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

## Chemicals Listed via the Labor Code Mechanism:

Hexafluoroacetone
Phenylphosphine

## Chemical Listed via the Authoritative Bodies Mechanism:

Chlorsulfuron

Office of Environmental Health Hazard Assessment
California Environmental Protection Agency
May 2014

# Hazard Identification Materials

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

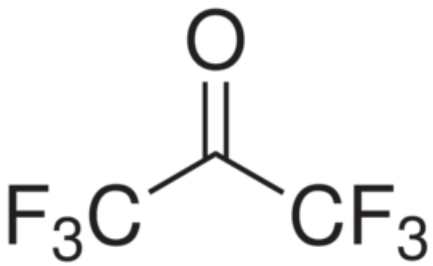
- Relevant data have been provided
  - summary tables
  - original study reports and published papers (whenever available)

# 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)

# Hexafluoroacetone (HFA)

CAS Number: 684-16-2




**A comprehensive literature search resulted in eight references with data on the potential reproductive toxicity of HFA in rats**

**Four references concern the potential developmental toxicity of HFA**

- Three conducted by the dermal route of exposure
- One conducted by the inhalation route of exposure

**Four references concern the potential male reproductive toxicity of HFA**

- Three conducted by the dermal route of exposure
- One conducted by the inhalation route of exposure

# Hexafluoroacetone (HFA)

Dermal developmental toxicity studies in rats (Britelli et al., 1979)

**Design and Methods**:

*Range-finding study*:

- N = 15 total pregnant females divided among 10 doses, ranging from 2.3 to 90 mg/kg-day; compared to vehicle (water) controls
- Exposure: daily on GD 6-16; HFA rubbed into shaved area on back
- Evaluated for viability and gross malformations

*Teratology study*:

- N = 14/group, doses of 1.0, 5.0, or 25 mg/kg-day; compared to vehicle controls daily on GD 6-16; HFA rubbed into shaved area on back
- Dams observed daily during treatment; body weights on GD 1, 6-16, 20, and 21
- Day 21 fetuses evaluated for all standard teratological endpoints

# Hexafluoroacetone (HFA)

Dermal developmental toxicity studies in rats (Britelli et al., 1979)

- **Range finding study**
  - Evidence of maternal toxicity at 90 mg/kg-day
  - At doses of 40 mg/kg-day and above, 35/36 fetuses resorbed
  - At lower doses, “high percentage of abnormal fetuses”
- **Teratology study** (effects noted differ from controls at $p < 0.05$)
  - 1 mg/kg-day -- Increased frequency of litters with hydrocephalus
  - 5 mg/kg-day – Increased frequencies of litters with stunted fetuses, pale fetuses, hemorrhage, hematoma, wavy ribs, and chondrodystrophy
  - 25 mg/kg-day – All effects seen at the lower doses as well as:
    - Decreased live fetuses per litter, and reduced mean fetal weight and crown rump length
    - Increased frequencies of dead fetuses, fetal resorptions per litter, and litters with resorptions
    - Increased litter-based frequencies of anasarca, anophthalmia, hydronephrosis, cleft palate, small kidneys, bipartite vertebral centra, unossified carpals and/or tarsals, and scoliosis
  - Reduced final maternal body weight at 5 and 25 mg/kg-day; 25% of treated dams showed skin irritation at application site

# Hexafluoroacetone (HFA)

Dermal developmental toxicity studies in rats (Becci et al., 1982 )

**Design and Methods**:

- HFA served as positive control for dermal effects on development
- Range-finding study: N = 5, dose = 0.5 mg/kg-day; compared to untreated controls
- Teratology study: N = 25, dose = 10 mg/kg-day; compared to tap water treated controls
- Exposure: Daily on GD 6-15; HFA in tap water applied to clipped area on back
- Maternal animals observed daily; body weights on days 0, 6, 9, 12, 15, and 20; sacrificed on GD 20
- Range-finding study endpoints: uterine pathology, litter endpoints of fertility and viability, fetal weight and sex
- Teratology study endpoints: above endpoints plus standard soft tissue and skeletal examinations

# Hexafluoroacetone (HFA)

Dermal developmental toxicity studies in rats (Becci et al., 1982)

