublished 1973b	DMAC Source and purity not described	Rats No information on strain or group size provided	Developmental toxicity study	Dermal	120, 250, 500 1000 mg/kg/d GD6-15 Control not stated	No information provided	No information provided	Decreased fetal weight, increased resorptions, skeletal defects, 1000 mg/kg/d	Original report not available. Described in Kennedy 1986 (ref 219b, p. 156) Text description only; no data; no statistics

N,N-Dimethylacetamide (DMAC) (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/ Concentrations		Parents	Offspring	
Monsanto unpublished 1973c	DMAC Source and purity not described	Rabbits, New Zealand white Group size not stated	Developmental toxicity	Dermal GD6-18	120, 250, 500 mg/kg/d Control not stated	No information provided	No maternal toxicity; NOEL 500 mg/kg/d	Decreased fetal body weight; decreased survival; increased sternal deviations; 2 gross malformations; 500 mg/kg/d; NOEL 250 mg/kg/d	Original report not available. Described in Kennedy 1986 (ref 219d, p.156 and OECD 2001 (ref 55, p. 78) Text description only; no data; no statistics
Stula and Krauss 1977	DMAC; Du Pont/ < 2% impurities	SD rats 220-250g 3-9 pregnancies/group	Developmental toxicity	Dermal; GD9, or 10&11, or 11&12, or 12&13;	0, 600, 1200, 2400 mg/kg-d;	Maternal weight change during dosing Fetal exam GD20 gross, visceral and skeletal	Maternal weight loss (1%) during dosing 1200 mg/kg-d GD 10&11; less weight gain, dose-dependent.	Increased embryomortality; dose dependent to 100% at 2400 mg/kg, GD10&11; 3 fetuses (1 litter) with encephalocele, 1 fetus with anasarca 1200 mg/kg, GD 10&11; lower fetal weight, dose dependent, GD10&11.	Results interpreted by authors as fetal effects with no maternal effects Data table; no statistics
		New Zealand rabbits, 4 kg 3-9 pregnancies/group	Developmental toxicity	Dermal GD 8-16.	200 mg/kg-d	Fetal exam GD30 gross, visceral and skeletal	No measures reported	No developmental effects	

N,N-Dimethylacetamide (DMAC) (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/ Concentrations		Parents	Offspring	
Merkle and Zeller 1980	DMAC BASF purity not stated	Rabbits 10-12/group 23-33 weeks old	Developmental toxicity FDA 1966 guidelines	Oral, gavage GD 6-18	0, 94, 282, 470 mg/kg	Maternal mortality, food intake; fetal mortality, weight, teratology exam	Mortality (17%) in dams at 470 mg/kg-d NOEL 282 mg/kg-d; Reduced food intake and weight gain dams NOEL 94 mg/kg-d	100% resorption, 470 mg/kg; decreased live fetuses, 5/35 fetuses with malformation (cleft palate, fused ribs, micro-ophthalmia), 282 mg/kg; NOEL 94 mg/kg-d	Dose calculation from OECD (2001); article in German
Miller et al. 1981	DMAC Aldrich purity not stated	Golden hamsters 6/group	Preimplantation embryotoxicity study; Fertility trial after previous preimplantation dosing	s.c. or oral single day treatment GD 1 to 8.	2.2, 1.8, 1.4, 1.1, 0.9 g/kg s.c. (lethal dose 6 mg/kg s.c.) 2.2, 1.1 g/kg oral	Implantation sites and ovary histopathology on GD8; Fertility trial 10 days after treatment	Not stated	Dose-dependent pregnancy termination 100% 2.2 g/kg to 0% 0.9 g/kg, s.c.; 100% pregnancy termination at 2.2 g/kg oral, 0% 1.1 g oral; histopathology corpora lutea; fertility 83% 10 days after treatment	Complex set of studies with good data reporting
McGregor 1981	DMAC Aldrich >99% pure	Rats 10/group	Dominant lethal 2 females/week, 9 weeks; Sperm abnormalities 5 weeks post dosing (epididymal); positive control	Inhalation 7h/day 5 days	0, 20, 700 ppm	Body weights during dosing	Not stated	No dominant lethal effects	Statistics; Dermal and i.p dominant lethal studies negative

N,N-Dimethylacetamide (DMAC) (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	
Dupont, 1983 Unpublished data	DMAC, source and purity not stated	Rats	Subchronic toxicity	Inhalation 6 h/day, 2 weeks	228 ppm (effective dose)	Not stated	Not stated	Testicular atrophy	Original report not available. Reviewed in Kennedy et al. 2012 Minimal information from table entry
Ferenz and Kennedy 1986	DMAC, DuPont/99.9 %pure	CD Rats 20 females 10 males 35 days old	One generation reproductive toxicity study Ending on pnd 21	Inhalation 6 h/day 10 weeks prebreed, 7 h/day breeding, gestation, lacation; Males and females; also males/females only at 300 ppm	0, 30, 100, 300 ppm	Mating, fertility, pregnancy outcome, postnatal growth and survival	Early weight effect in males only, 1 female death 300 ppm; Parental increased liver weight NOEL 30 ppm	Enlarged testes males 30 ppm	Haskell labs; complex results from M, F and M&F exposures
							Parents	Offspring	
							As above	2 pups with no tails; decreased postnatal weights; Increased liver weight, decreased pup weight pnd 21 NOEL 100 ppm	

N,N-Dimethylacetamide (DMAC) (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	
Johannsen et al. 1987	DMAC, Monsanto/99. 72% pure	CD Rats 12 weeks old 22-25 group	Developmental toxicity	Gavage GD 6-19 Water vehicle	0, 65, 160, 400 mg/kg-d	Body weight GD 1,6,9,12,16,20, Clinical signs. Sacrifice and fetal exam GD20; gross, visceral, skeletal	No mortality; reduced maternal body weight gain, corrected NOEL 160 mg/kg-d	Reduced fetal weight,; Increased post-implantation loss, increased malformation; reduced ossification; 400 mg/kg/d; distinctive cardiovascular malformation NOEL 160 mg/kg/d	Full report with statistics
Wang et al. 1989	DMAC/ Monsanto/99. 8% pure	Rats male 12/group	Male fertility and developmental toxicity	Inhalation 6 h/day, 5 days/week prior to and during mating; 43 exposures prior to mating, 69 total exposures	0, 40, 116, 386 ppm	Fertility, GD20 dam necropsy for litter size, resorptions, fetal weight, gross malformation	Increased liver weight NOEL40 ppm	No developmental effects	
							Systemic Toxicity	Reproductive Toxicity	
							As above	No fertility effects	
Solomon et al. 1991	DMAC, du Pont >99.9% pure	CD rats Females 60 days old, males 90 days old; 25 pregnancies/ group	Developmental toxicity; sacrifice and fetal exam GD 21	Inhalation 6 h/day GD 6-15	0, 30, 100, 300 ppm	Body weight GD 1,6,9,13,16,21; clinical signs daily; corpora lutea and implantation sites; fetal weight, gross and visceral, skeletal exam.	Parents	Offspring	Full statistics reported; table of individual malformations
							Reduced body weight gain 300 ppm, NOEL 100 ppm; no effects on corrected maternal body weight gain.	Reduced fetal weights, 300 ppm; no statistically significant malformation increase NOEL 100 ppm	

N,N-Dimethylacetamide (DMAC) (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/ Concentrations		Parents	Offspring	
DuPont Haskell Labs 1997	DMAC >99% pure	SD rats 24-25/group Females 64 days old, males, 76 days old	Developmental toxicity; exam GD22	Gavage GD7-21	0, 20, 65, 150, 400 mg/kg bw Vehicle HPLC water	Maternal body weight, food intake during pregnancy; uterine liver kidney weight at necropsy; fetal weight, gross, visceral, skeletal exam	Reduced maternal weight gain 150, 400 mg/kg; reduced corrected maternal weight gain 400 mg/kg; reduced food intake 400 mg/kg; increased liver and kidney weight, clin chem effects 400 mg/kg NOEL 65 mg/kg-d	Increased resorption, reduced litter size, 400 mg/kg; reduced fetal weight, 150 and 400 mg/kg; anasarca, cardiovascular and cerebral malformation, 400 and 150 mg/kg; NOEL 65 mg/kg-d	
Klimisch and Hellwig 2000	DMAC >99.9%pure	Rabbits (Himalayan) 23-27 weeks old 15/group main study 5/group satellite study	Developmental toxicity Sacrifice and fetal exam GD29	Inhalation GD7-19 Main 6 h/day “Satellite” 16 h/day	0, 57, 200, 570 ppm	Maternal body weight GD0,3,7; clin signs; gross and histopathology, blood chemistry,	No maternal toxicity	Decreased fetal and placental weights, all doses, no NOEL. Increased skeletal and soft tissue variations including cardiovascular, statistically significant at 570 ppm; NOEL 57 ppm	

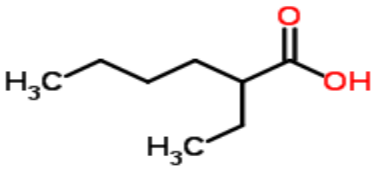
N,N-Dimethylacetamide (DMAC) (continued)

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/ Concentrations		Systemic Toxicity	Reproductive Toxicity	
Valentine et al. 1997	DMAC, Dupont Fibers, 99.8% pure	CD-1 mice Male 35 days old 5/group mated after exposure 10/group mated after 14 day recovery.	Sub Chronic Toxicity	Inhalation 6 h/day, 5 days/wk for 2 weeks.	0, 30, 100, 310, 490, 760 ppm	Body weights, Hematology, organ weights and histopathology	Increased mortality, pubescent mice 490 (20%), 760 ppm (80%); reduced body weight 700 ppm; clinical signs 490, 760 ppm; increased liver weights 490 ppm; decreased lung weights 490 ppm; hepatocellular, lymphoid and adrenal pathology.	Reduced relative testes weights 490 ppm; testicular lesions, atrophy 310, 490, 700 ppm; epididymal and seminiferous tubule lesions	
	As above	CD-1 mice 7 weeks old 9-13/group	As above	As above	0, 52, 150, 300 480, ppm (target doses)	Gross lesions at necropsy plus epididymal and testes histopathology, testicular sperm counts	No mortality or clinical signs.	Reduced testes weights at 480 ppm; seminiferous tubule atrophy at 480 ppm; no sperm effects	
	As above	CD rats 47 days old 9-13 group	As above	As above	0, 52, 150, 300 480, ppm (target doses)	Gross lesions at necropsy plus epididymal and testes histopathology, testicular sperm counts	No mortality or clinical signs; reduced weight gain 480 ppm.	No effects reported	

N,N-Dimethylacetamide (DMAC) (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/ Concentrations		Parents	Offspring	
Okuda et al. 2006	DMAC Wako Pure Chemical Industries >99.9% pure	CD rats female 9 weeks old 10/group	Developmental toxicity	Inhalation 6h/day GD 6-19	0, 100, 300,450, 600 ppm (v/v); vapor 214, 321, 428 mg/kg-d for 300, 450 and 600 ppm	Maternal weights GD 6,7,9,13,17,20; necropsy GD20, liver enzymes and hisopath; fetal exam, gross, visceral, skeletal	Decreased maternal body weight 450 and 600 ppm; increased maternal relative liver weight 300, 450, 600 ppm; maternal liver histopathology 450, 600 ppm. NOEL 100 ppm	Decreased fetal weight 300, 450, 600 ppm, decreased live male fetuses 600 ppm, Increased visceral and skeletal malformation, 450, 600 ppm; gross, (anasarca) 600 ppm; cardiovascular, malformation (ventricular septal defect, persistent truncus arteriosus) 450 and 600 ppm; skeletal (fused vertebrae, skull) 450, 600 ppm; NOEL 100 ppm	

2-Ethylhexanoic Acid



Molecular Formula: $C_8H_{16}O_2$

2-Ethylhexanoic acid is used as a chemical intermediate and for manufacture of resins used for baking enamels, lubricants, detergents, flotation aids, and corrosion inhibitors. The chemical is also used as a catalyst for polyurethane foaming, for solvent extraction, and for dye granulation.

Relevant Studies

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2-Ethylhexanoic Acid (EHXA)

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	
Ritter et al. 1987	2-Ethylhexanoic acid (EHXA) 6.25 and 12.5 mmol solutions	Wistar rats Pregnant females At least 7 litters/group Control: 7 litters 6.25 mmol/kg: 7 litters 12.5 mmol/kg: 10 litters	Pregnant rats were killed on gestation day (GD) 20 and following C-section, implantation sites counted and fetuses processed for teratogenic examination Potentiation of effects with caffeine also examined.	Oral gavage on GD 12 Vehicle not stated	EHXA: 0, 6.25, or 12.5 mmol/kg (6.25 mmol/ kg EHXA is equivalent to 1.0 mL/kg) Positive control valproic acid (an isomer of EHXA): 6.25 mmol/ kg EHXA 6.25 mmol/kg plus intra-peritoneal (i.p.) injection of 150 mg/kg caffeine	Number of dead or resorbed fetuses, living fetuses weighed and examined for external malformations. Skeletal and visceral effects examined.	No information reported on dams	↓ Fetal weight noted at 12.5 mmol/kg ↑ % of dead and resorbed fetuses at 12.5 mmol/kg ↑incidence of survivors with hydronephrosis - 20.9 % at 12.5 mmol/kg; 14.4% in valproic acid group. Malformed fetuses: 0.8% and 67.8% for EHXA at 6.25 and 12.5 mmol/kg; 31.5% for caffeine plus EHXA at 6.25 mmol/kg.	EHXA alone had teratogenic effect Caffeine further potentiated EHXA effects, increasing the incidence of fetal malformations ↑ fetal malformations observed in additional groups dosed with DEHP or 2-ethylhexanol (2-EHXO) Caffeine also potentiated the effects of these chemicals.
Hauck et al. 1990	3 different isomers tested: (R)-EHXA 93% (S)-EHXA 90% (±)-EHXA 90%	NMRI Mice Pregnant females Group size: Control: 10 (R)-EHXA: 17 (S)-EHXA: 9 (±)-EHXA: 20 (±)-EHXA: 14	Pregnant mice were killed on GD 18 and number of implantations, resorptions, live and dead fetuses examined.	I.p. injection each morning and evening on GD 7 and 8 for (R)-, (S)- and first (±)-EHXA groups. Single i.p. injection on GD 8 for second (±)-EHXA group.	500 mg/kg per i.p. injection for both single-dose and multiple dose groups Controls received 3.0 mmol NaCl/kg	Number of implantations, embryolethality (resorptions, live and dead fetuses). Living fetuses were weighed and examined for exencephaly	No information reported on dams	(S)-EHXA: No teratogenicity or embryolethality (R)-EHXA: Highly teratogenic and embryotoxic, 59% with exencephaly,↓ fetal weight (±)-EHXA: Multiple dose -teratogenic, 32% with exencephaly; Single dose – 5% with exencephaly and no effect on fetal weight	

2-Ethylhexanoic Acid (EHXA) (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	
Collins et al. 1992	EHXA administered as sodium 2-ethylhexanoate (Na EHA) Na (±) EHA >99% pure	SWV mice no controls, 19 litters for 4 dose groups	Pregnant females dosed on specific days of gestation to investigate neural tube closure and exencephaly in offspring.	Single subcutaneous (s.c.) injection Na (±) EHA on GD 8 or 8.5 Vehicle: Physiological saline (0.9% NaCl)	807, 864, or 1037 mg/kg on GD 8 864 mg/kg on GD 8.5.	Maternal lethality, embryolethality, malformations	↑ Maternal lethality at ≥ 864 mg/kg	Low incidence of exencephaly (about 10%) on GD8 at 807 and 864 mg/kg 39.5% resorptions on GD 8.5 at 864 mg/kg	Dilution details of racemic solutions provided
	EHXA administered as sodium 2-ethylhexanoate (Na EHA) Na (±) EHA >99% pure	SWV mice. Untreated Controls: 19 litters; Treated: 10 litters C57BL mice Untreated Controls: 22 litters Treated: 11 litters	Pregnant females of two strains were dosed on specific days of gestation to investigate the induction of exencephaly and embryolethality	Multiple i.p. injections given at various one-half day intervals during some of GDs 7-10 (presumptive time of neural tube closure) Vehicle: Physiological saline (0.9% NaCl)	SWV & C57BL: 576 mg/kg x 4 (on GD 7.5, 8, 8.5, 9)	Maternal lethality, embryolethality, malformations	No information reported on dams	SWV: ↑% dead or resorbed (21%); ↑exencephaly (49%) statistically significant C57BL: ↑% dead or resorbed fetuses; ↑ exencephaly (7.3%), though not statistically significant. Resorption rate was 21%	SWV more sensitive strain than C57BL for induction of exencephaly
	EHXA administered as sodium 2-ethylhexanoate (Na EHA) Na (±) EHA >99% pure	SWV mice No Controls 7-10 litters/group	Pregnant females dosed on specific days of gestation to investigate the most sensitive gestational times to induce exencephaly	Multiple i.p. injections at various one-half day intervals during GDs 7-10 (presumptive time of neural tube closure) Vehicle: Physio-logical saline (0.9% NaCl)	SWV: 576 mg/kg x 3 (on either GD 7, 7.5, 8 or 7.5, 8, 8.5 or 8, 8.5, 9 or 8.5 9, 9.5 or 9, 9.5, 10)	Maternal lethality, embryolethality, malformations	No information reported on dams	The most sensitive time for induction of exencephaly was GDs 8.0, 8.5 and 9.0 Incidence of exencephaly was 44% Resorption rate was 14%	GD 8.0, 8.5, and 9.0 most sensitive period.

