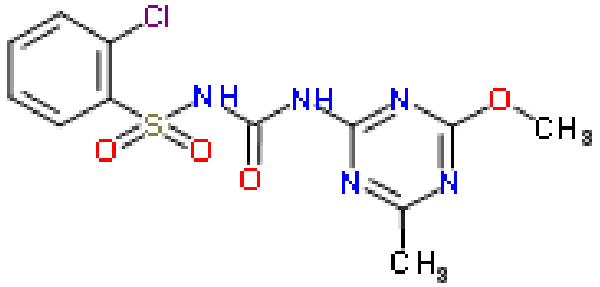
Chlorsulfuron

CAS Number: 64902-72-3



- A DART literature search was conducted, and proprietary studies were provided by DuPont Crop Protection requesting delisting of chlorsulfuron from Proposition 65
  - Developmental toxicity (teratogenicity)
  - Reproductive toxicity

Chlorsulfuron
Developmental Toxicity (Teratogenicity) Studies

- Hoberman, A. (1980). Chlorsulfuron (DPX - W4189) Technical: Teratology Study in Rabbits. E.I. du Pont de Nemours and Company. HLO 534–80, Laboratory Project ID 201 – 536. Haskell Laboratory for Toxicology and Industrial Medicine, Newark, DE 19714. USA.
  - 3 supplements
- Alvarez, L. (1991a). Teratogenicity Study of DPX-W4189-165 (Chlorsulfuron) in Rabbits. E.I. du Pont de Nemours and Company. Laboratory Project ID 306-90. Haskell Laboratory for Toxicology and Industrial Medicine, Newark, DE 19714. USA.
  - 3 supplements
- Alvarez L. (1991b). Teratogenicity Study of DPX-W4189-165 (Chlorsulfuron) in Rats. E.I.du Pont de Nemours and Company. Laboratory Project ID 734-90. Haskell Laboratory for Toxicology and Industrial Medicine, Newark, DE 19714. USA.

Chlorsulfuron: Hoberman, 1980

Teratogenicity study using pregnant New Zealand white rabbits

- **Exposure**: Oral intubation on gestation day (GD) 6-19
  - Doses: 0 (vehicle, corn oil), 10, 25, or 75 mg/kg-d
    - N=16 to 17 per group
- **Results:**
  - Reduced maternal body weight gain in treated groups GD 6-19
  - Increased resorptions at all doses
    - Significant at 75 mg/kg-d, but p-value was not reported
- Three study supplements

Chlorsulfuron: Supplements to Hoberman, 1980

Hoberman, 2010 - Supplement #1

- Re-analyzed fetal resorption data
  - Compared to MARTA historical control data (1993)
  - Excluded 100% resorbed litters

Hoberman, 2011 – Supplement #1, Revision #1

- Provided all data as individual animal data, corrected a calculation error, and presented group means that both include and exclude animals with total resorptions

Munley, 2014 – Supplement #1, Revision #2

- Corrected calculations and table entries
  - Excluded data from a female found in group 3 (25 mg/kg-d) that was dead on GD 18 from all litter mean calculations
  - Excluded data from two females in group 4 (high dose, 75 mg/kg-d) for misclassification

Chlorsulfuron: Hoberman, 1980 and supplements

Percent resorptions per litter				
	0 (vehicle)	**10 mg/kg-d**	**25 mg/kg-d**	**75 mg/kg-d**
Hoberman, 1980	11.6%	23.9%	13.8%	31.3%* (p = 0.01)
Hoberman, 2010 (excludes 100% resorptions)	[11.6%]	[17.3%]	[9.7%]	[14.8%]
Hoberman, 2011 (all pregnancies)	11.6%	23.9%	13.9%	35.9%* (p = 0.01)
Hoberman, 2011 (excludes 100% resorptions)	11.6%	18.0%	8.5%	26.0%* (p = 0.02)
Munley, 2014 (excludes 100% resorptions)	11.6%	18.0%	8.5%	28.2%* (p = 0.01)