- **Maternal endpoints**
  - No effects on pregnancy rate or gestational weight gain in either the range finding or the teratology study
- **Litter endpoints**
  - *Range finding study* – No significant difference from controls in live litter size, implantation frequency, resorption frequency, or mean fetal weight
  - *Teratology study*
    - No significant difference from controls in mean live litter size, sex ratio, or implantation frequency
    - Significant increase in mean resorption frequency per litter ($p \leq 0.05$)
    - Significant decrease in mean fetal weight ($p \leq 0.05$)
- **Malformations and variations** (assessed in teratology study only)
  - No significant increase in the frequency of any soft tissue anomaly
  - Increases in certain skeletal anomalies: missing sternebrae, wavy ribs, rudimentary ribs, extra ribs, incompletely ossified vertebrae, incomplete closing of the skull, incomplete ossification of extremities, and missing or incomplete hyoid bones ($p \leq 0.05$ in each case)

# Hexafluoroacetone (HFA)

Haskell Laboratory, E.I. Dupont de Nemours & Co. Inc. (1988) FYI-OTS-0189-0662
Hoechst Celanese Corporation. (1991) 8EHQ-1191-1521

Two developmental toxicity studies were submitted to US EPA under the Toxic Substances Control Act (TSCA) Section 8(e); Substantial Risk Notifications (http://www.epa.gov/oppt/tsca8e/)

- Section 8(e) of the Toxic Substances Control Act (TSCA) requires chemical companies to provide US EPA with any evidence they hold that a product may pose a risk of harm
- In addition, EPA established a voluntary “For Your Information” (FYI) program for chemical risk submissions by persons or organizations not subject to the reporting requirements of TSCA Section 8(e).

# Hexafluoroacetone (HFA)

Dermal rat teratology study

Hoechst Celanese Corporation (1991) 8EHQ-1191-1521

This is the same rat dermal teratology study reported by Britelli et al. (1979)

# Hexafluoroacetone (HFA)

Inhalation rat teratology study

Haskell Laboratory, (1988) FYI-OTS-0189-0662

- **Methods -** Pregnant rats, inhalation 6 hr/day, daily GD 7-16, evaluation on GD 22
  - *Range finding study*: N = 6 per group, 0, 0.1, 10, 30, or 60 ppm
  - *Teratology study*: N = 24 per group, 0, 0.1, 1.0, or 7 ppm
- **Maternal results**
  - *Range finding study*: maternal mortality 100% at 60 ppm, 67% at 30 ppm
  - *Teratology study* – No effect on body weight, adjusted weight change, or food consumption. Increased liver weight at 1 and 7 ppm (absolute and relative to bw)
- **Litter and fetal results** (pairwise differences significant at $p < 0.05$)
  - *Range finding study*: resorptions 100% at 30 ppm, 3 surviving fetuses (from 2 litters) at 10 ppm. No effects observed at 0.1 ppm
  - *Teratology study*
    - <u>0.1 ppm</u>: decreased weight of female fetuses
    - <u>1.0 ppm</u>: decreased fetal weights, increased late resorptions; increased % of litters with variations
    - <u>7 ppm</u>: same as 1.0 ppm, plus decreased mean litter size, increased total malformations, and increased variations

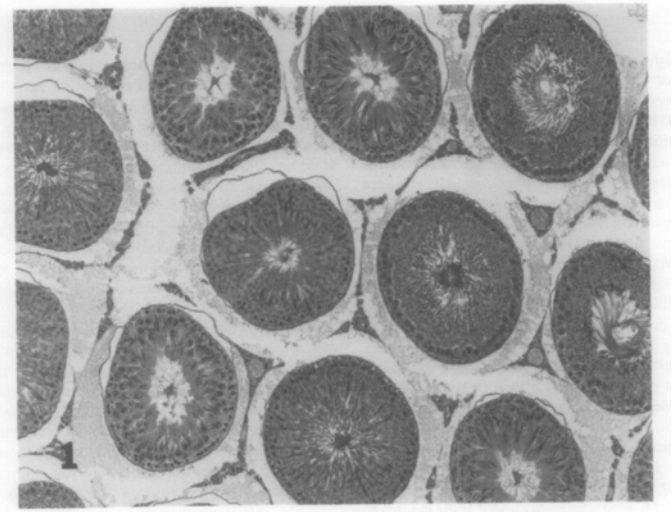
# Hexafluoroacetone (HFA)