2-Ethylhexanoic Acid (EHXA) (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	
Collins et al. 1992 (continued)	EHXA administered as sodium 2-ethylhexanoate (Na EHA) Na (±) EHA Na (S)-EHA Na (R)-EHA >99% pure	SWV mice No Controls 6-10 litters/group	Pregnant females dosed to investigate the most sensitive enantiomer to induce exencephaly and embryolethality	I.P injections at various doses at 3 gestational times- GD 8.0, 8.5 and 9.0 Vehicle: Physiological saline (0.9% NaCl)	Na (±) EHA; 3X 576 mg/kg Na (S)-EHA; 3X 864 mg/kg 3X 576 mg/kg Na (R)-EHA; 3X 403 mg/kg 3X 518 mg/kg 3X 576 mg/kg	Maternal lethality, embryolethality, malformations	Maternal lethality at 3X 864 mg/kg	Na (±) EHA; Resorptions -14% Exencephaly- 44% Na (S)-EHA; Resorptions -12.5% Exencephaly- 0% Na (R)-EHA; Resorptions – 9-20% Exencephaly - 0 - 50% Highest incidence of exencephaly (50%) noted in Na (R)-EHA group	R-enantiomer most potent
Pennanen et al. 1992	EHXA administered as Na EHA 99.5% pure	Wistar rats 20-21 pregnant females/dose group	Developmental toxicity study. Fetuses examined on GD 20	In drinking water on GD 6-19 Controls received de-ionized water	0, 100, 300 or 600 mg/kg/d	Maternal BW, fetuses examined for external, visceral, and skeletal malformations and variations.	Marginally toxic to the dams at 600 mg/kg/d with body weight ↓by 11%	Fetotoxic at 600 mg/kg/d with a 5-8% ↓ fetal BW in males and females. No effect on number of implantations or live fetuses. Dose dependent ↑ in variations (affected fetuses:2.4%,4.9%, 8.9% and 15.3%). ↑skeletal malformations ≥ 100 mg/kg/d	According to the authors, the skeleton appears to be the main target

2-Ethylhexanoic Acid (EHXA) (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	
Pennanen et al. 1993	EHXA administered as Na EHA 99.5% pure	Wistar rats, males and females 24 rats/sex/ dose group	Preconception, gestational, and lactational exposure.	In drinking water. Male rats were exposed for 10 weeks and females for 2 weeks prior to mating, both sexes during the mating period and females during the entire gestation and lactation period.	0, 100, 300, or 600 mg/kg/d. Controls received plain water.	Examined for external, visceral, and skeletal malformations and variations.	No information reported	Average litter size ↓ by 16% at 600 mg/kg/d. Birth weights of the pups unaffected but BW gain was transiently slower during lactation at 600 mg/kg/d. Several pups appeared abnormal (kinky tail, lethargic, slightly paralyzed legs) and the physical development assessed by several landmarks (opening of eyes, eruption of teeth, hair growth) and reflexes (grip reflex, cliff avoidance) was delayed at 300 and 600 mg/kg/d.	
	As above	Wistar rats, males and females Group size: GD4 - 4 GD5 - 6 GD6 – 6 GD7 - 12	Prenatal dosing.	Single gavage on either GD 4, 5, 6, or 7. Water vehicle	0, 600 mg/kg Controls received plain water.	Examined for external, visceral, and skeletal malformations and variations. Number of implantations counted on GD 10.	No information reported	Delayed postnatal development in pups. GD 6 most critical for implantation, with resorptions in 80% of pregnant animals (4/5) Less severe effects seen with exposure on GD 7	

2-Ethylhexanoic Acid (EHXA) (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	
Hendrickx et al. 1993	EHXA 99.4% pure	Fischer F344/Crl/Br rat Pregnant females (147-174g) 25/dose group	Developmental toxicity study Dams killed on GD 21 Litter was the unit of comparison	Oral gavage GD 6-15 Corn oil vehicle	0, 100, 250, 500 mg/kg-d	Parents Clinical signs, mortality; Maternal BW Food consumption. Gross examination of reproductive organs, corpora lutea count, uterine weight, number fetuses live/dead, resorption sites, maternal liver weight Offspring Fetal external examination, fetal weight, skeletal and visceral examination of fetuses (half of the offspring in each category)	Clinical signs of toxicity & ocular discharge in dams at 500 mg/kg-d only Maternal LOEL = 500 mg/kg-d; NOEL = 250 mg/kg-d	↓ossification in fetuses at 250 mg/kg-d ↓ fetal body weight at 500 mg/kg-d Fetal LOEL = 250 mg/kg-d; NOEL = 100 mg/kg-d	
	As above	New Zealand White rabbits Pregnant females (2.5-3.5 kg) 15/dose group	Developmental toxicity study Does killed on GD 29 Litter was the unit of comparison	Oral gavage GD 6-18 Corn oil vehicle	0, 25, 125, 250 mg/kg-d	Parents Clinical signs, mortality; Maternal BW (GD 0, 6, 9, 12, 15,18 and 29); Food consumption daily. Gross examination of reproductive organs, corpora lutea count, uterine weight, number fetuses live/dead, resorption sites, maternal liver weight Offspring Fetal external examination, fetal weight, skeletal and visceral examination of fetuses	↓weight gain at 250 mg/kg-d (after treatment period). Maternal LOEL = 125 mg/kg-d (death and abortion) NOEL = 25 mg/kg-d	No teratogenic effects Some ↓ fetal BW at 250 mg/kg-d (not statistically signifi-cant) Developmental NOEL = 250 mg/kg-d	

2-Ethylhexanoic Acid (EHXA) (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	
Narotsky et al. 1994	EHXA 99+% pure	Sprague-Dawley rats Pregnant females Control: 20 Treated:15/ dose group	Chernoff/ Kavlock assay. Dams allowed to deliver and the pups examined postnatally	Oral gavage, once daily on days 6-15 of gestation Corn oil vehicle	0, 900, 1200 mg/kg/d	Mortality, clinical signs (rales, dyspnea, motor depression), BW	↑Mortality in treated groups (27% and 40%) ↑motor depression	↓ Number of live pups on PND 1 (p<0.05 at 900 mg/kg/d; not assessed in 1200 mg/kg/d grp) ↓ Number of live pups on PND 6 (p<0.01 at 900 mg/kg/d; p<0.001at 1200 mg/kg/d) ↑ perinatal loss (p<0.01 at 900 mg/kg/d; p<0.001at 1200 mg/kg/d) ↓ pup weight on PND 1 (p<0.001 at 900 mg/kg/d; not assessed in 1200 mg/kg/d grp) ↓ pup weight on PND 6 (p<0.001 at 900 mg/kg/d; not statistically significant at 1200 mg/kg/d)	According to authors, maternal effects not responsible for developmental effects.

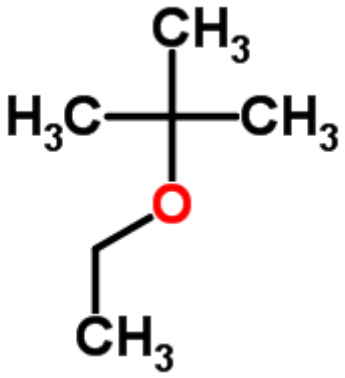
2-Ethylhexanoic Acid (EHXA) (continued)

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/ Concentrations		Parents	Offspring	
Bui et al. 1998	EHXA 99.4% pure	Sprague-Dawley rats Pregnant females N=7-10	Dams dosed at 1400 hr on GD 11.5, followed by 32 µCi ^{65}Zn at 2200 hr, and killed at 0800 hr on GD 12.5	Single gavage dose at 1400 hr on GD 11.5, followed at 2200 hr by gavage with 32 µCi ^{65}Zn.	0, 3.13, 6.25, 9.38 or 12.5 mmol/kg (0, 451, 902, 1355 or 1804 mg/kg Controls received 1 ml/kg corn oil	Maternal food intake, maternal liver Zn, ^{65}Zn and metallothio-nein (MT). Fetal weight, crown-rump length, encephalocele	Maternal food intake not affected. ↑Maternal liver Zn, MT, and ^{65}Zn accumulation, dose-related.	At the higher doses, ↓ ^{65}Zn retained in embryos. .	EHXA-associated changes in ^{65}Zn distribu-tion were associated with increased maternal liver MT. According to the authors, these results support the hypothesis that EHXA, which induces maternal toxicity, may influence embryonic Zn metabolism and trigger abnormal development
		As above	Dams fed diets containing various levels of Zn from GD 0-16, dosed with EHXA on GD 8-15, and killed on GD 16 or 19	Zn administered in the diet from GD 0 -16 and EHXA administered by gavage on GD 8-15 Corn oil vehicle	0, 3.5 mmol EHXA/kg/d; Zn in the diet at 1, 25 or 97 µgZn/g	Maternal BW gain, maternal liver Zn and MT. Fetal weight, crown-rump length, malformations	Lower dietary Zn intake ↓ mater-nal liver MT and Zn and plasma Zn. Lower dietary Zn intake ↓ maternal BW gain in EHXA-treated and control groups. Adequate and supplemental Zn intake ↓ maternal BW gain in EHXA-treated group but not controls	↑ encephalocele and tail defects in GD 16 fetuses of EHXA-treated dams fed low or adequate Zn diet; highest in low Zn group. In GD 19 fetuses, ↑ tail defects with EHXA; highest incidence in the low Zn EHXA group. Encephalocele only observed in the low Zn EHXA-treated group. ↓ Fetal weight and crown-rump lengths by EHXA and low dietary Zn. ↑ incidence of rib anomalies in EHXA-groups	
		As above	*in vitro* GD 10.5 embryos collected from control dams cultured for 48 hr in serum from control or EHXA-treated (9.38 mmol EHXA/kg) male rats fed diets containing various levels of Zn.	*in vitro* Embryos exposed in culture to serum from male rats fed diets with various levels of Zn and treated with EHXA. Supplemental Zn was added to some cultures.	Serum from males treated with 0 or 9.38 mmol EHXA/kg fed diets containing 4.5 or 25 µg Zn/g Zn added to cultures yielded Marginal (10.4 µM Zn); Adequate (20.1 µM Zn).(Sera measured for Zn before and after culture. EHXA conditioned: 10.5 µM; Marginal Zn + Zn repletion: 19.4 µM; EHXA conditioned + Zn repletion: 19.8 µM	Crown-rump length, head length, number of somites, developmental score		Embryodevelopmental toxicity effects. Embryos cultured in serum from males with either low Zn diets or treatment with EHXA exhibited delayed development; addition of Zn to these sera eliminated the developmental toxicity effects.	