*: indicates significant pairwise differences from vehicle control group; *p*-values from the Wilcoxon rank sum (Mann-Whitney) test are given in parentheses

- MARTA historical control data
  - Resorption rate range 0 – 29.2%

Chlorsulfuron: Alvarez, 1991a

Teratogenicity study in pregnant New Zealand white rabbits

- Two-part study: Main and supplemental parts
- **Exposure:** Gavage once a day, GD 7-19
  - Doses:
    - *Main:* 0 (vehicle, aqueous 0.5% methyl cellulose (w/v) solution), 25, 75, 200, or 400 mg/kg-d
      - N=20/group
    - *Supplemental:* 0 (vehicle, aqueous 0.5% methyl cellulose (w/v) solution), 400, or 1000 mg/kg-d
      - N=20/group

Chlorsulfuron: Alvarez, 1991a

(continued)

- **Results:**
  - Maternal toxicity

    Main study: none reported

    Supplemental study:

    o Decreased mean maternal weight gain on days 7-29 in 400 mg/kg-d group

    o Maternal toxicity in 1000 mg/kg-d group
      - significant incidence of mortality
      - reduced maternal weight gain
      - increased clinical signs
  - Fetal toxicity

    o Significant increase in minor fetal skeletal defects and total fetal malformations at 400 mg/kg-d

    o Increased incidence of unossified sternebra at 1000 mg/kg-d

    o Reduced fetal weight at 400 mg/kg-d
- Three study supplements

Chlorsulfuron: Supplements to Alvarez, 1991a

Mylchreest, 2005a – Supplement #1

- Done to add one row of information, percent resorptions per litter, to the reproductive outcome tables for the main and supplemental studies

Lewis, 2008 – Supplement #2

- Re-tabulated data and performed a retrospective statistical analysis of maternal and developmental findings in the context of historical control data
  - Skeletal variation (fetal sternebrae and fetal skull findings)

Munley, 2012a – Supplement #3

- Provided additional statistical analysis to support the interpretation of the fetal body weight data from the original Alvarez study

Percent resorptions per litter
(Mylchreest, 2005a: supplement to Alvarez, 1991a)

	0 (vehicle)	25 mg/kg-d	75 mg/kg-d	200 mg/kg-d	400 mg/kg-d	1000 mg/kg-d
Main study	2.5%	10.5%	8.8%	9.6%	5.6%	---
Supplemental study	9.6%	---	---	---	4.1%	9.4%

Incidence of skeletal variations
(Lewis, 2008: supplement to Alvarez, 1991a)

		0 (vehicle)	25 mg/kg-d	75 mg/kg-d	200 mg/kg-d	400 mg/kg-d	1000 mg/kg-d
unossified sternebrae fetuses (litters)	*Main*	0	0	1 (1)	2 (1)	3 (3)	---
	Supplemental	0	---	---	---	2 (2)	0
partially ossified sternebrae fetuses (litters affected/total)	*Main*	13 (6/16)	5 (4/17)	10 (7/15)	7 (4/16)	13 (5/16)	---
	Supplemental	5 (1/10)	---	---	---	12 (5/13)	4 (2/4)
partially ossified skull bone fetuses (litters)	*Main*	0	0	0	1 (1)	2 (2)	---
	Supplemental	0	---	---	---	0	0

Fetal Malformations
(Alvarez, 1991a)

	0 (vehicle)	25 mg/kg-d	75 mg/kg-d	200 mg/kg-d	400 mg/kg-d	1000 mg/kg-d
Main Study						
Visceral	0(0)	0(0)	0(0)	0(0)	1(1) absent gallbladder 1(1) doubled aorta 1(1) ventricular septal defect	---
Hemivertebra	0(0)	0(0)	0(0)	1(1)	2(2)	---
Total	0(0)	2(2)	1(1)	1(1)	5(5)*	---
Supplemental Study						
Visceral	0(0)	---	---	---	0(0)	0(0)
Hemivertebra	2(2)	---	---	---	0(0)	0(0)
Total	2(2)	---	---	---	0(0)	0(0)

Fetal Weights
(Munley, 2012a: supplement to Alvarez, 1991a)

Fetal weights (g: mean ± SD)

	0 (vehicle)	25 mg/kg-d	75 mg/kg-d	200 mg/kg-d	400 mg/kg-d	1000 mg/kg-d
***Main study*:**						
males	46.49 ± 4.1	48.73 ± 6.2	46.93 ± 4.5	45.36 ± 5.9	45.62 ± 6.6	---
females	**49.15 ± 6.4⁺**	48.66 ± 6.4#	**44.46 ±4.9##**	46.43 ± 7.1 (46.59)	43.81 ± 5.3# **(44.04)***	---
both	49.39	49.66	45.90	46.88	45.19	
***Supplemental study*:**						
males	47.07 ± 5.0	---	---	---	44.72 ± 2.8	46.83 ± 6.4
females	46.89 ± 8.3	---	---	---	42.22 ± 3.8	**43.68 ± 8.6** #
both	48.47				**43.50***	46.12

Alvazez (1991a) statistical significance:
*****p<0.05 pairwise comparison (Mann-Whitney U); ⁺ trend significance p<0.05 (Jonckheere's test)
Munley (2012a) analysis of covariance:
0.05<p<0.1 pairwise comparison; ##p=0.0007 pairwise comparison
Statistical analysis not performed by Munley on combined gender data.

Chlorsulfuron: Rats

Teratogenicity Study in Rats (Alvarez, 1991b)

- **Exposure:** Oral gavage at 0, 55, 165, 500 or 1500 mg/kg-d (Gestation Days 7-16)
  - N=25 inseminated female rats/group
  - Strain - Crl:CD*BR

Chlorsulfuron: Rats

Teratogenicity Study in Rats (Alvarez, 1991b)

- **Maternal toxicity results:**
  - Increased vaginal discharge during treatment at dose-levels of 500 and 1500 mg/kg-d
  - Reduced body weights and food consumption at 1500 mg/kg-d ($p \leq 0.05$); Less severe reduction in maternal body weight gain at 500 mg/kg-d
- **Developmental toxicity results:**

Fetal Body Weights

	0 (vehicle)	55 mg/kg-d	165 mg/kg-d	500 mg/kg-d	1500 mg/kg-d
Total	5.44	5.58	5.48	5.34	4.91*
Male	6.67	5.73	5.62	5.48	5.02*
Female	5.27	5.44	5.28	5.16	4.77*

*$p \leq 0.05$

Chlorsulfuron
Multi-generation Reproductive Toxicity Studies

- Mylchreest, E. (2005b) Chlorsulfuron (DPX-W4189) Technical: Multigeneration Reproduction Study in Rats. Laboratory Project ID: DuPont-13475. E.I. du Pont de Nemours and Company, Wilmington, Delaware 19898, U. S. A.

- Wood, C.K. (1981). Long-Term Feeding Study with 2-Chloro-N-[(4-methoxy-6-methyl-1,3,5-triazin-2-yl) aminocarbonyl] benzenesulfonamide (INW-4189) in Rats. E.I.du Pont de Nemours and Company. Laboratory Project ID 557-81. Haskell Laboratory for Toxicology and Industrial Medicine, Newark, DE 19714. USA.

  - 2 Supplements

Chlorsulfuron: Rats

Two-generation Reproduction Study in Rats (Mylchreest, 2005b)

- **Exposure:** 0, 100, 500, 2500, 7500 ppm (in the diet); guideline study; exposure about 70 days premating, during mating, gestation and through the study for two generations (1 litter/generation)
  N= 30/Sex/Group

- **Systemic toxicity results:**
  - Reduction in body weight and body weight gain at ≥ 500 ppm
- **Reproductive toxicity results:**
  - No adverse effects reported

Chlorsulfuron: Rats

Three-generation Reproduction Study in Rats (Wood, 1981)

- **Exposure:** 0, 100, 500, 2500 ppm (in diet) in a guideline study; exposure about 103 days premating, during mating, gestation and through the study for three generations (2 litters/generation)
  Animals from F0 → F1A & F1B litters
  animals from F1b →F2A & F2B litters
  animals from F2b →F3A & F3B litters
  N= 20/sex/group

- Two Supplements
  - Supplement 3, Revision 1 (Munley, 2011)
  - Supplement 3, Revision 2 (Munley, 2012b)

Chlorsulfuron: Rats

Three-generation Reproduction Study in Rats (Wood, 1981)

- **Systemic toxicity results:**
  - Reduction in body weight and body weight gain in males at 2500 ppm
  - Hematological effects at 500 ppm and 2500 ppm
- **Reproductive toxicity results:**
  - Decrease in fertility index at 2500 ppm for both F2b matings
  - **95% in controls vs. 79% for both F3A and F3B litters**

Wood (1981) and Munley (2011 and 2012b) Supplements

Mating Success for F2b animals

Dose group (ppm)	0	100	500	2500	Exact tests for trend (p)	
Generation 3A					Munley Step Down (SD):	0.058
With Litter	18	20	18	15	OEHHA unadjusted:	0.037
Without Litter	1	0	2	4	OEHHA SD Bonferroni:	
Fertility Index (%)	95	100	90	79	scores = doses:	0.021
					scores = ranks:	0.072
					OEHHA SD permutation:	
					scores = doses:	0.029
					scores = ranks:	0.049
Generation 3B					Munley SD :	0.090
With Litter	18	20	19	15	OEHHA unadjusted:	0.047
Without Litter	1	0	0	4	OEHHA SD Bonferroni:	
Fertility Index (%)	95	100	100	79	scores = doses:	0.012
					scores = ranks:	0.047
					OEHHA SD permutation:	
					scores = doses:	0.012
					scores = ranks:	0.046

Historical Control Data: Rats

Munley (2011) - Supplement 3, Revision 1 to Wood (1981)

Text Table 1: Historical Control Data from 1974 to 1983 - Fertility Index

Report	Year of In-Life Conduct	F1A	F1B	F2A	F2B	F3A	F3B
HL-432-85	1983	90	75	70	63.2	...	...
HL-524-84	1983	60	60	60	75	...	...
HL-281-84	1983	90	75	94.7	78.9	...	...
HL-422-85	1983	80	77.8	...	...	...	...
HL-436-84	1982	90	85	100	95	89.5	...
HL-294-90	1981	91.4	...	94.7	...	...	...
HL-367-84	1981	95	90	85	95	...	...
HL-557-81	**1978**	**95**	**100**	**95**	**100**	**95**	**95**
HL-25-78	1978	90	90	85	75	90	95
HL-353-77	1974	80	...	95	...	100	...
HL-24-74	1974	100	...	100	...	100	...
HL-24-74	1974	95	...	100	...	100	...
	Mean:	88.0	81.6	89.0	83.2	95.8	95.0
	S.D.:	10.13	11.51	12.56	12.61	4.60	0
	Minimum:	60	60	60	63.2	89.5	95
	Maximum:	100	100	100	100	100	95

Overall Mean:	87.7
S.D.:	11.63
Minimum:	60
Maximum:	100

Issues Addressed in Supplemental Analyses (Munley 2011 and 2012b, supplements to Wood,1981)

- **Male Fertility index data showing individual matings for F2b animals → F3A and F3B**
  - Females unsuccessful in first pairing were successful in subsequent pairing
  - For males: 3 individuals unsuccessful in both pairings
- **Problems in longevity and reproductive performance in Sprague-Dawley rats**
  - Result of inbreeding practices that were in place around the time of the conduct of the study
  - Study conducted prior to proven breeder program
- **Comparison with subsequent two-generation reproductive toxicity study at dietary concentrations of up to 7500 ppm (i.e., Mylchreest, 2005b)**

Overall Summary of DART Studies with Chlorsulfuron

Developmental Toxicity Studies:

- Rabbit
  - