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

Chemicals Listed via the Labor Code Mechanism:

n-Butyl glycidyl ether

Diglycidyl ether

Phenyl glycidyl ether

Methyl n-butyl ketone

Methyl isopropyl ketone

α-Methyl styrene

March 19, 2014

Office of Environmental Health Hazard Assessment
California Environmental Protection Agency

Hazard Identification Materials

- Six 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)

Glycidyl Ethers

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

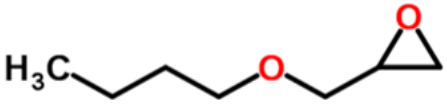
Chemical Listed via the Labor Code Mechanism

n-Butyl Glycidyl Ether

Office of Environmental Health Hazard Assessment
California Environmental Protection Agency

n-Butyl Glycidyl Ether (BGE)

CAS Number: 2426-08-6



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

n-Butyl Glycidyl Ether

Subchronic toxicity study in male rats (Anderson et al., 1957)

- **Exposure:** Inhalation at 0, 0.2, 0.4, 0.8, 1.6 g/m^3; 7 h/day; 5 days/week for 10 weeks. N=10 males/group
- **Endpoints:** Organ weight and pathology at end of the experiment

➢ Systemic toxicity results:

- Increased mortality: (none for 0 - 0.4 g/m^3), 10% at 0.8 g/m^3 and 50% at 1.6 g/m^3
- Reduced weight gain at 0.8 ($p<0.01$) and 1.6 ($p<0.05$) g/m^3
- Increased lung and liver weights at 1.6 g/m^3 ($p<0.05$)
- Bronchopneumonia in 1 rat at 0.4, and 5 rats at 0.8 g/m^3

n-Butyl Glycidyl Ether

Subchronic toxicity study in male rats (Anderson et al., 1957)

- **Exposure:** Inhalation at 0, 0.2, 0.4, 0.8, 1.6 g/m^3; 7 h/day; 5 days/week for 10 weeks. N=10 males/group

➢Reproductive toxicity results:

- Atrophic testes in 4/5 surviving animals (and 1 animal that died after 40 exposures) at 1.6 g/m^3
- Very small testes in 1/10 at 1.6 g/m^3
- Slight patchy testes atrophy in 1/10 at 0.4 g/m^3
- Only 1 case with testes atrophy had no other organ pathology

n-Butyl Glycidyl Ether

Dominant lethal study in mice (Pullin and Legator, 1977)

- **Exposure:** Dermal, 0 or 1.5 g/kg, 3 times/week for 8 weeks. N=10 males/group
- **Endpoints at 13-14 days post mating:**
  - Pregnancy rate
  - Implantations
  - Fetal mortality

➢ **Results**:

- Lower pregnancy rate at one and two weeks after exposure (p=0.05)
- Greater fetal mortality and postimplantation loss (p=0.04)

n-Butyl Glycidyl Ether

Dominant lethal study in mice (Whorton et al., 1983)

- **Exposure:** Dermal, 0 (saline), 0.375, 0.75, 1.5 g/kg-d, 3 times/week for 8 weeks. N= 36-44 males/group
- **Endpoints:**
  - Males: weekly body weight, testicular pathology
  - Females (evaluated 13-14 days post mating): pregnancy, implantations, and fetal death

➢ **Results**:

- No significant dose-related testicular changes
- Greater fetal death rate at 1.5 g/kg-d, after 1st week of mating ($p<0.05$)

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

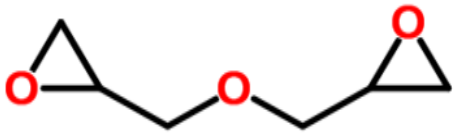
Chemical Listed via the Labor Code Mechanism

Diglycidyl Ether

Office of Environmental Health Hazard Assessment
California Environmental Protection Agency

Diglycidyl Ether (DGE)

CAS Number: 2238-07-5



- A comprehensive literature search produced one reference regarding male reproductive toxicity of DGE in laboratory animals.