Dermal rat male reproductive toxicity study (Gilles and Lee, 1983)

- **Methods**
  - Dermal application of 0, 13, 39, or 130 mg/kg-day, daily for 14 days
  - Each dose group had its own control group; control rats pair fed to treated rats
  - N = 8 per group; sacrifice 24 hours following last dose
  - Right testis from each rat prepared for light microscopy; left testis incubated with radiolabeled glucose and acetate for biochemical studies
- **Results**
  - Decrease in weight gain with increasing dose
  - Chromodacryorrhea observed at 39 and 130 mg/kg-day
  - Testicular atrophy: minimal at 39 mg/kg-day, severe at 130 mg/kg-day
  - Increased incorporation of radiolabeled glucose and acetate into triacylglycerol and phospholipid at 39 and 130 mg/kg-day
  - Decreased incorporation of radiolabeled acetate into sterols
  - No effect on levels of vitamin A or zinc

# Hexafluoroacetone (HFA)

Dermal rat male reproductive toxicity study (Gilles and Lee, 1983)



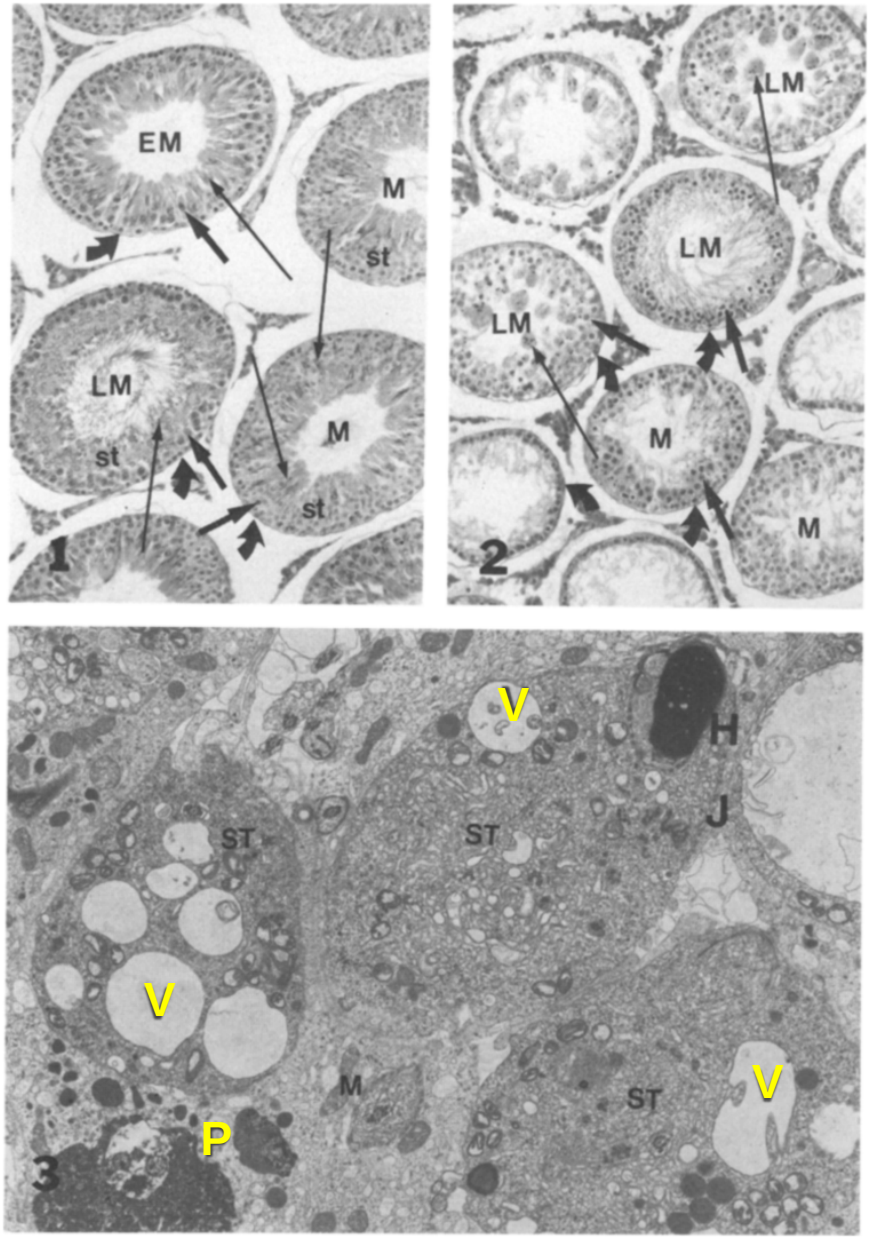

Seminiferous tubules of a testis from a control rat

Atrophic seminiferous tubules from a rat treated with 130 mg/kg-day HFA

# Hexafluoroacetone (HFA)

Dermal rat male reproductive toxicity study (Lee and Gilles, 1984)

- **Methods**
  - Dermal application of 0, 13, 39, or 130 mg/kg-day, daily for 14 days
  - Control rats dose with water (vehicle) and pair-fed to treated rats
  - N = 10 per group; sacrifice 24 hours following last dose
  - Testes examined grossly and prepared for light microscopy; testes from high dose group prepared for electron microscopy
- **Results**
  - No discussion of systemic toxicity, body weights or organ weights
  - Testicular atrophy:
    - Slight in 50% of rats at 39 mg/kg-day
    - Severe in all rats at 130 mg/kg-day
  - Light microscopy: effects on spermatids and spermatocytes, no effects noted on spermatogonia, Sertoli cells or Leydig cells
  - EM: Increased lipid droplets in Sertoli cells, and ultrastructural changes in Leydig cells




**Lee and Gilles, 1984**

Fig 1. Normal seminiferous tubules
X 80

Fig 2. Degenerative tubules
130 mg/kg-day HFA, X 80

Fig 3. EM level showing spermatids and Sertoli cells
130 mg/kg-day HFA, X 4200

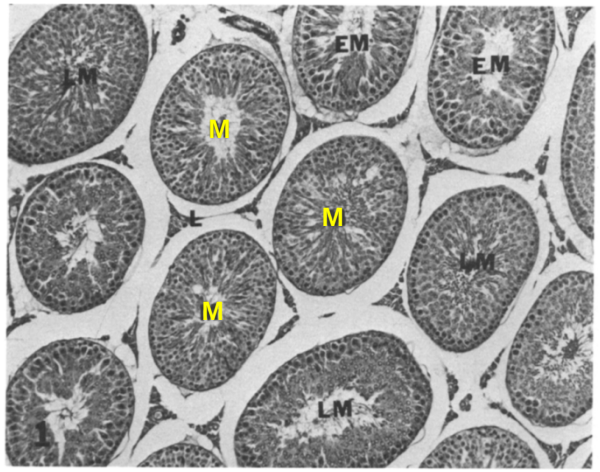
# Hexafluoroacetone (HFA)

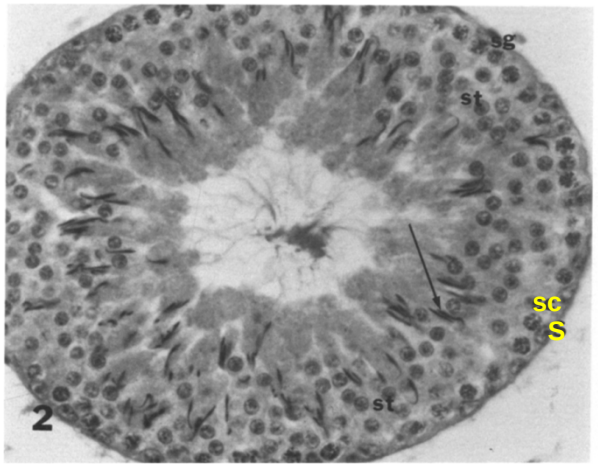
Dermal rat male reproductive toxicity study (Gilles and Lee,1985)

- **Methods**
  - In Vivo: Dermal application of 0 or 130 mg/kg-day, daily for 14 days. N = 7 exposed and 8 controls. Testicular weights, histology, hormone levels, uptake of radiolabeled acetate and mevalonate.
  - In Vitro: Testes from untreated rats incubated with 1.0 mM HFA plus 10 pCi/ml [$^{14}$C]acetate, 3 hours at 37.5° C
- **Results**
  - In Vivo:
    - No effect on final body weight, or serum levels of LH or testosterone
    - 48% increase in serum FSH
    - 43% reduction in testicular weights, 50% reduction in testicular testosterone
    - Degenerative changes in spermatocytes
    - Inhibition of Leydig cell $C_{19}$ steroidogenesis
  - In Vitro: No effect on steroidogenesis

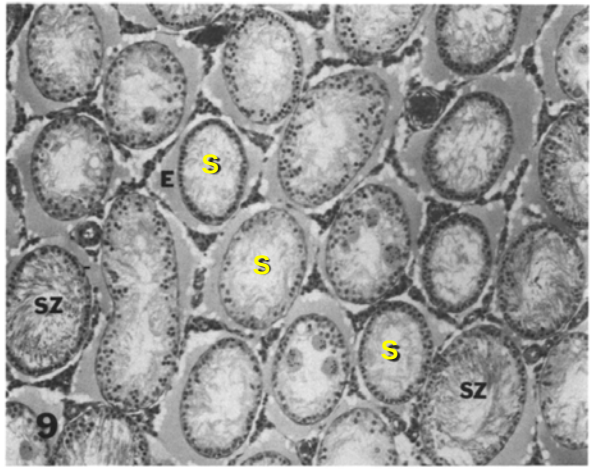
# Dermal rat male reproductive toxicity study (Gilles and Lee, 1985)

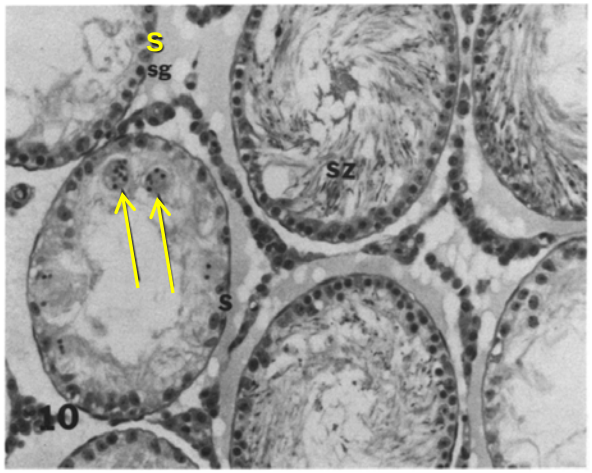
Control seminiferous tubules




Seminiferous tubules after 14 days HFA exposure



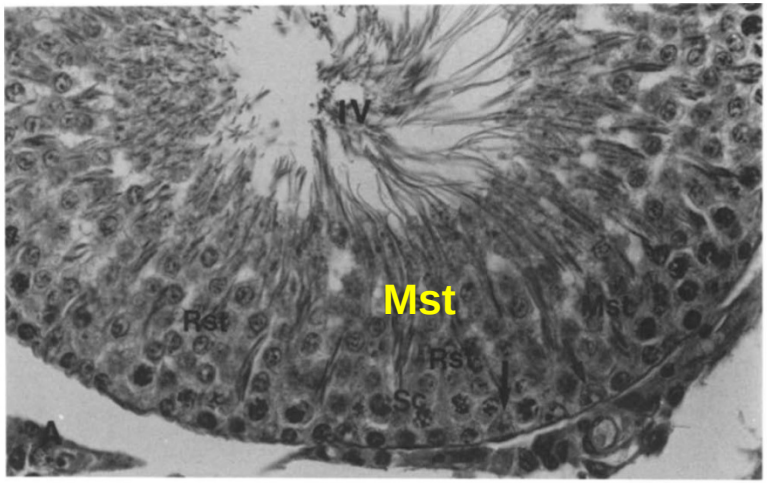
# Hexafluoroacetone (HFA)

Inhalation rat male reproductive toxicity study (Lee and Kennedy, 1991)

