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)	p,p'-Oxybis(benzenesulfonyl hydrazide)
2-Chloropropionic acid	1,3,5-Triglycidyl-s-triazinetrione
N,N'-Dimethylacetamide (DMAC)	4-Vinyl-cyclohexene (VCH)
2-Ethylhexanoic acid	Vinyl cyclohexene dioxide (VCD)
Ethyl-tert-butyl ether (ETBE)	

Office of Environmental Health Hazard Assessment
California Environmental Protection Agency

Hazard Identification Materials

- Nine chemicals being presented to the DART IC
  - decision as to whether they have been "clearly shown through scientifically valid testing according to generally accepted principles to cause reproductive toxicity".

- Relevant data have been provided
  - summary tables
  - original study reports and published papers (whenever available)

Hazard Identification Materials

- Publications identified through literature searches covering the three major reproductive toxicity endpoints: developmental, male reproductive and female reproductive toxicity.
  - Conducted through a contract with the Public Health Library at the University of California, Berkeley
  - Search protocol described in the Hazard Identification Document (Appendix A)

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

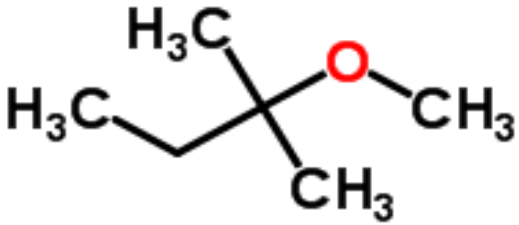
Chemical Listed via the Labor Code Mechanism

tert-Amyl Methyl Ether (TAME)

Office of Environmental Health Hazard Assessment
California Environmental Protection Agency

tert-Amyl Methyl Ether (TAME)

CAS Number: 994-05-8



- A comprehensive literature search resulted in three references with data on the potential reproductive toxicity of TAME in mice and rats.

tert-Amyl Methyl Ether (TAME)

Developmental toxicity studies in two species of rodents (Welsch et al., 2003)

- Effects at 3500 ppm
  - Reduced maternal body weight in mice and rats
  - Reduction in fetal body weight in mice and rats
  - Increased incidence of fetal death in CD mice but not in rats
  - Increased incidence of skeletal malformations in mice

tert-Amyl Methyl Ether (TAME)

Two-generation reproductive study (Tyl et al., 2003)

- **Endpoints:**
  - Dam toxicity: survival, organ and body weight, feed consumption
  - Fetal survival, body weight, vaginal patency and preputial separation for F1
  - Anogenital distance at birth for F2
  - Histopathological evaluation of reproductive organs

- **Results**:
  - Reduced body weight of dams during lactation at 3000 ppm
  - F0: Increased % abnormal sperm at 3000 ppm
  - Reduced body weight in F1 and F2 at 1500 ppm
  - Decrease survival of F2 at 3000 ppm
  - Reduced estrous cycle length at 1500 ppm
  - Increased gestational length at 1500 ppm.

tert-Amyl Methyl Ether (TAME)

Study of female reproductive toxicity (Berger and Horner, 2003)

- **Results**:
  - Reduced percentage of oocytes fertilized
  - Decreased penetrated sperm/oocyte

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

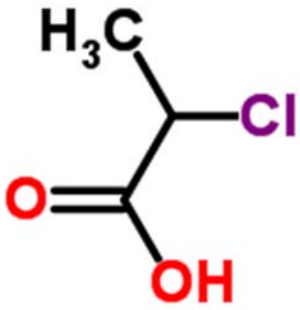
Chemical Listed via the Labor Code Mechanism

2-Chloropropionic Acid

Office of Environmental Health Hazard Assessment
California Environmental Protection Agency

2-Chloropropionic Acid

CAS Number: 598-78-7



- A comprehensive literature search produced two references regarding male reproductive toxicity

2-Chloropropionic Acid

- 2-Chloropropionic acid was converted to its neutral sodium salt, 2-chloroproprionate, for administration

- Two publications from the same laboratory (Yount et al., 1982a,b)
  - Metabolic studies conducted in Wistar rats

2-Chloropropionic Acid

- *In vitro* study (Yount et al., 1982a)
  - Isolated testicular cells
    - Testes from one adult rat
    - Pooled testes from 8 or more rats, 24-27 day old
    - Pooled testes from 40 rats,14-day old
  - Unspecified concentration for 60-minute incubation
  - Isolated testicular cells produced ketone bodies

2-Chloropropionic Acid

- *In vivo* study (Yount et al., 1982b)
  - 6 weanling rats/group
  - 12 week oral exposure
    - 0 (control), 0.04 mol/kg feed
      - Approximately 4 to 2.5 mmol/kg-d
  - Testicular damage
    - Testicular maturation arrest
    - Degeneration of germ cells
  - Mean testes plus epididymis weights of treated rats were significantly less than control values

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

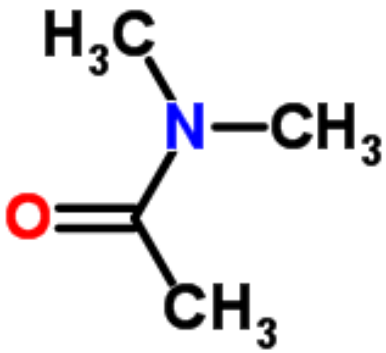
Chemical Listed via the Labor Code Mechanism

N,N-dimethylacetamide (DMAC)

Office of Environmental Health Hazard Assessment
California Environmental Protection Agency

N,N-Dimethyl Acetamide (DMAC)

CAS Number: 127-19-5



- Belongs to the “amide-type solvent” group with similar toxicity
- Inhalation and dermal concerns in the workplace
- Recent regulatory reviews: OECD (2001), Health Canada (2009), EU (2011)
- Emphasis on developmental toxicity

DMAC developmental toxicity studies

1960s-70s

Thiersch, 1962
Anderson and Morse, 1966
Von Kreybig et al., 1969
Monsanto, 1973a,b,c
Stula and Krauss, 1977

1980s-1990s

Merkle and Zeller, 1980
Johanssen et al., 1987
Solomon et al., 1991
Dupont, 1997

Oral and inhalation "guideline" studies

2000s

Klimisch and Hellwig, 2000
Okuda et al., 2006

Updated inhalation "guideline" studies

-Basic researchers uncover developmental toxicity of amide solvents including DMAC
-Published and unpublished dermal studies also found developmental toxicity

Route	rat	rabbit
Dermal	xx	xx
Oral	xx	x
Inhalation	xx	x

DMAC: Developmental toxicity endpoints

- Early injection studies:
  - Embryolethal, delayed embryo development; digit and tail malformations
- Early dermal studies:
  - Decreased litter size and fetal weight; skeletal deviations, particularly sternum; individual fetuses with encephalocele and anasarca

- Later oral guideline studies:
  - Fetal loss and reduced fetal weights
  - Anasarca (whole body edema)
  - Skeletal defects and reduced ossification, often sternebrae
  - Individual cleft palate, microophthalmia
  - Distinctive cardiovascular malformations
- Later inhalation guideline studies:
  - Major malformations not seen in inhalation studies

- Recent inhalation guideline studies:
  - Cardiovascular malformations
  - Anasarca, skeletal malformations and variations

DMAC: cardiovascular malformations

Rat Inhalation Toxicology Study (Okuda et al., 2006)

0, 100, 300, 450, 600 ppm

- Decreased pregnancy weight gain 450, 600 ppm

- Increased dam relative liver weight 300, 450, 600 ppm
- Hepatocyte swelling 450, 600 ppm
- No elevation of liver enzymes

- Increased fetal loss 600 ppm
- Decreased fetal weights 300, 450, 600 ppm

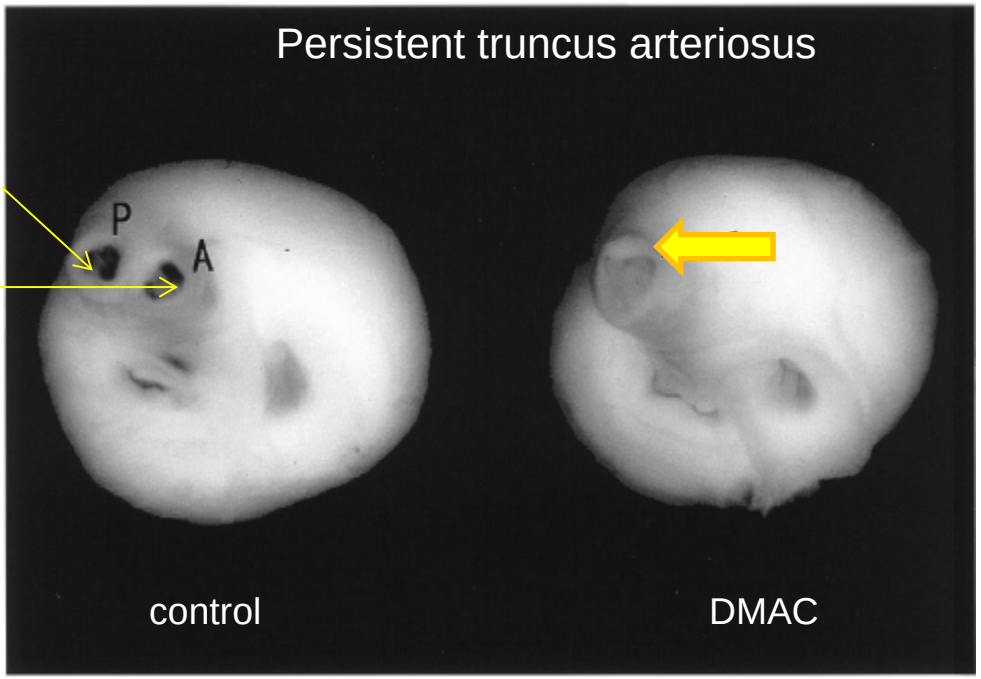
DMAC: cardiovascular malformations

	0 ppm	100 ppm	300 ppm	450 ppm	600 ppm
Ventricular septal defect	0(0)	0(0)	2(2)	7##(6)##	22##(8)##
Persistent truncus arteriosus	0(0)	0(0)	0(0)	2(1)	12## (7) ##
Malpositioned subclavian branch	0(0)	0(0)	0(0)	0(0)	4#(3)#
Retroesophageal subclavian	0(0)	0(0)	0(0)	0(0)	3#(3)#

(Okuda et al., 2006)

Fetal heart:
P=pulmonary artery

A=aorta



DMAC: Additional reproductive toxicity studies

- Dominant lethal studies in rats (McGregor, 1981; Kennedy, 1986)
  - Two inhalation studies produce inconsistent, marginal findings
  - Unpublished dermal and i.p. studies described as negative
- Subchronic inhalation (Valentine et al., 1997)
  - Unpublished data described as showing testicular atrophy in rats
  - Pubescent mice: testicular atrophy in the presence of mortality; epididymal and seminiferous tubule lesions
  - Adult mice: lower testes weight, seminiferous tubule atrophy; no sperm count effects
  - Adult rats; no effects on weight or pathology of testes, epididymis or sperm count
- Male fertility inhalation study in rats (Wang et al., 1989)
  - No fertility or developmental effects at end of pregnancy

DMAC: Additional reproductive toxicity studies

- One generation inhalation study with male and female exposure of rats (Ferenz and Kennedy, 1986)
  - No fertility effects
  - Two pups with external malformation at birth
  - Decreased postnatal weight gain

- Hamster studies (Miller et al., 1981)
  - Oral and s.c. treatment
  - Preimplantation embryotoxicity study
    - Dose-dependent pregnancy loss
    - Corpora lutea pathology
  - Delayed fertility trial
    - No fertility effect

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

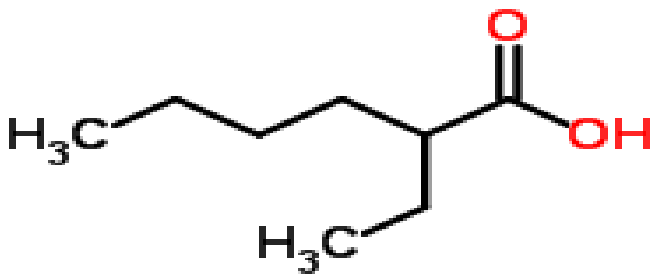
Chemical Listed via the Labor Code Mechanism

2-Ethylhexanoic Acid (EHXA)

Office of Environmental Health Hazard Assessment
California Environmental Protection Agency

2-Ethylhexanoic Acid (EHXA)

CAS Number: 149-57-5



- A primary metabolic product of the plasticizer di(2-ethylhexyl) phthalate (DEHP).

2-Ethylhexanoic Acid (EHXA)

- Eight studies examined the effects of EHXA on development subsequent to prenatal exposure in
  - Rat (Wistar, Fischer F344/Crl/Br, Sprague – Dawley),
  - Mouse (NMRI, SWV, C57BL) and
  - Rabbit (NZW)
- 3 studies examined the effects of EHXA using *in vitro* systems (embryo culture, cell-culture, FETAX)

2-Ethylhexanoic Acid (EHXA)

Wistar Rats

- Prenatal exposure (oral gavage) (Ritter et al, 1987)
  - Increase in % of dead and resorbed fetuses
  - Reduced fetal weight, Reduced litter size
  - Increase in fetal malformations (hydronephrosis, cardiovascular, tail and limb defects)
- Prenatal exposure (drinking water) (Pennanen et al., 1992)
  - No effects in number of implantations or live fetuses
  - Caused dose-related increase in skeletal malformations
- Pre-mating, prenatal & postnatal exposure (drinking water) (Pennanen et al., 1993)
  - Slight decrease in sperm quality, decrease in spermatozoa motility, increase in abnormal sperm
  - Reduced litter size
  - Delay in developmental landmarks (opening of eyes, eruption of teeth) and reflexes (grip reflex, cliff avoidance)
  - Single dose administration
    - GD 6: Reduced number of implantations, resorptions (80%)
    - GD 7: Less severe effects

2-Ethylhexanoic Acid (EHXA)

Fischer F344/Crl/Br

- Prenatal exposure (oral gavage) (Hendrickx et al., 1993)
  - Reduced fetal weight
  - Reduced ossification in fetuses

Sprague-Dawley Rats

- Prenatal exposure (oral gavage) (Narotsky et al., 1994)
  - Delayed parturition (GD 22 or later)
  - Reduced pup weight
  - Decreased progeny viability
  - Malformations: Syndactyly, vestigial tail, fused ribs, extra presacral vertebrae, increased incidence of cervical ribs, lumbar ribs
- Prenatal exposure (oral gavage) (Bui et al., 1998)
  - Increase in incidence of hydrocephaly, encephalocele and tail defects and rib anomalies in fetuses from EHXA-treated animals fed low and adequate Zn diet. Decrease in fetal weight and crown-rump length by EHXA treatment and low dietary Zn.
  - At the higher doses: Reduced ^{65}Zn retained in embryos compared to controls

2-Ethylhexanoic Acid (EHXA)

NMRI Mice

- Prenatal exposure (i.p. injection) (Hauck et al., 1990)
  - Embryotoxic (embryolethality, reduced fetal weight)
  - Teratogenic (exencephaly)

SWV Mice & C57BL Mice

- Prenatal exposure (i.p. injection) (Collins et al., 1992)
  - Increase in % dead or resorbed fetuses
  - Increase in exencephaly
  - SWV more sensitive strain than C57BL for induction of exencephaly
  - GDs 8.0, 8.5 and 9.0: Most sensitive time for exencephaly
  - Other malformations noted include ablepharon (open eyes), hydronephrosis, skeletal effects (axial skeleton and skull)

2-Ethylhexanoic Acid (EHXA)

New Zealand White Rabbits (NZW)

- Prenatal exposure(oral gavage) (Hendrickx et al., 1993)
  - No teratogenic effects
  - Some decrease in fetal BW at high dose level (250 mg/kg-d) but not statistically significant

2-Ethylhexanoic Acid (EHXA)

Embryo culture (Bui et al., 1998)

- Delayed development (Sera from low Zn diets or treatment with EHXA)
- Addition of Zn to these sera eliminated the developmental toxicity effects.

Cell-culture (pituitary cells) (Svechnikova et al., 2007)

- Directly enhanced sensitivity of gonadotropes to GnRH
- No effect on basal production of luteinizing hormone (LH)

Frog Embryo Teratogenesis Assay: *Xenopus* (FETAX)
(Dawson 1991)

- Increased malformations (microcephaly, abnormal gut coiling, eye edema, skeletal kinking and general edema)

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

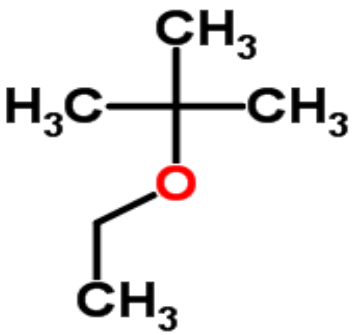
Chemical Listed via the Labor Code Mechanism

Ethyl-tert-Butyl Ether (ETBE)

Office of Environmental Health Hazard Assessment
California Environmental Protection Agency

Ethyl-tert-Butyl Ether (ETBE)

CAS Number: 637-92-3



- A comprehensive literature search resulted in six references with data on the potential reproductive toxicity of ETBE in laboratory animals.

Ethyl-tert-Butyl Ether (ETBE)

Toxicological studies in rats and mice (Medinsky et al., 1999)

- **Endpoints:**
  - Body weight
  - Pituitary, testes, epididymis, prostate, seminal vesicles, ovaries, vagina, uterus.
- **Results:**
  - Rats: significant effects in the testes such as increased percentage of seminiferous tubules with spermatocyte degeneration and decreased spermatocytes in tubules at 1750 and 5000 ppm
  - No reported effects in female rats
  - Mice: no reported effects

Ethyl-tert-Butyl Ether (ETBE)

Toxicological studies in rats (de Peyster et al., 2009)

- **Results:**
  - Fischer F344 rats, adult males:
    - Increased circulating estradiol at 1200 and 1800 mg/kg/day
  - Sprague Dawley rat isolated Leydig cells *in vitro:*
    - Low testosterone production at 50 and 100 nM

Ethyl-tert-Butyl Ether (ETBE)

Developmental study in rabbits (Asano et al., 2011)

- **Endpoints:**
  - Number of corpora lutea
  - Embryo-fetal deaths
  - 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

- **Results:**
  - No significant differences in the number of corpora lutea or implantations
  - No differences in fetal external malformations.

Ethyl-tert-Butyl Ether (ETBE)

One generation reproduction study (Fujii et al., 2010)

- **Results:**
  - Gestation was significantly prolonged
  - 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 or delivery
  - Normal estrous cycle in all groups
  - No significant difference in the number of pups delivered

Ethyl-tert-Butyl Ether (ETBE)

Developmental toxicity study in SD rats (Gaoua, 2004)

- **Endpoints**:
  - Clinical signs and mortality
  - Body weight and food consumption
  - Weight of gravid uterus
  - Number of corpora lutea, implantation sites, early and late resorptions, dead and live fetuses
  - The fetuses were weighed, sexed, soft tissue and skeletal examination
- **Results:**
  - Lower maternal body weight gain over the treatment period
  - No treatment-related effects on gestational parameters or fetuses

Ethyl-tert-Butyl Ether (ETBE)

Study of female reproductive toxicity (Berger and Horner, 2003)

- **Results**:
  - No effect on percentage of oocytes fertilized
  - No effect on number of penetrated sperm/oocyte

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

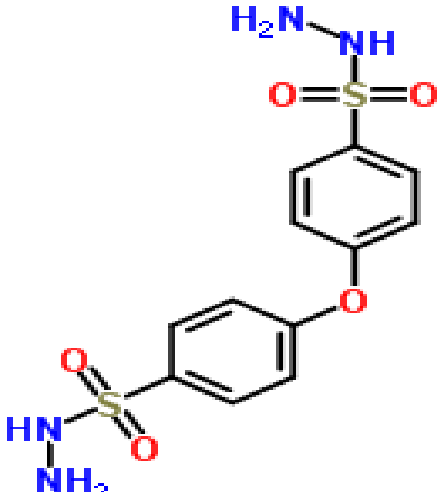
Chemical Listed via the Labor Code Mechanism

p,p’-Oxybis(benzenesulfonyl hydrazide)

Office of Environmental Health Hazard Assessment
California Environmental Protection Agency

p,p’-Oxybis(benzenesulfonyl hydrazide)

CAS Number: 80-51-3



- No relevant studies were identified for this chemical

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

Chemical Listed via the Labor Code Mechanism

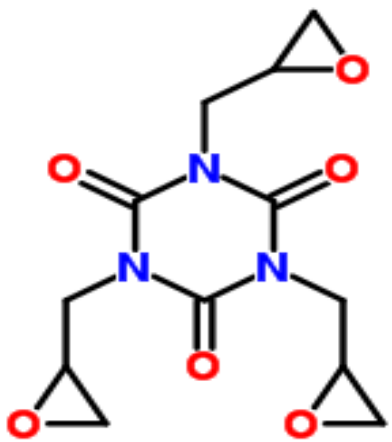
1,3,5-Triglycidyl-s-triazinetrione (TGIC)

Office of Environmental Health Hazard Assessment

California Environmental Protection Agency

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

CAS Number: 2451-62-9



- An epoxy compound
- Recent regulatory reviews:

  Australian Government (NICNAS, 1994) and Nordic Expert Group (2001)

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

- Several chromosome studies on male mice germinal epithelium (spermatogonia, spermatocytes)
- Dominant Lethal Assays (mice)
- Toxicity and Fertility study in the rat

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

- **Cytogenetic assays** evaluating chromosomal damage in male germinal epithelium in spermatogonia/spermatocytes
  - 10 studies in several strains of mice via varied routes of exposure (6 oral, 4 inhalation)
  - Most with chromosomal damage
  - Increase in frequencies of chromosomal aberrations (chromatid gaps, breaks and SCE)
  - 3 studies - No chromosomal damage was noted

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

Dominant Lethal Assay Design

- 4 studies in mice (2 oral, 2 inhalation)

  (Ciba-Geigy, 1986; Hazleton, 1989b; Bushy Run, 1992a,b)

Effects on development

- One oral study: (Ciba-Geigy, 1986)
  - Increase in number of embryonic deaths compared to control (for first mating period but not in second or third mating periods).
- Other 3 studies - No effects on embryos
  - No effect on number of resorptions/litter, total number of implants, number of viable implants or % postimplantation loss.