2-Ethylhexanoic Acid (EHXA) (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	
Svechnikova et al. 2007	EHXA obtained from Sigma, purity not stated	*In vitro* study using primary cultures of pituitary cells	Cells from anterior pituitary glands of 20-day–old female rats were cultured with EHXA for 24 hr at 37 ºC, and then for an additional 3 hr in fresh medium containing EHXA and 1 ng/ml GnRH.	Cell culture media, 24 hr plus 3 hr	0, 1 µM EHXA in culture medium	LH released by cultured pituitary cells into the cell culture medium	N/A	EHXA enhanced by 30% ($p \leq 0.05$) the GnRH-stimulated production of LH by cultures of pituitary cells isolated from untreated 20-day-old female rats. EHXA had no effect on basal production of LH.	EHXA can directly enhance the sensitivity of gonadotropes to GnRH
Dawson 1991	EHXA ≥98% pure	*Xenopus* embryos 75 embryos/ group	A modified FETAX (Frog Embryo Teratogenesis Assay: *Xenopus*) assay. Embryos were exposed in covered glass Petri dishes (25 embyos/dish; 3 replicates per treatment) containing 10 ml of solution for 96 hours, with solution renewal every 24 hours. Embryos were then fixed and evaluated for gross malformations	FETAX solution, 96 hr exposure	0, 22, 44, 55, 66 or 88 mg/L	Number and type of malformations were noted for each dish	N/A	Number of survivors malformed: 5 (7%) in controls 8 (11%) at 22 mg/L 28 (37%) at 44 mg/L 49 (66%) at 55 mg/L 63 (84%) at 66 mg/L 75 (100%) at 88 mg/L EC_{50} = 47.9 mg/L Malformations induced included: microcephaly, abnormal gut coiling, eye edema, skeletal kinking and general edema	

Ethyl-tert-Butyl Ether (ETBE)



Molecular Formula: $C_6H_{12}O$

Ethyl-tert-Butyl Ether is an oxygenate gasoline fuel additive.

Relevant Studies

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Ethyl-tert-Butyl Ether (ETBE)

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	
Medinsky et al. 1999	ETBE (ARCO Chemical Co. Newtown Square, PA) 97.5% pure	Fischer 344 rats, males and females, 5 weeks old 10-15 rats/ sex/grcoup	Gross pathology/ histopathology study Euthanized/ necropsied on day after last exposure. All retained tissues fixed in 10% formalin. Tissues were embedded in paraffin for microscopy evaluation	Inhalation; 6h/day for 5 consecutive days/ week for at least 13 weeks; total of 65 exposure days.	0 (filtered air), 500, 1750, 5000 ppm	Body weight (BW) and clinical signs prior to treatment and weekly through the exposure period. Tissues of relevance were: Pituitary, testes, epididymis, prostate, seminal vesicles, ovaries, vagina, uterus. Testes examined in all dosed males.	No effect on mortality. 25% decrease in BW gain for males and females at 1750 and 5000 ppm. Significantly increased BW in female rats at 5000 ppm. Transient ataxia in male rats at 5000 ppm	Testes were the "only tissues with significant microscopic findings". Increased percentage of seminiferous tubules with spermatocyte degeneration at 1750 and 5000 ppm. Decreased spermatocytes in tubules of stage I-VIII. No treatment effect at 500 ppm (=NOEL).	
	As above	CD·1 mice, males and females, 5 weeks old 10-15 mice/ sex/group	As above	As above	As above	As above	No effect on mortality or BW. Transient ataxia in male and female mice at 5000 ppm	No reported effects.	

Ethyl-tert-Butyl 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	
de Peyster et al. 2009	ETBE from Lyondell Chemical Company (Houston, TX). The purity of the individual batch tested was not provided. The accompanying MSDS listed ETBE composition as 90–98%	F344 rats, Adult males 12/group	14-Day *in vivo* experiment Animals euthanized by carbon dioxide sedation followed by decapitation 1 h after the last dose on day 14.	Gavage daily for 14 days Corn oil vehicle	0 (vehicle), 600, 1200, or 1800 mg/kg/ day	Plasma concentrations of testosterone and estradiol (assessed by radioimmuno-assay). Organ weights (testes, accessory sex organs), testis fixed for histopathology.	All rats were lethargic for up to 30 min after dosing. Three rats died: 1 on treatment day (TD) 10 in the 1200 mg/kg and 2 on TD 13-14 in the 1800 mg/kg group. Depressed mean group BW at 1200 and 1800 mg/kg/day ($p < 0.05$). No statistically significant differences in organ weights.	Testosterone reduced to 66% of control at 1800 mg/kg (NS). Estradiol was significantly increased in both the 1200 and 1800 mg/kg dose groups relative to control ($p < 0.05$). NOEL = 600 mg/kg/day	The study report stated that "gavage errors could not be ruled out as the cause of death" for animals in the treatment groups
		Isolated Leydig cells from adult Sprague–Dawley rats	*In vitro*: isolated Leydig cells (viability >90%; purity >85%) incubated in the presence of hCG (2.0 IU/ml) to stimulate testosterone production	Direct exposure into RPMI culture medium for 3 h.	0, 50, 100 mM	Testosterone release into the culture medium.		50 mM ETBE inhibited testosterone production to 67% of control ($p<0.05$) (LOEL).	

Ethyl-tert-Butyl Ether (continued)

Reference	Experimental Parameters					Endpoints Assessed Parents/ Offspring	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	
Asano et al. 2011	ETBE (Tokyo Chemical Industry Co., Ltd. Tokyo, Japan), 99% pure	New Zealand white rabbits Pregnant females 14 weeks old 24 copulating females/group	Pregnant rabbits were killed on GD 28. Ovaries, uterus and live fetuses were evaluated at necropsy.	Oral (by catheter), daily from GD 6 to 27 Olive oil vehicle	0 (vehicle), 100, 300, or 1,000 mg/kg/ day in 1.67 mL/kg BW	Number of corpora lutea, live fetuses and embryo-fetal deaths as well as their stage were counted and recorded after the weighing of gravid uterus. Live fetuses and their placentas were observed for external malformations and gross abnormalities. Live fetuses were weighed and observed macroscopically for organ abnormalities and skeletal malformations. Body weight and food consumption was measured in parents	No deaths among dams. Mean BW of dams in the 1,000 mg/kg/day group was slightly lower than that of controls; the difference was statistically significant on GDs 12, 14 and 16. No differences in BW at lower doses. No differences in food consumption. At necropsy on GD 28 no abnormalities were observed in the main organs or tissues of the thoracic or abdominal cavities.	Four dams aborted: One on GD 28 in the control group; 2 in the 300 mg/kg/day group (one each on GD 19 and 26) and 1 on GD 25 in the 1000 mg/kg/day group. No significant differences were noted in the number of corpora lutea or implantations. No significant differences in the index of external malformations or incidence of skeletal malformations or variations External observation of fetuses revealed 1 fetus with acephaly and gastroschisis at 100 mg/kg/day and 1 fetus with brachyury at 1,000 mg/kg/day.	

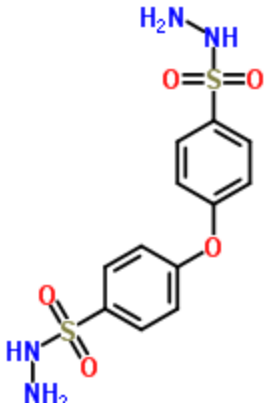
Ethyl-tert-Butyl Ether (continued)

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	
Fujii et al. 2010	ETBE (Tokyo, Japan) lot no. 2ZGTA, 99% purity	SD rats; males and females; 5 weeks old 24 animals/sex/ group	One generation reproduction study Animals pretreated daily for 10 weeks, mated and then treated for an additional 16 weeks (males) or 17 weeks (females). BW and food consumption for F0. F1 necropsy at PND 21, except for 1 F1 weanling/sex/ litter selected for observation of sexual development.	Oral F0 males and females dosed daily for 10 weeks prior to mating. After mating, F0 males and females treated daily for approximately 16 and 17 weeks, respectively. F1 weanling males and females dosed daily for approximately 4 and 2 weeks, respectively. Oive oil vehicle	0 (vehicle), 100, 300, or 1,000 mg/kg/ day in 5 mL/ kg BW	F0: BW and food consumption. At necropsy, recorded the number of implantation sites. Examined for sperm parameters. F1: During lactation, daily examination for clinical signs and mortality. 1 animal/sex/litter was selected to observe sexual development (preputial separation or vaginal opening), one testis and epididymis per male was fixed with Bouin's solution and preserved in 70% ethanol.	No changes in BW or food consumption. No treatment-related clinical signs of toxicity in any F0 rats during the dosing period. 2 F0 females in the 1000 mg/kg group were moribund and euthanized on lactation day (LD) 2 and LD 4 (all their pups died).	Gestation length significantly prolonged by 0.4 days in the 1000 mg/kg group ($p \leq 0.05$) No differences were found in any of the studied parameters for the F1 generation. No statistically significant differences in the indices of copulation, fertility, gestation and delivery. Normal estrous cyclicity in all groups.	
							Parents	Offspring	
							As above	No significant difference in the number of pups delivered. One full litter died from one animal in the F0 females 1000 mg/kg group without clinical signs	

Ethyl-tert-Butyl Ether (continued)

Reference	Experimental Parameters					Endpoints Assessed Parents/ Offspring	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	
Gaoua 2004	ETBE (TOTAL France S.A., Paris-la-Défense, France), batch Nos. S02-08-159-I3 and S02-08-159-I3/2, Purity >98%	Sprague-Dawley female rats, 11 weeks-old 24/group	Developmetnal toxicity study Animals treated daily, sacrificed at day 20 post mating. Macroscopic examination of dams and fetuses. Litter parameters recorded	Gavage, once/day; from day 5 to 19 after mating Corn oil vehicle	0 (vehicle), 250, 500 or 1000 mg/kg/ day	Clinical signs and mortality. Body weight and food consumption. Litter parameters: weight of gravid uterus, number of corpora lutea, implantation sites, early and late resorptions, dead and live fetuses. The fetuses were weighed, sexed. Half of the fetuses from each treatment group were subjected to a detailed examination of soft tissue, while the remainder underwent a detailed skeletal examination.	At 1000 mg/kg/day significantly lower maternal BW gain (-11%, $p<0.05$) and net BW gain (-17%, $p<0.01$) were recorded over the treatment period. NOEL for maternal toxicity is 500 mg/kg/day.	No treatment-related effects on gestational parameters or fetuses. The NOEL for embryo-fetal development is 1000 mg/kg/day.	

p,p’-Oxybis(benzenesulfonyl hydrazide)



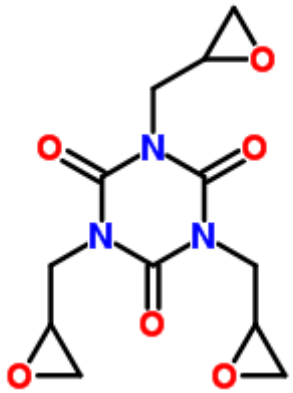
Molecular Formula: $C_{12}H_{14}N_4O_5S_2$

p,p’-Oxybis(benzenesulfonyl hydrazide) is a blowing agent for sponge rubber and expanded plastics.

Relevant Studies

None identified

1,3,5-Triglycidyl-s-triazinetrione



Molecular Formula: $C_{12}H_{15}N_3O_6$

1,3,5-Triglycidyl-s-triazinetrione is a curing agent used in powder coatings.

Synonyms: TGIC; Triglycidyl isocyanurate; 1,3,5-Triglycidyl-s-triazinetrione. There are two main technical grades of TGIC used in the manufacture of powder coatings worldwide. These are 'Araldite PT 810' (also known as 'TK 10622') manufactured by Ciba-Geigy Ltd, Switzerland, with purity > 97% TGIC and 'TEPIC', manufactured by Nissan Chemical Industries Pty Ltd, Japan with purity of approximately 90% TGIC (NICNAS., 1994).

Relevant Studies

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1,3,5-Triglycidyl-s-triazinetrione (TGIC)

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	
Ciba-Geigy 1986 No 850067	TK10622 (Commercial grade Araldit PT 810) > 97% TGIC pure	Tif MAGf (SPF) Mouse N= 15/dose group; 12/control	Chromosome studies on male germinal epithelium (spermatogonia) Male mice were dosed on 5 consecutive days. Killed on the day following the final treatment.	Oral Arachis oil	0, 43, 128 mg/kg	Chromosomal Aberrations	Not reported	Cytotoxicity observed at 128 mg/kg. Chromosomal aberrations induced	Statistical analysis was not carried out on the data
Ciba-Geigy 1986 No 850068	TK 10622 > 97% TGIC pure	Tif MAGf (SPF) Mouse 12/Negative control	Chromosome studies on male germinal epithelium (spermatocytes) Killed 3 days after the final dose	Oral gavage on days 0, 2, 3, 5 and 9. in arachid oil	0, 32 or 96 mg/kg	Chromosomal Aberrations in primary and secondary spermatocytes	N/A	Authors of review concluded the results of this study were negative.	Primary source not available Cited in NICNAS 1994
Ciba-Geigy 1986 No 850069	TK 10622 > 97% TGIC pure	Tif MAGf (SPF) Mouse 20 males & 40 females/dose group	Dominant Lethal Assay Mice mated over 3 periods of 6 days Females killed on GD 14 to determine number of live and dead fetal resorptions	Single oral gavage in arachid oil	0, 160, or 480 mg/kg	Females killed on GD14 and numbers of live and dead fetal resorptions noted	Parents No information provided	Offspring Significant ↑in number of embryonic deaths compared to control at 480 mg/kg for first mating period but not in the second and third mating periods	Primary source not available Cited in NICNAS 1994

1,3,5-Triglycidyl-s-triazinetrione (TGIC) (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	
Hazelton 1989a (James Ivett) No 10386-0-474	PL88-810 > 97% TGIC pure	ICR mice 10/group	Cytogenetics assay-Mutagenicity test in spermatogonial cells Killed 6 hours after the final dosing for extraction of testes	Oral gavage (in peanut oil), for 5 consecutive days	0 30, 125 or 350 mg/kg; Positive control (triethylene-melamine, 1.3 mg/kg via intraperitoneal injection)	Body weight and weight change; Chromosomal aberrations (simple, not computed, complex) in spermatogonia	No toxic effects were noted	↑ Frequencies of chromosomal aberrant cells at 125 and 350 mg/kg.	
Hazelton 1989b (James Ivett) No 10386-0-471	PL88-810 > 97% TGIC pure	ICR mice 20/group	Dominant Lethal Assay 18 hours after exposure male mice bred to first group of two females (for up to 5 days) and similarly mated to different females for a total of 3 weeks. Females killed around 14 days after copulation and uteri examined for implantations.	Oral gavage (in peanut oil), Triethylene-melamine, 0.3 mg/kg was positive control (intra-peritoneal injection).	0 137.5, 275 or 550 mg/kg	Mortality, toxic effects in males Females were killed around 14 days after copulation. At necropsy uteri examined for the number of live or dead implantations (early or late).	Parents No toxic effects in males after dosing	Offspring No significant effects at any dose on fertility, total number of implantations, frequency of dead implantations, proportion of females with either one or more or two or more dead implantations, or frequency of dead implants relative to total implants/female.	Positive control treatment induced large and significant effects on all parameters examined.

1,3,5-Triglycidyl-s-triazinetrione (TGIC) (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	
Hazelton 1991a (R. Marshall)	TK10622 (PT 810 [TGIC, 97% pure])	B6D2F1 male mice (3/group in range finding study); 5 in control group 5/group 10/group for high dose of 115 mg/kg in case replacements were needed	In vivo cytogenetics assay - Chromosome damaging potential on spermatogonial cells. Treated animals killed and sampled 6 hours after final dose. Positive control animals killed 24 hours after treatment. Where possible, ≥ 50 metaphases from each testis were analyzed	Oral for 5 consecutive days; Vehicle control - 0.5% (w/v) carboxymethyl cellulose	28.75, 57.5 or 115 mg/kg Mitomycin C at 0.3 mg/kg was positive control (intra-peritoneal)	Slides from all dose groups examined for chromosomal aberrations	No deaths or toxic effects were observed	Significant ↑ numbers of chromosomal aberrant cells at 28.5 and 115 mg/kg; several chromatid exchanges (rarely seen in untreated animals) observed at the mid-dose of 57.5 mg/kg.	Chromosomal aberrations and the frequency of aberrant cells in the positive control group were significantly greater than that in concurrent controls.
Hazelton 1991b (R. Marshall)	TK10622, a polyester formulation containing 60.2 % polyester (P 2400 DSM Resins) [4.8% TGIC, and 35% TiO_2 (CL 2310, KRONOS)	B6D2F1 male mice 5/group; 10 for high dose (5000 mg/kg) in case replacements were needed	In vivo cytogenetics assay - Chromosome damaging potential on spermatogonial cells. Treated animals killed and sampled 6 hours after final dose Positive control animals killed 24 hours after treatment. Where possible, 50 metaphases from each testis were analyzed.	Oral for 5 consecutive days Vehicle control - 0.5% (w/v) carboxymethyl cellulose	185.2, 555.6, 1667 or 5000 mg/kg Mitomycin C at 0.4 mg/kg was positive control (intra-peritoneal).	Slides from all dose groups examined for chromosomal aberrations.	No deaths or toxic effects were observed	No significant ↑ numbers of chromosomal aberrant compared to vehicle control. NOEL = 5000 mg/kg	The positive control induced a clear increase in the incidence of cells with structural aberrations.