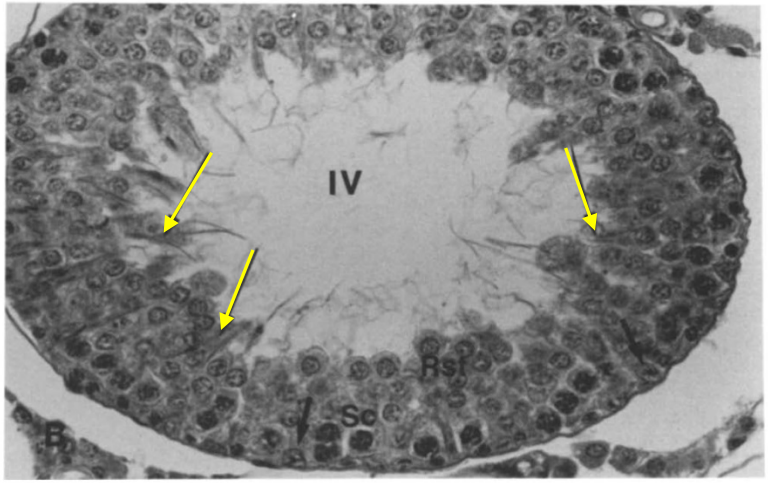
- **Methods**
  - Inhalation exposure to 0, 0.1, 1.0, or 12 ppm, 6 hr/day, 5 days/week for 90 days
  - Sacrificed after 30 or 90 days exposure, or at 28 or 84 days post-exposure (PE)
  - N = 40 per group; daily clinical observations; weighed weekly
  - Testes and epididymides weighed and prepared for light microscopy
- **Results**
  - 12 ppm, all time points: body weight decreased, clinical symptoms; decreased absolute testes weights
  - 12 ppm, 30 days exposure: testicular atrophy, and oligospermia or aspermia, effects on spermatids and spermatocytes
  - 12 ppm, 90 days exposure: severe testicular atrophy, disappearance of mature and immature spermatids from the seminiferous tubules, spermatozoa absent from epididymal tubules
  - 12 ppm, 28 days PE: evidence of variable regeneration
  - 12 ppm, 84 days PE: partial restoration of spermatogenesis

# Hexafluoroacetone (HFA)

Inhalation rat male reproductive toxicity study (Lee and Kennedy, 1991)




A seminiferous tubule at stage IV, control testis, 28 days PE, X 400




Seminiferous tubule at stage IV, 12 ppm, 28 days PE, X 400

# Summary of Data on Developmental Toxicity of HFA

## Rat developmental toxicity studies, dermal route

| Reference | Findings* | Effective doses |
|---|---|---|
| **Britelli et al., 1979; Hoechst Celanese Corp, 1991** | Reduced viability, reduced growth of surviving fetus, increased soft tissue and skeletal anomalies | 1, 5 and 25 mg/kg-day – increased severity and frequency of effects with increasing dose |
| **Becci et al., 1982** | Increased resorptions and skeletal anomalies, decreased mean fetal weight | 10 mg/kg-day |

*all listed findings significant at $p \leq 0.05$

## Rat developmental toxicity study, inhalation route

| Reference | Findings* | Effective doses |
|---|---|---|
| **Haskell Labs, 1988** | Decreased viability, reduced weights of surviving fetuses, increased soft tissue and skeletal anomalies | 0.1, 1.0, 7.0, 10 and 30 ppm – increased severity and frequency of effects with increasing dose |

*all listed findings significant at $p \leq 0.05$

# Summary of Data on the Male Reproductive Toxicity of HFA

Male rat reproductive toxicity studies, dermal route

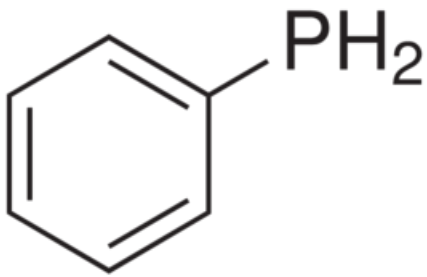
| Reference | Findings | Effective doses |
|---|---|---|
| Gilles & Lee, 1983 | Testicular atrophy | 39 and 140 mg/kg-day |
| Lee & Gilles, 1984 | Testicular atrophy<br>Damaged spermatids and spermatocytes<br>EM level effects on Sertoli and Leydig cells | 39 and 130 mg/kg-day |
| Gilles & Lee, 1985 | Increased serum FSH<br>Reduced: testicular weights, testicular testosterone, steroidogenesis<br>Degenerative spermatocytes | 130 mg/kg-day |

Male rat reproductive toxicity studies, inhalation route

| Reference | Findings | Effective dose |
|---|---|---|
| Lee & Kennedy, 1991 | Decreased body and testicular weights<br>Testicular atrophy and degenerative effects on germline cells | 12 ppm |

# Phenylphosphine

CAS Number: 638-21-1




**A comprehensive literature search identified two references with data on the potential reproductive toxicity of phenylphosphine in animals:**

Du Pont (1992). "Initial submission: 90-day inhalation toxicity study of phenylphosphine in rats and dogs with cover letter dated 101592." 8(e) submission to U.S EPA EPA/OTS **920009737**: #88-920009737.