Diglycidyl Ether

Toxicological studies in male rats, rabbits and dogs (Hine et al., 1961)

- **Endpoints:**
  - Peripheral blood, bone marrow, body weight, and mortality
  - Physical observation and histology for testes, skin, lymphoid, kidney, adrenal medulla and pancreas at necropsy
  - Males: weekly body weight, testicular pathology
  - Females: pregnancy, implantations, fetal death

Diglycidyl Ether

Rat Study #1 (Hine et al.,1961)

- **Exposure:** cutaneous at 0, 125, 250, 500 mg/kg; daily 5 days/week for 4 weeks*. N=5 males/group

➢ **Systemic toxicity results:**

  – 2 deaths at 125 mg/kg

  – 2 deaths each at 250 and 500 mg/kg after six applications (*treatment stopped in these groups)

  Effects seen at all doses:

  – Weight loss

  – Reduced leukocyte count

  – Necrosis of the skin, lymphoid tissue and kidney

  – Hemorrhage of the adrenal medulla

Diglycidyl Ether

Rat Study #1 (Hine et al.,1961)

- **Exposure:** cutaneous at 0, 125, 250, 500 mg/kg; daily 5 days/week for 4 weeks*. N=5 males/group

➢ Reproductive toxicity results:

  – Focal necrosis of the testes at all doses. No specific findings for the different dose groups were provided (p values not provided)

Diglycidyl Ether

Rat Study #2 (Hine et al.,1961)

- **Exposure:** cutaneous at 0, 15, 30, 60 mg/kg; daily 5 days/week for 4 weeks. N=5 males/group

➢ Systemic toxicity results:

- Weight gain reported to be significantly retarded at 30 and 60 mg/kg (no data provided)
- No deaths; no visceral abnormalities

➢ Reproductive toxicity results:

- It was reported that there was no adverse effect on testes to body weight ratio

Diglycidyl Ether

Rat Study #3 (Hine et al.,1961)

- **Exposure:** Inhalation at 0, 3 ppm for 4 h/day, 5 days/week for 29 day period. N=30 (DGE), N=10 (control) males [only 15 treated males evaluated – basis for selection not stated]

➢ **Systemic toxicity results:**

  – 5 animals died (pneumonia, bronchopneumonia, necrosis of the pancreas and spleen)

  – Reduced percentage body weight gain (reported to be significant; p value not provided)

  – Reduction in total leukocyte count, percentage of polymorphonuclear cells and number of nucleated cells in the femoral marrow

Diglycidyl Ether

Rat Study #3 (Hine et al.,1961)

- **Exposure:** Inhalation at 0, 3 ppm for 4 h/day, 5 days/week for 29 day period. N=30 (DGE), N=10 (control) males [only 15 treated animals evaluated – basis for selection not stated]

➢ Reproductive toxicity results:

- In 15 treated animals: one case of necrosis of the tubules of the testes.
- Apparent increase (10%, NS) in relative testes weight

Diglycidyl Ether

Rat Study #4 (Hine et al.,1961)

- **Exposure:** Inhalation at 0, 0.3 ppm for 4 h/day, 5 days/week for 90 day period. N=30 (DGE), N=15 (control) males [only 10 treated and 5 control animals evaluated – basis for selection not stated]

➢ Systemic toxicity results:

- One animal had acute peribronchiolitis. Not reported if it was one of the 5 showing reproductive toxicity.
- No other systemic toxicity reported

➢ Reproductive toxicity results:

- Five rats had poorly defined focal degeneration of the germinal epithelium

Diglycidyl Ether

Rabbit Study (Hine et al.,1961)

- **Exposure:** Inhalation at 0, 3, 6, 12, 24 ppm for 24 hours. N=3 males/group

➢ Systemic toxicity results:

- Two rabbits in the 24 ppm group died with 30% and 35% weight loss. One had confluent bronchopneumonia and hepatitis. The other had focal atelectasis, peribronchiolitis, and focal hemorrhage in the kidneys and lungs
- The third rabbit in the 24 ppm group died 2 days later, with 35% weight loss, and was not necropsied
- Rabbits exposed to lower levels showed no gross changes at necropsy and were not studied histologically

Diglycidyl Ether

Rabbit Study (Hine et al.,1961)

- **Exposure:** Inhalation at 0, 3, 6, 12, 24 ppm for 24 hours. N=3 males/group

➢ Reproductive toxicity results:

- The first two animals that died at 24 ppm had greatly atrophied testes
- No additional testicular effects were reported

Diglycidyl Ether

Dog Study (Hine et al.,1961)

- **Exposure:** Single weekly intravenous injection at 25 mg/kg for 3 weeks. N=3 (treated) males, no control group reported

➢ Systemic toxicity results:

- Low leukocyte count ($p < 0.01$)
- 2 dogs died: one 7 days after the second injection (apparently of pneumonia), the other 6 days after the third weekly injection

➢ Reproductive toxicity results:

- Hyaline degeneration of the testicular tubules seen in the dog that died 7 days after the second injection

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

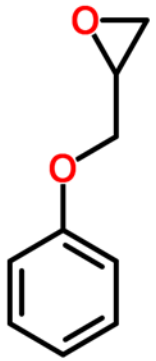
Chemical Listed via the Labor Code Mechanism

Phenyl Glycidyl Ether

Office of Environmental Health Hazard Assessment
California Environmental Protection Agency

Phenyl Glycidyl Ether (PGE)

CAS Number: 122-60-1



- A comprehensive literature search resulted in two references with data on the potential reproductive toxicity of PGE in rats and dogs.

Phenyl Glycidyl Ether

Rat Study #1 (Terrill and Lee, 1977)

Exposure: Inhalation at 0, 29 ppm for 4 h/day, 5 days/week, for 2 weeks. N=6 males/group

- **Systemic toxicity results:**
  - Depressed weight gain (p value not provided)

- **Reproductive toxicity results:**
  - "Atrophic changes" in various organs including testes (p value not provided)

Phenyl Glycidyl Ether

Rat Study #2 (Terrill and Lee, 1977)

Exposure: Inhalation at 1, 5, 12 ppm for 6 h/day, 5 days/week, for 90 days. N=32/sex/group

- **Systemic toxicity results:**
  - No adverse effects reported
- **Reproductive toxicity results:**
  - No significant changes in histological examinations of relevant tissues

Phenyl Glycidyl Ether

Dog Study (Terrill and Lee, 1977)

Exposure: Inhalation at 1, 5, 12 ppm for 6 h/day, 5 days/week, for 90 days

- **Systemic toxicity results:**
  - No adverse effects reported
- **Reproductive toxicity results:**
  - No significant changes in histological examinations of relevant tissues

Phenyl Glycidyl Ether

Inhalation toxicity studies in rats (Terrill et al., 1982)

Study #1: Two-generation rat reproduction and dominant lethal study

Exposure: Inhalation at 0, 2, 6, 11 ppm for 6 h/day, 19 consecutive days. N=8 males/group

- **Endpoints:**
  - Fertility parameters
  - On GD 18: gross examination of uterine content
  - Corpora lutea (CL), implantations and resorptions
  - Gross pathology of females that did not conceive at GD23 and some F1 males and females at 12 weeks post weaning
  - Histopathology on testes of the F0 males

Phenyl Glycidyl Ether

Study #1 Two-generation rat reproduction and dominant lethal study (Terrill et al., 1982)

Exposure: Inhalation at 0, 2, 6, 11 ppm for 6 h/day, 19 consecutive days. N=8 males/group

- **Systemic toxicity results:**
  - No increase mortality in F0

Phenyl Glycidyl Ether

Study #1 Two-generation rat reproduction and dominant lethal study (Terrill et al., 1982)

Exposure: Inhalation at 0, 2, 6, 11 ppm for 6 h/day, 19 consecutive days. N=8 males/group