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

Dominant Lethal Assay Design

- 4 studies in mice (2 oral, 2 inhalation)

  (Ciba-Geigy, 1986; Hazleton, 1989b; Bushy Run, 1992a,b)

Effects on male reproductive system

- One inhalation study: (Bushy Run, 1992a)
  - Male fertility effects
  - Reduction in number of sperm-positive and pregnant females
  - High dose: Week 1, 2, 3 and Week 6
  - Low dose: Week 3

- Other 3 studies - No effects on male fertility

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

13-Week Toxicity & Fertility Study (SD-Rat) (CIT, 1995)

- Dose-related decrease in sperm count
- Reduced mean number of spermatozoa (5, 13 and 23% compared to controls – reported by Nordic Expert Group, 2001)

- No effect on spermatozoa viability
- Mating index = 100%; No impact on fertility
- No effects on pre- and post-implantation losses, number of live fetuses, fetal body weights, sex ratios, external anomalies or malformations

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

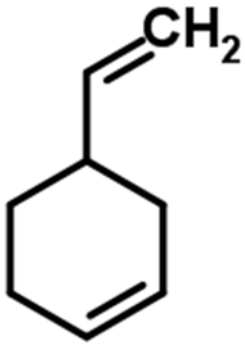
Chemicals Listed via the Labor Code Mechanism

4-Vinyl-Cyclohexene (VCH)
Vinyl-Cyclohexene Dioxide (VCD)

Office of Environmental Health Hazard Assessment
California Environmental Protection Agency

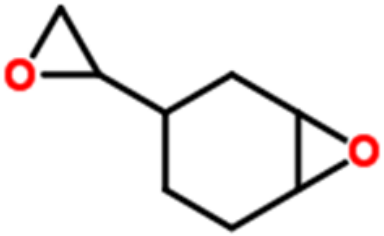
4-Vinyl-Cyclohexene (VCH)