Waritz, R. S. and R. M. Brown (1975). "Acute and subacute inhalation toxicities of phosphine, phenylphosphine and triphenylphosphine." Am Ind Hygn Asscn J **36**(6): 452-458.

# Phenylphosphine

Inhalation rat 90-day toxicity study
(Dupont de Nemours, 1992 - Submitted to US EPA)

- **Methods**
  - Inhalation, 6 hr/day, 5 days/week, 59 exposures; N = 20/sex/group
  - 0, 0.6, and 2.2 ppm (average per exposure)
  - Sacrifices, 5/sex/group at test days 30 and 90, and 28 days post exposure; remainder at 65 days post exposure
- **Results**
  - Systemic effects at 2.2 ppm:
    - Clinical and neurological effects, decreased body weight and weight gain
    - Symptoms resolved during post exposure period
  - Reproductive effects:
    - Irreversible, severe testicular degeneration at terminal sacrifice of 5/5 males in the 2.2 ppm group

# Phenylphosphine

## Inhalation toxicity study in male beagle dogs,

(Dupont de Nemours, 1992 - Submitted to US EPA)

- **Methods**
  - Inhalation, 6 hr/day, 5 days/week, 59 exposures; N = 4 group
  - 0, 0.6, and 2.2 ppm (average per exposure)
  - Sacrifices, 2 dogs/group at test day 90; remainder at 28 days post exposure
- **Results**
  - Systemic effects:
    - 0.6 ppm – clinical symptoms during treatment, resolved post exposure
    - 2.2 ppm – severe clinical symptoms, decreased hematological values and evidence of moderate anemia starting at 1 month of exposure and resolving post exposure
  - Reproductive effects:
    - Testicular degeneration – focal in 1/4 controls and 1/4 dogs at 0.6 ppm; diffuse in 3/4 dogs at 2.2 ppm
    - Oligospermia – 1/4 dogs at 0.6 ppm, 2/4 dogs at 2.2 ppm

# Phenylphosphine

Inhalation male rat acute and subacute toxicity study
(Waritz and Brown, 1975)

- **Methods**
  - *Acute study* — 4 hour exposure to 0, 19, 32, or 44 ppm; N = 6/group; 2 rats/group sacrificed at each of days 1, 2, and 7 post exposure to 19 ppm; 2 rats/group sacrificed at 14 days post exposure to 44 ppm; gross pathology
  - *Subacute study* — 4 hours/day for 10 days exposure to 0.31 µM/liter (ppm not provided, calculated by OEHHA to be 6.8 ppm); 3 rats/group sacrificed immediately post exposure; remaining 3 rats/group sacrificed 14 days post exposure; gross pathology
- **Results**
  - Systemic effects:
    - *Acute study* — respiratory irritation during exposure; no other evidence of toxicity reported
    - *Subacute study* — respiratory irritation during exposure; temporary dermatitis; foci of red blood cell formation in the spleen; decreased weight gain
  - Reproductive effects:
    - *Acute study* — none
    - *Subacute study* — “mild depression” of spermatogenesis 2/3 rats immediately post exposure and 1/3 rats 14 days post exposure

# Summary of Data on the Male Reproductive Toxicity of Phenylphosphine, Inhalation Route

Male rat and dog reproductive toxicity studies, inhalation route

| Reference | Findings | Effective doses |
|---|---|---|
| **Dupont, 1992 (90-day rat)** | Testicular degeneration | 2.2 ppm |
| **Dupont, 1992 (90-day dog)** | Testicular degeneration, oligospermia | 0.6, 2.2 ppm |
| **Waritz and Brown, 1975 (rat)** | Mild depression of spermatogenesis | 0.31 µM/liter (calculated by OEHHA to equal 6.82 ppm) |