- **Reproductive toxicity results:**
  - No increase in resorptions
  - No difference in number and survival of pups
  - Lower number of pregnant females in week 1 at 11 ppm ($p<0.05$)
  - Low fertility indices in F1a and F2a in all groups
  - No evidence of dominant lethal response

Phenyl Glycidyl Ether

Study #2 Rat teratogenicity study (Terrill et al., 1982)

Exposure: Inhalation at 0, 1, 5, 12 ppm for 6 h/day, GD4 to GD15. N=25 females/group

➢ **Results:**

- **Dams**
  - No changes in clinical signs or body weight gain
- **Offspring**
  - Number of implantations, fetuses, and resorptions were similar in all groups
  - Fetuses had similar length and weight, and all appeared normal upon gross exam

Ketones

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

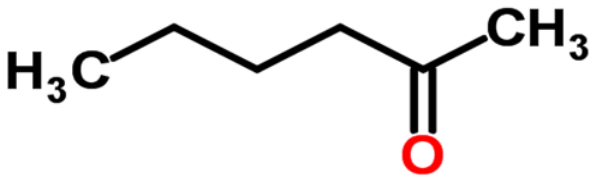
Chemical Listed via the Labor Code Mechanism

Methyl n-Butyl Ketone

Office of Environmental Health Hazard Assessment
California Environmental Protection Agency

Methyl n-Butyl Ketone (MnBK)

CAS Number: 591-78-6



- A comprehensive literature search resulted in three references with data on the potential reproductive toxicity of methyl n-butyl ketone in rats.

Methyl n-Butyl Ketone

Developmental neurotoxicity study in rats (Peters et al., 1981)

Exposure: Inhalation at 0, 500, 1000, 2000 ppm for 6 h/day, GD0 to GD20. N=25 females/group

- **Endpoints:**
  - Daily maternal weights; pregnancy outcome at birth and 1-3 weeks old
  - PND2 behavior observation; postnatal developmental indices; week 4, 8, 12 and month 18-20 gross and histopathology and behavioral test battery

Methyl n-Butyl Ketone

Developmental neurotoxicity study in rats (Peters et al., 1981)

Exposure: Inhalation at 0, 500, 1000, 2000 ppm for 6 h/day, GD0 to GD20. N=25 females/group

- **Parents results:**
  - Decreased maternal weight gain at 1000 ppm (10%), and 2000 ppm (14%)
  - Clinical signs at 2000 ppm: hair loss, incoordination; statistics not given

Methyl n-Butyl Ketone

Developmental neurotoxicity study in rats (Peters et al., 1981)

Exposure: Inhalation at 0, 500, 1000, 2000 ppm for 6 h/day, GD0 to GD20. N=25 females/group

- **Offspring results:**
  - Decreased litter size & birth weight (2000 ppm)
  - Decreased postnatal & adult weights (males at 1000, 2000 ppm)
  - Grip strength, maze latency, activity (1000, 2000 ppm male &/or female, at least one age)
  - Pentobarbital increased sleeping time (2000 ppm, males at puberty)
  - Decreased testes weights in weanlings
  - Ovarian cysts at 18 months

Methyl n-Butyl Ketone

Adult neurotoxicity study in rats (Katz et al., 1980)

Exposure: Inhalation at 0, 700 ppm for 72 h/week for 81 days (two 20-hour and two 16-hour exposure periods/week). N=5 males/group

- **Endpoints**:
  - Body weight
  - Clinical chemistry
  - Gross and histopathology
  - Neurotoxicity

Methyl n-Butyl Ketone

Adult neurotoxicity study in rats (Katz et al., 1980)

Exposure: Inhalation at 0, 700 ppm for 72 h/week for 81 days (two 20-hour and two 16-hour exposure periods/week). N=5 males/group

- **Systemic toxicity results:**
  - Markedly reduced weight gain
  - Decreased white cell counts
- **Reproductive toxicity results:**
  - Decreased absolute and relative testes weights
  - Atrophy of testicular germinal epithelium described (no data presented)

Methyl n-Butyl Ketone

Adult neurotoxicity study in rats (Krasavage et al., 1980)

Exposure: Gavage at 0, 660 mg/kg for 5 days/week for 90 days. N=5 males/group

- **Endpoints:**
  - Body weight
  - Gross and histopathology
  - Neurotoxicity

Methyl n-Butyl Ketone

Adult neurotoxicity study in rats (Krasavage et al., 1980)

Exposure: Gavage at 0, 660 mg/kg for 5 days/week for 90 days. N=5 males/group

- **Systemic toxicity results:**
  - Reduced body weight gain
- **Reproductive toxicity results:**
  - Atrophy of the testicular germinal epithelium described

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

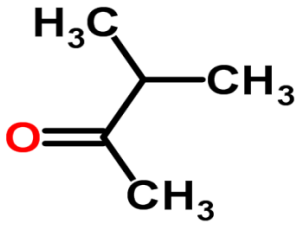
Chemical Listed via the Labor Code Mechanism

Methyl Isopropyl Ketone

Office of Environmental Health Hazard Assessment
California Environmental Protection Agency

Methyl Isopropyl Ketone (MIPK)

CAS Number: 563-80-4



- A comprehensive literature search resulted in one reference with data on the potential reproductive toxicity of MIPK in rats.
- An additional relevant guideline study for regulatory submission (unpublished) conducted in rats was submitted during the public comment period.

Methyl Isopropyl Ketone

Reproductive and developmental toxicity screening study in rats (Bernard, 2001)

Exposure: Inhalation at 0, 1, 2.5, 5 mg/L for 6 h/day; 7 days/week, from 2 weeks premating to GD19. N=12/sex/group

- **Endpoints:**
  - Systemic toxicity: body weight, food consumption
  - Fertility
  - Sperm parameters (epididymal number, morphology, motility)
  - Pregnancy outcome
  - Postnatal growth and mortality PND 0-4

Methyl Isopropyl Ketone

Reproductive and developmental toxicity screening study in rats (Bernard, 2001)

Exposure: Inhalation at 0, 1, 2.5, 5 mg/L for 6 h/day; 7 days/week, from 2 weeks premating to GD19. N=12/sex/group

- **Systemic toxicity results:**
  - Decreased paternal food intake & body weight at 1 mg/ml
  - Decreased maternal food intake premating and 1st week of gestation at all doses
  - Decreased maternal body weight 2nd premating week and last week of pregnancy, NOEL 1 mg/L
  - Maternal clinical signs during exposure, NOEL 1 mg/L

Methyl Isopropyl Ketone

Reproductive and developmental toxicity screening study in rats (Bernard, 2001)

Exposure: Inhalation at 0, 1, 2.5, 5 mg/L for 6 h/day; 7 days/week, from 2 weeks premating to GD19. N=12/sex/group

- **Reproductive toxicity results:**
  - None reported
- **Offspring results:**
  - Decreased number of live pups on PND 0 and 4, at 5 mg/L, $p \leq 0.05$
  - Increased number of dead pups on PND 0 at 2.5 mg/L, $p \leq 0.05$
  - Increased pups dying PND 0 to 4, at 5 mg/L, $p \leq 0.05$

Methyl Isopropyl Ketone

Study submitted during comment period

Developmental toxicity study in rats (Edwards, 2012)

Exposure: Inhalation at 0, 300, 750, 1500 ppm for 6 h/day; 7 days/week, from GD0 to GD19. N=25 females/group

- **Endpoints:**
  - Systemic toxicity: clinical observations, body weight, food consumption
  - Laparohysterectomy on GD20: uteri, placentae, ovaries, numbers of fetuses, resorptions, implantations, and corpora lutea

Methyl Isopropyl Ketone

Study submitted during comment period

Developmental toxicity study in rats (Edwards, 2012)

Exposure: Inhalation at 0, 300, 750, 1500 ppm for 6 h/day; 7 days/week, from GD0 to GD19. N=25 females/group

- **Systemic toxicity results:**
  - Decreased food intake and body weight at 750 and 1500 ppm
- **Reproductive toxicity results:**
  - Significant reduction in fetal weight at 750 ppm only ($p<0.05$)
  - No significant effect on fetal survival in any group

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

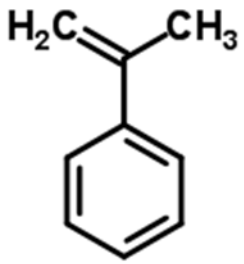
Chemical Listed via the Labor Code Mechanism

α-Methyl Styrene

Office of Environmental Health Hazard Assessment
California Environmental Protection Agency

α-Methyl Styrene (AMS)

CAS Number: 98-83-9



- A comprehensive literature search resulted in two references with data on the potential reproductive toxicity of α-methyl styrene in rats and mice.

α-Methyl Styrene

Developmental toxicity study in rats (Hardin et al., 1981)

Exposure: Intraperitoneal injection at 0, 250 mg/kg from GD1 to GD15. N=10-15 inseminated rats/group

- **Parents Endpoints:**
  - Gross examination of internal organs
  - Brain, heart, lungs, liver, spleen, kidneys, adrenals and ovaries weighed and preserved for histopathological examination

α-Methyl Styrene

Developmental toxicity study in rats (Hardin et al., 1981)

Exposure: Intraperitoneal injection at 0, 250 mg/kg from GD1 to GD15. N=10-15 inseminated rats/group

- **Offspring Endpoints:**
  - Weighed, measured for crown-rump length, sexed, and examined for externally visible malformations
  - One half to two-thirds of each litter used for internal examination
  - The rest of each litter preserved in ethanol for skeletal staining

α-Methyl Styrene

Developmental toxicity study in rats (Hardin et al., 1981)

Exposure: Intraperitoneal injection at 0, 250 mg/kg from GD1 to GD15. N=10-15 inseminated rats/group

- **Parents results:**
  - No treatment-related weight changes
  - No histopathological changes
- **Offspring results:**
  - Significantly increased incidence of fetal resorptions ($p < 0.05$)
  - Altered fetal sex ratio ($p < 0.05$) with a deficit of female fetuses

α-Methyl Styrene

3-month inhalation exposure studies in mice and rats (NTP, 2007)

Exposure: 0, 75, 150, 300, 600, and 1,000 ppm for 6h/day, 5 days/week for 14 weeks. N=10/sex/group

- **Endpoints:**
  - Body weights: initially, weekly, and at the end of the studies
  - Epididymal sperm concentration and motility
  - Cauda epididymis and testis weights
  - Vaginal cytology

α-Methyl Styrene

3-month inhalation exposure studies in mice and rats (NTP, 2007)

Exposure: 0, 75, 150, 300, 600, and 1,000 ppm for 6h/day, 5 days/week for 14 weeks. N=10/sex/group

Mouse studies

- **Systemic toxicity results:**
  - 5 to 17% decrease in body weight ($p<0.05$) for both males and females at 300, 600 and 1000 ppm
- **Reproductive toxicity results:**
  - Decreased cauda epididymal weights at 600 and 1000 ppm ($p<0.05$)
  - No effect on other reproductive endpoints in males
  - Longer estrous cycle at 600 and 1,000 ppm: 3.9 days (control) vs. 4.8 and 5.2, respectively ($p<0.05$ for 600 ppm and $p<0.01$ for 1000 ppm)

α-Methyl Styrene

3-month inhalation exposure studies in mice and rats (NTP, 2007)

Exposure: 0, 75, 150, 300, 600, or 1,000 ppm for 6h/day, 5 days/week for 14 weeks. N=10/sex/group

Rat studies

- **Systemic toxicity results:**
  - No difference in body weights at any dose
  - Kidney toxicity

- **Reproductive toxicity results:**
  - No observable adverse reproductive effects reported in treated rats of either sex