CAS Number: 100-40-3

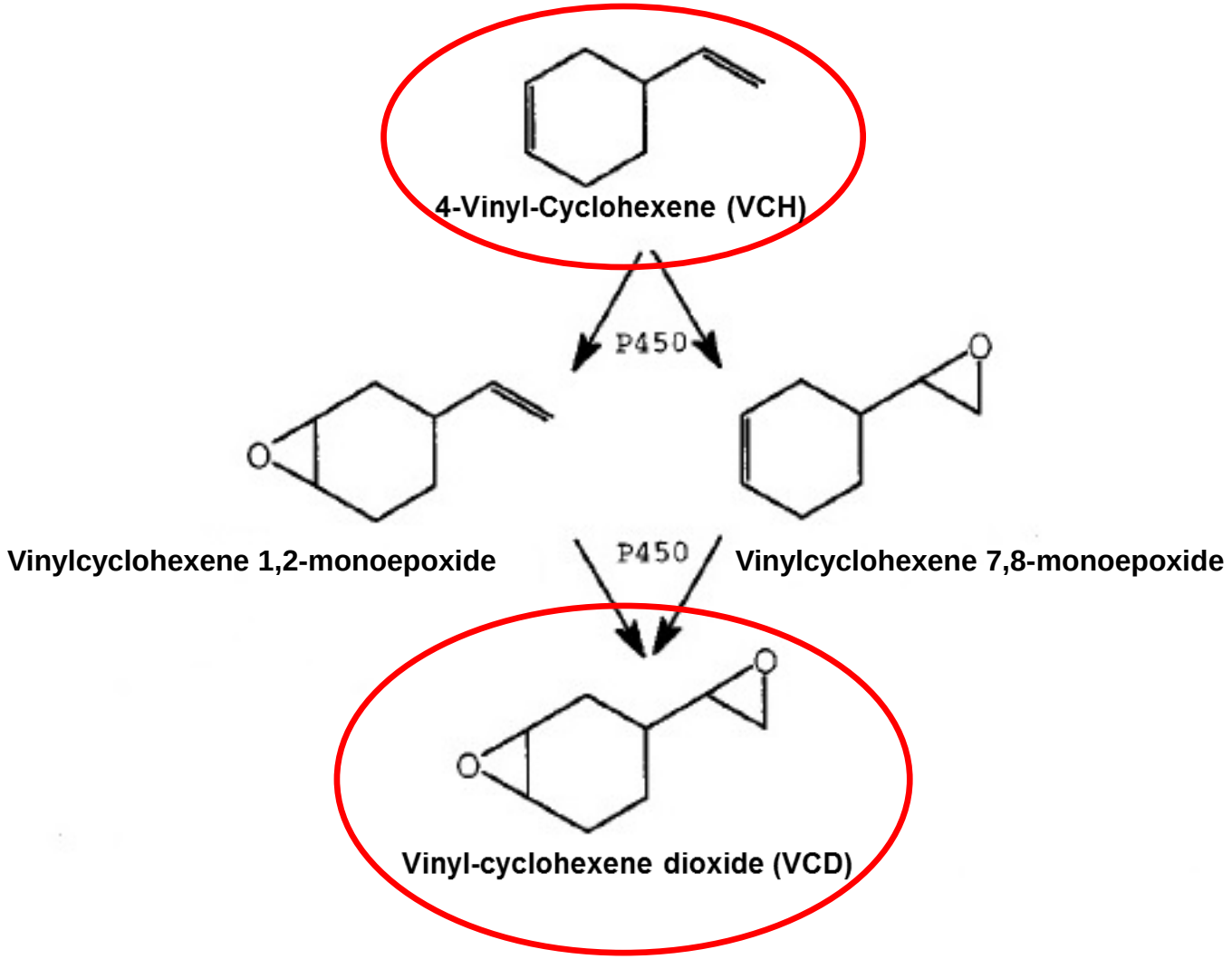


Vinyl-Cyclohexene Dioxide (VCD)

CAS Number: 106-87-6

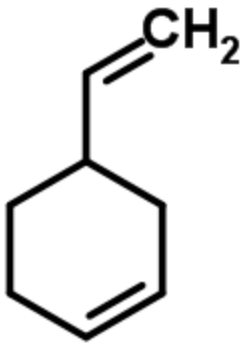


4-Vinyl-Cyclohexene Metabolism



4-Vinyl-Cyclohexene (VCH)

CAS Number: 100-40-3



- A comprehensive literature search produced
  - A few references on male reproductive toxicity and developmental toxicity
  - Numerous references on female reproductive toxicity

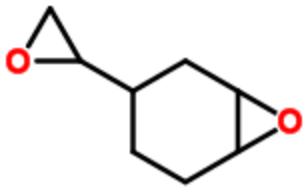
4-Vinyl-Cyclohexene (VCH)

- Studies on male reproductive and developmental toxicity
  - One study in mice suggests effects on testes (Grizzle et al., 1994; George et al., 1991)
    - Reduced spermatid heads per mg of testicular tissue
  - No effects on mating and fertility indices, or pregnancy outcome endpoints (Grizzle et al., 1994; George et al., 1991)
  - No developmental effects reported (Grizzle et al., 1994; George et al., 1991)

- Female reproductive toxicity
  - Extensive data because VCH is a model compound for ovarian toxicity in mice
    - References show VCH causes loss of small pre-antral (primordial and primary) follicles
  - Mice are more susceptible to VCH than rats
    - Mice are more capable of metabolic bioactivation of VCH

Vinyl-Cyclohexene Dioxide (VCD)

CAS Number: 106-87-6



- Vinyl cyclohexene dioxide is used commercially, as well as being a metabolite of VCH

- A comprehensive literature search resulted in
  - One reference on male reproductive toxicity
  - Extensive references on female reproductive toxicity

Vinyl-Cyclohexene Dioxide (VCD)

- Male reproductive toxicity (Hooser et al., 1995)
  - Reduced testicular weight
  - Testicular degeneration

- Female reproductive toxicity
  - Extensive data because VCD is a model compound for ovarian toxicity
    - VCD induces apoptosis of primordial and primary follicles in mice and rats
    - Rodent model of menopause