1,3,5-Triglycidyl-s-triazinetrione (TGIC) (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	
Hazelton 1991c (R. Marshall)	TK10622/1, a polyester formulation containing 91% polyester (E2514, UCB) [8.92% TGIC]	B6D2F1 male mice 5/group; 10 for high dose of 800 mg/kg in case replacements were needed	In vivo cytogenetics assay Chromosome damaging potential on spermatogonial cells. Treated animals killed and sampled 6 hours after final dose. Positive control animals killed 24 hours after treatment.	Oral for 5 consecutive days; In vehicle control - 0.5% (w/v) carboxymethylcellulose; Mitomycin C at 0.4 mg/kg was positive control (intra-peritoneal)	29.63, 88.89, 266.7 or 800 mg/kg	Slides from all dose groups examined for chromosomal aberrations. Where possible, ≥ 50 metapases from each testis were analyzed.	No deaths were observed in animals	Significant ↑in frequency of chromosomal aberrant cells compared to vehicle control,at high dose of 800 mg/kg in two animals. This effect was onserved at a dose which caused a large decrease in cell proliferation in the testis.	The positive control induced a clear increase in the incidence of cells with structural aberrations.
Bushy Run 1991	PL90-810 Test article purity 10% TGIC powder coating	CD-1 male mice 10/group	Chromosomal Aberrations Assay Animals killed 6 hours after end of last exposure or 30 hours after receiving positive control.	Inhalation 6hrs/day for 5 consecutive days Cyclo-phosphamide 50mg/kg was positive control (intraperitoneal)	0, 100,1000, 1700 mg/m^3	Slides from all dose groups examined for chromosomal aberrations.	No information provided	% aberrant cells in animals with >50 scorable cells: 0.3,0.3, 1.6 and 2.5 for increasing doses with 6.9 for the positive control	↓ number of animals with ≥50 scorable cells at low dose – possibly because of cytotoxicity Primary source not available. Cited in NICNAS 1994 Large quantity of dust deposited on chamber wall - grooming may have affected dose.

1,3,5-Triglycidyl-s-triazinetrione (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	
Bushy Run (May) 1992a PL 90-810	Inhaled dust > 97% TGIC pure particle size 2.5-3.5 µm	CD-1 mouse 30 males /group	Dominant Lethal Assay About 24 hours after last exposure males bred to naïve females (1M: 2 F), with females replaced every week for 8 weeks	Inhalation of PL90-810 dust 6hrs/day for 5 consecutive days.	0, 2.5, 10 or 50 mg/m^3 6hrs/day for 5 consecutive days. Positive control [(triethylene-melamine (TEM), 0.3 mg/kg via intraperitoneal injection]	Male fertility, sperm-positive and pregnant females	General toxicity at high dose NOEL = 10 mg/m^3	↓Male fertility for first 3 weeks and week 6 at high dose; at 10 mg/m^3 for the 3rd week ↓sperm-positive ↓pregnant females NOAEL for fertility effects = 2.5 mg/m^3 Effects on mature sperm, maturing spermatids and Type B spermatogonia at 50 mg/m^3 and early spermatids at 10 mg/m^3	Sperm and spermatid stages (weeks 1, 2 and 3 of mating) were most sensitive to effects of positive control TEM Spermatocyte and spermatogonial stages were highly resistant to TEM induced dominant-lethal mutations
							Parents	Offspring	
							As above	No effect on number of resorptions/litter, total number of implants, number of viable implants or % postimplantation loss. No dominant-lethal effects. NOEL = 50 mg/m^3	

1,3,5-Triglycidyl-s-triazinetrione (TGIC) (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	
Bushy Run (June) 1992b	PL 90-810PC Inhaled dust purity not stated, presumed > 97% TGIC particle size 2.5-3.5 µm	Mouse CD-1 30 males/group	Dominant Lethal Assay About 24 hours after last exposure males bred to naïve females (1M: 2 F), with females replaced every week for 8 weeks.	Inhalation of PL90-810 dust	0, 100, 1000 or 1700 mg/m^3 6hrs/day for 5 consecutive days. Positive control (triethylene-melamine, 0.3 mg/kg via intraperitoneal injection)	Body weight and general toxic effects Male fertility, sperm-positive, and pregnant females	↓ Body weight on males at 1000 and 1700 mg/m^3 NOEL for general toxicity = 100 mg/m^3	No effect on male fertility, on number of resorptions/litter, total number of implants, number of viable implants or % postimplantation loss. No dominant-lethal effects. NOEL = 1700 mg/m^3	Sperm and spermatid stages (weeks 1, 2 and 3 of mating) were most sensitive to effects of positive control TEM; spermatocyte and spermatogonial stages were highly resistant to TEM induced dominant-lethal mutations
Bushy Run 1992c	PL90-810 Test article purity Technical grade TGIC presumed > 97% TGIC	Mouse 10/group	Chromosomal Aberrations Assay Killed 6 hours end of last exposure or 30 hours after given positive control	Inhalation; Cyclophosphamide 50mg/kg was positive control (intra-peritoneal)	0, 2.5, 10,or 50 mg/m^3 6hrs/day for 5 consecutive days	Slides from all dose groups examined for chromosomal aberrations	Systemic Toxicity No deaths or adverse clinical signs observed	Reproductive Toxicity % aberrant cells in animals with >50 scorable cells: 4.7, 5.1, 2.1,and 8.3 for dose levels 0, 2.5, 10 or 50 mg/m^3 with 12.7 for the positive control Low number of scorable cells at 10 and 50 mg/m^3 dose groups and mean number of aberrant cells in control group was higher than expected	Large quantity of dust deposited on chamber wall - grooming may have affected dose Primary source not available. Cited in NICNAS 1994

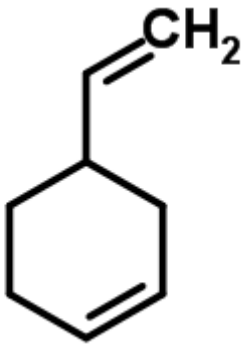
1,3,5-Triglycidyl-s-triazinetrione (TGIC) (continued)

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/ Concentrations		Systemic Toxicity	Reproductive Toxicity	
Safepharm 1992	TEPIC technical (approximately 90% TGIC) or 10% powder coating	CD-1 mouse 10/group for groups 1-5; Group 6&7 was positive control with 5/group	Chromosomal analysis in spermatogonia. Animals killed 6 hours after last exposure or 24 hours after exposure to positive control	Nose-only Inhalation study Oral administration of TEPIC included for comparison	0, 7.8 (TEPIC), 95.3, 255.3 mg/m^3, (as 10% powder) 115 mg/kg - oral (TEPIC) for 5 consecutive days. 50 mg/kg cyclo-phosphamide (oral) and 3.0 mg/kg mitomycin C (intra-peritoneal)	Body weight and clinical signs Cytotoxicity, mortality	Hunched posture and piloerection noted on day 5 at 115 mg/kg.	Slight cytotoxicity in one animal at 255.3 (10% TEPIC). No significant ↑ in chromosomal damage in spermatogonia noted at any of the inhalation dose groups. Significantly ↑ in cytotoxicity and chromosomal aberrations at 115 mg/kg.	Primary source not available Cited in NICNAS 1994 Cyclo-phosphamide positive control did not ↑ chromosomal damage. According to authors effects of cyclophoshamide cannot be detected at a 24 hour kill-time.
Hazleton Microtest Laboratory 1993	4.6% TGIC powder coating U.60092.100G	B6D2F1 Mice 6 males/group	Chromosome damaging potential on spermatogonial cells. Killed 6 hours after last exposure or 24 hours after exposure to positive control	Inhalation	0, 250, 500, 1000 or 2000 mg/m^3; Positive control was 0.3 mg/kg mitomycin C (intra-peritoneal)	Slides from all dose groups examined for chromosomal aberrations	Body weights of animals exposed to powder coating remained the same or↓ slightly during the period of exposure.	Significant ↑ numbers of spermatogonial cells with chromosomal aberrations (mainly due to chromosome damage in one animal) at 2000 mg/m^3	Primary source not available Cited in NICNAS 1994 The positive control showed a significant ↑ in number of cells with chromosomal aberrations.

1,3,5-Triglycidyl-s-triazinetrione (TGIC) (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	
Centre International de Toxicologie 1995 Study No. 11099	Test article purity TGIC PT810 > 97% TGIC pure (concentration in dietary mixture provided)	Sprague-Dawley rats 10 treated males /group 20 untreated females per group	Toxicity & Fertility Study Toxicity study: males killed after 13 weeks of exposure, selected organs weighed, sperm sampling performed on day of sacrifice for concentration and viability of spermatozoa. Fertility study: Female rats received untreated diet. After 64 days of treatment, each male was placed with 2 untreated females for mating. On day 19 females were allocated to hysterectomy or delivery subgroup	13 weeks in diet at 0, 10, 30 or 100 ppm	0, 0.7, 2.1, 7.3 mg/kg	Clinical signs, mortality, body weight ↓ Mean spermatozoa viability Fertility outcome Embryonic/pup development Pre and post-implantation loss, number of live fetuses, fetal body weight, sex ratio, number live born, viability at PND 4 and PND 21, external anomalies, malformations, reflex development Females: Bodyweight on days 0, 6, 9, 12, 15 or 20 of pregnancy and Day 1, 7, 14, 21 post-partum (delivery group)	Slightly ↓body weight gain in first 6 weeks at 100 ppm group. Male NOEL= 30 ppm (2.1 mg/kg-day)	Dose-related decrease in sperm count (linear trend test p=0.015); ↓Mean number of spermatozoa (5, 13 and 23% compared to controls), No impact on fertility	For the treated groups; the mean number of spermatozoa was lower than the lowest value of controls as follows: 1/10, 2/10 and 4/10 animals
							Parents	Offspring	
							As above	No impact on embryonic or pup development	

4-Vinyl-Cyclohexene (VCH)



Molecular Formula: C_8H_{12}

4-Vinyl-cyclohexene has been used as a solvent and in the production of flame retardants, polyolefins and vinylcyclohexene dioxide.

Relevant Studies

A relatively large body of relevant data was identified because the compound is a model compound for ovotoxicity. There are fewer publications on male reproductive and developmental toxicity for VCH. Below, the publications on male reproductive and developmental toxicity are listed first, followed by tabulations of study design parameters and results for those studies. Then the publications on female reproductive toxicity are listed. Because of the large number of studies, a recent review of female reproductive toxicity of VCH published in a peer-reviewed scientific journal is provided (in Appendix B), rather than data tabulations. All listed publications on the compound have been provided on CD to the DART IC and are available to the public upon request.

1. *Male Reproductive and Developmental Toxicity (Summarized in Table[4])*

Bevan, C., J. C. Stadler, G. S. Elliott, S. R. Frame, J. K. Baldwin, H. W. Leung, E. Moran and A. S. Panepinto (1996). "Subchronic toxicity of 4-vinylcyclohexene in rats and mice by inhalation exposure". Fundam Appl Toxicol **32**(1):1-10.

[4] Some of these papers also provide data on female reproductive toxicity of 4-vinyl-cyclohexene (VCH) (see Relevant Studies 2. *Female Reproductive Toxicity*); however, only data relevant to male reproductive and developmental toxicity are summarized in the table.

George, J. D., P. A. Fail, T. B. Grizzle, J. J. Heindel and R. E. Chapin (1991). "Final report on the reproductive toxicity of 4-vinylcyclohexene (CAS no. 100-40-3) in CD-1-Swiss mice: final study report and appendices I-II". NTIS Technical Report **211250**(155).

Grizzle, T. B., J. D. George, P. A. Fail, J. C. Seely and J. J. Heindel (1994). "Reproductive effects of 4-vinylcyclohexene in Swiss mice assessed by a continuous breeding protocol". Fundam Appl Toxicol **22**(1): 122-9.

Hooser, S. B., D. G. DeMerell, D. A. Douds, P. Hoyer and I. G. Sipes (1995). "Testicular germ cell toxicity caused by vinylcyclohexene diepoxide in mice". Reprod Toxicol **9**(4): 359-67.

4-Vinyl-Cyclohexene (VCH) : Studies Reporting on Male Reproductive or Developmental Effects

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	
Grizzle et al. 1994; George et al. 1991	VCH > 99% pure	CD-1 (Swiss) mice, male and female, 11 weeks old Control: 40 mice/sex/ group; Treated: 20 mice/sex/ group	Reproductive Assessment by Continuous Breeding (RACB) study F0: Both sexes treated 1 week then cohabited for 14 weeks; F1: control and high dose offspring of both sexes treated from weaning to 74 days of age, mated for one week, with necropsy after birth of F2 litters.	Gavage, daily F0 dosing: 15 weeks (males); 18 weeks (females) F1 dosing: weaning through 74 days of age plus 1 week mating and 3 weeks gestation Corn oil	0 (corn oil), 100, 250, 500 mg/kg-d	F0: BW, food and water intake, fertility measures per pair; pregnancy outcome and pup growth per litter F1 (control and high-dose only): BW, liver weight, food and water intake, fertility, pregnancy outcome, estrus cyclicity, sperm evaluations, ovarian cell counts, histopathology	F0: No effect on mortality or food and water intake. 8% decrease in BW of 500 mg/kg/d females at conclusion of dosing. F1 (500 mg/kg-d males and female): Decreased BW post weaning. Sporadic increased food and water intake post weaning. Increased liver weights at conclusion of dosing.	F0: No effects on mating index, fertility index, or pregnancy outcome endpoints. F1 (500 mg/kg-d): No effects on mating index, fertility index or pregnancy outcome endpoints. F1 females: No effects on estrous cycle. Reduced numbers of ovarian primordial, growing and antral follicles at conclusion of dosing. F1 males: No effect on weights of testis, epididymis, or prostate. Reduced absolute weight of seminal vesicles and counts of spermatid heads per mg of testicular tissue.	
							Parents	Offspring	
							As above	No developmental effects reported	

4-Vinyl-Cyclohexene (VCH): Studies Reporting on Male Reproductive or Developmental Effects (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	
Bevan et al. 1996	VCH purity 99.6%	B6C3F1 mice, male and female, 7-8 weeks old 10 mice/sex/ group	Subchronic toxicity study Clinical, hematological, serum/urine analysis, and necropsy.	Inhalation; 6h/day, 5 days/ Week, for 13 weeks (65 total exposure days)	0, 50, 250, 1000 ppm	BW, clinical, hematological, and serum/urine parameters Relative organ weights, routine histopathological evaluation of all major organs.	1000 ppm: 10/10 males and 5/10 females died on Test days 11 or 12. No treatment-related mortality in other groups. No effects on BW, organ weights, or hematological, serum, or urine parameters. Ovarian atrophy in 1000 ppm females. No other histopathological findings in any dose groups.	No effect on relative testis weight or histopathology. No data on ovarian weight. (Ovarian atrophy in 5/10 females at 1000 ppm)	

4-Vinyl-Cyclohexene (VCH): Studies Reporting on Male Reproductive or Developmental Effects (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	
Hooser et al. 1995	VCH 99% pure Vinylcyclo-hexene diepoxide (VCD) 97% pure Vinylcyclo-hexene 1,2-monoepoxide (VCM) 98% pure	B6C3F1 male mice, 4 weeks old 8-10 per group	VCH, VCD, and VCM testicular toxicity comparison	Intraperitoneal (i.p.) injection daily for 30 days sesame oil	0 (sesame oil), VCH: 800 mg/ kg-d VCD: 160 or 320 mg/kg-d VCM: 200 mg/ kg-d	Weights of testis, seminal vesicles Necropsy of testis, epididymis	No data reported	VCH: No effect on weights of testis or seminal vesicles VCD: Reduced weights of testis and seminal vesicles in both dose groups. VCM: No effect on weights of testis or seminal vesicles	
		B6C3F1 male mice, 4 weeks old 5 mice per group	VCD time-course study	i.p.injection daily for 5, 10, 15, 20, 25, or 30 days sesame oil	0 (sesame oil), 320 mg/kg-d	As above	No data reported	VCD: dosing for ≥ 5 days caused reduced testis weight and testicular degeneration.	
		B6C3F1 male mice, 4 weeks old 10 mice per group	VCD dose-response testicular toxicity study	i.p. injection daily for 30 days sesame oil	0 (sesame oil), 40, 80, 160 or 320 mg/kg-d	As above	No data reported	VCD: dosing at ≥ 80 mg/kg-d for 30 days caused reduced testis weight and testicular degeneration.	

2. *Female Reproductive Toxicity*

The scientific literature on the female reproductive toxicity of 4-vinyl cyclohexene (VCH) and its primary bioactive metabolite 4-vinyl-1-cyclohexene diepoxide (vinyl cyclohexane dioxide; VCD) are extensive. Studies have established that bioactivation of VCH to epoxides is required for its ovotoxicity, with VCD being the most potent epoxide of VCH in terms of follicular depletion. A literature search conducted by OEHHA resulted in 147 unique references on the topic of female reproductive toxicity to VCH and VCD. Of those 147 references, there are 21 female reproductive toxicity references on VCH, and 56 female reproductive toxicity references on VCD. VCD is a model chemical used to induce perimenopause in rodents (Frye et al., 2012). The remaining references resulting from the literature search are abstracts, reviews, theses, and grants on VCH and VCD.

Due to the extensive literature available on female reproductive toxicity, a recent review of female reproductive toxicity of VCH published in a peer-reviewed scientific journal is provided (in Appendix B), rather than data tabulations. Citations for the review and studies are provided below, and the full publications have been provided on CD to the DART IC and are available to the public upon request.

Review (provided in Appendix B)

Hoyer, P. B. and I. G. Sipes (2007). "Development of an animal model for ovotoxicity using 4-vinylcyclohexene: a case study". Birth Defects Research. Part B, Developmental Reprod Toxicol 80(2): 113-25.

Studies of Female Reproductive Toxicity of VCH

Anonymous (1991). "Final report on the reproductive toxicity of 4-vinylcyclohexene (CAS no. 100-40-3) in CD-1-Swiss mice: laboratory supplement". NTIS Technical Report **211268**(315).

Bevan, C., J. C. Stadler, G. S. Elliott, S. R. Frame, J. K. Baldwin, H. W. Leung, E. Moran and A. S. Panepinto (1996). "Subchronic toxicity of 4-vinylcyclohexene in rats and mice by inhalation exposure". Fundam Appl Toxicol **32**(1): 1-10.

Borman, S. M., P. J. Christian, I. G. Sipes and P. B. Hoyer (2000). "Ovotoxicity in female Fischer rats and B6 mice induced by low-dose exposure to three polycyclic aromatic hydrocarbons: comparison through calculation of an ovotoxic index". Toxicol Appl Pharmacol **167**(3): 191-8.

Cannady, E. A., C. A. Dyer, P. J. Christian, I. G. Sipes and P. B. Hoyer (2002). "Expression and activity of microsomal epoxide hydrolase in follicles isolated from mouse ovaries". Toxicol Sci **68**(1): 24-31.

Cannady, E. A., C. A. Dyer, P. J. Christian, I. G. Sipes and P. B. Hoyer (2003). "Expression and activity of cytochromes P450 2E1, 2A, and 2B in the mouse ovary: the effect of 4-vinylcyclohexene and its diepoxide metabolite". Toxicol Sci **73**(2): 423-30.

Collins, J. J. and A. G. Manus (1987). "Toxicological evaluation of 4-vinylcyclohexene. I. Prechronic (14-day) and subchronic (13-week) gavage studies in Fischer 344 rats and B6C3F1 mice". J Toxicol Environ Health **21**(4): 493-505.

Collins, J. J., R. J. Montali and A. G. Manus (1987). "Toxicological evaluation of 4-vinylcyclohexene. II. Induction of ovarian tumors in female B6C3F1 mice by chronic oral administration of 4-vinylcyclohexene". J Toxicol Environ Health **21**(4): 507-24.

Doerr, J. K., S. B. Hooser, B. J. Smith and I. G. Sipes (1995). "Ovarian toxicity of 4-vinylcyclohexene and related olefins in B6C3F1 mice: role of diepoxides". Chem Res Toxicol **8**(7): 963-9.

Doerr, J. K. and I. G. Sipes (1996). "Ovarian toxicity and metabolism of 4-vinylcyclohexene and analogues in B6C3F1 mice: structure-activity study of 4-vinylcyclohexene and analogues". Adv in Exp Med Biol **387**: 377-84.

Doerr-Stevens, J. K., J. Liu, G. J. Stevens, J. C. Kraner, S. M. Fontaine, J. R. Halpert and I. G. Sipes (1999). "Induction of cytochrome P-450 enzymes after repeated exposure to 4-vinylcyclohexene in B6C3F1 mice". Drug Metab Dispos **27**(2): 281-7.

Fontaine, S. M., P. B. Hoyer, J. R. Halpert and I. G. Sipes (2001). "Role of induction of specific hepatic cytochrome P450 isoforms in epoxidation of 4-vinylcyclohexene". Drug Metab Dispos **29**(9): 1236-42.

George, J. D., P. A. Fail, T. B. Grizzle, J. J. Heindel and R. E. Chapin (1991). "Final report on the reproductive toxicity of 4-vinylcyclohexene (CAS no. 100-40-3) in CD-1-Swiss mice: final study report and appendices I-II". NTIS Technical Report **211250**(155).

Grizzle, T. B., J. D. George, P. A. Fail, J. C. Seely and J. J. Heindel (1994). "Reproductive effects of 4-vinylcyclohexene in Swiss mice assessed by a continuous breeding protocol". Fundam Appl Toxicol **22**(1): 122-9.

Hooser, S. B., D. P. Douds, D. G. DeMerell, P. B. Hoyer and I. G. Sipes (1994). "Long- term ovarian and gonadotropin changes in mice exposed to 4-vinylcyclohexene". Reprod Toxicol **8**(4): 315-23.

Hooser, S. B., L. R. Parola, E. M. D. Van and I. G. Sipes (1993). "Differential ovotoxicity of 4-vinylcyclohexene and its analog, 4-phenylcyclohexene". Toxicol Appl Pharmacol **119**(2): 302-5.

Keller, D. A., S. C. Carpenter, S. Z. Cagen and F. A. Reitman (1997). "In vitro metabolism of 4-vinylcyclohexene in rat and mouse liver, lung, and ovary". Toxicol Appl Pharmacol **144**(1): 36-44.

Rajapaksa, K. S., E. A. Cannady, I. G. Sipes and P. B. Hoyer (2007). "Involvement of CYP 2E1 enzyme in ovotoxicity caused by 4-vinylcyclohexene and its metabolites". Toxicol Appl Pharmacol **221**(2): 215-21.

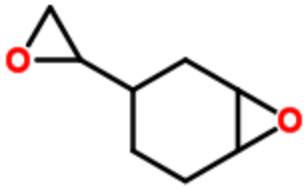
Smith, B. J., D. E. Carter and I. G. Sipes (1990). "Comparison of the disposition and in vitro metabolism of 4-vinylcyclohexene in the female mouse and rat". Toxicol Appl Pharmacol **105**(3): 364-71.

Smith, B. J., D. R. Mattison and I. G. Sipes (1990). "The role of epoxidation in 4-vinylcyclohexene-induced ovarian toxicity". Toxicol Appl Pharmacol **105**(3): 372-81.

Smith, B. J., D. R. Plowchalk, I. G. Sipes and D. R. Mattison (1991). "Comparison of random and serial sections in assessment of ovarian toxicity". Reprod Toxicol **5**(4): 379-83.

Smith, B. J., I. G. Sipes, J. C. Stevens and J. R. Halpert (1990). "The biochemical basis for the species difference in hepatic microsomal 4-vinylcyclohexene epoxidation between female mice and rats". Carcinogenesis **11**(11): 1951-7.

Vinyl Cyclohexene Dioxide
(4-Vinyl-1-Cyclohexene Diepoxide; VCD)



Molecular Formula: $C_8H_{12}O_2$

4-Vinyl-1-cyclohexene diepoxide is used as a reactive diluent for other diepoxides and for epoxy resins derived from bisphenol A and epichlorohydrin.

Relevant Studies

A relatively large body of relevant data was identified because the compound is a model compound for ovotoxicity. There is one publication on male reproductive and there are no publications on developmental toxicity. Below the publication on male reproductive toxicity is provided first, followed by the tabulation of that study's design parameters and results. Then the publications on female reproductive toxicity are listed. Because of the large number of studies, a recent review of female reproductive toxicity of VCD published in a peer-reviewed scientific journal is provided (in Appendix B), rather than data tabulations. Citations for the review and studies are provided below, and the full publications have been provided on CD to the DART IC and are available to the public upon request.

1. *Male Reproductive Toxicity (Summarized in Table)*

Hooser, S. B., D. G. DeMerell, D. A. Douds, P. Hoyer and I. G. Sipes (1995). "Testicular germ cell toxicity caused by vinylcyclohexene diepoxide in mice". Reprod Toxicol 9(4): 359-67.

Vinyl Cyclohexene Dioxide (VCD) : Study Reporting on Male Reproductive Effects

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	
Hooser et al. 1995	VCH 99% pure Vinylcyclo-hexene diepoxide (VCD) 97% pure Vinylcyclo-hexene 1,2-monoepoxide (VCM) 98% pure	B6C3F1 male mice, 4 weeks old 8-10 per group	VCH, VCD, and VCM testicular toxicity comparison	Intraperitoneal (i.p.) injection daily for 30 days sesame oil	0 (sesame oil), VCH: 800 mg/ kg-d VCD: 160 or 320 mg/kg-d VCM: 200 mg/ kg-d	Weights of testis, seminal vesicles Necropsy of testis, epididymis	No data reported	VCH: No effect on weights of testis or seminal vesicles VCD: Reduced weights of testis and seminal vesicles in both dose groups. VCM: No effect on weights of testis or seminal vesicles	
		B6C3F1 male mice, 4 weeks old 5 mice per group	VCD time-course study	i.p.injection daily for 5, 10, 15, 20, 25, or 30 days sesame oil	0 (sesame oil), 320 mg/kg-d	As above	No data reported	VCD: dosing for ≥ 5 days caused reduced testis weight and testicular degeneration.	
		B6C3F1 male mice, 4 weeks old 10 mice per group	VCD dose-response testicular toxicity study	i.p. injection daily for 30 days sesame oil	0 (sesame oil), 40, 80, 160 or 320 mg/kg-d	As above	No data reported	VCD: dosing at ≥ 80 mg/kg-d for 30 days caused reduced testis weight and testicular degeneration.	

2. *Female Reproductive Toxicity*

The scientific literature on the female reproductive toxicity of 4-vinyl-1-cyclohexene diepoxide (vinyl cyclohexane dioxide; VCD), the primary bioactive metabolite of 4-vinyl cyclohexene (VCH), is extensive. Studies have established that bioactivation of VCH to epoxides is required for its ovotoxicity, with VCD being the most potent epoxide of VCH in terms of follicular depletion. VCD is a model chemical used to induce perimenopause in rodents (Frye et al., 2012). A literature search conducted by OEHHA identified 55 female reproductive toxicity studies on VCD. Seven abstracts regarding female reproductive toxicity of VCD were also identified.

Due to the extensive literature available on female reproductive toxicity, a recent review of female reproductive toxicity of VCD published in a peer-reviewed scientific journal is provided (in Appendix B), rather than data tabulations. Citations for the review and studies are provided below, and the full publications have been provided on CD to the DART IC and are available to the public upon request.

Review (provided in Appendix B)

Kappeler, C. J. and P. B. Hoyer (2012). "4-vinylcyclohexene diepoxide: a model chemical for ovotoxicity". Sys Biol in Reprod Med **58**(1): 57-62.

Studies of Female Reproductive Toxicity of VCD

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Wright, L. E., J. B. Frye, A. L. Lukefahr, S. L. Marion, P. B. Hoyer, D. G. Besselsen and J. L. Funk (2011). "4-Vinylcyclohexene diepoxide (VCD) inhibits mammary epithelial differentiation and induces fibroadenoma formation in female Sprague Dawley rats". Reprod Toxicol **32**(1): 26-32.

Appendix A

Parameters for Computerized 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 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.

Appendix B

Reviews of the Female Reproductive Toxicity of 4-Vinyl-Cyclohexene and its Metabolite, Vinyl Cyclohexene Dioxide

The DART IC has been provided in this appendix the published reviews listed below. The public will be provided these reviews upon request.

Hoyer, P. B. and I. G. Sipes (2007). "Development of an animal model for ovotoxicity using 4-vinylcyclohexene: a case study". Birth Defects Research. Part B, Developmental and Reproductive Toxicology 80(2): 113-25.

Kappeler, C. J. and P. B. Hoyer (2012). "4-vinylcyclohexene diepoxide: a model chemical for ovotoxicity". Systems Biology in Reproductive Medicine **58**(1): 57-62.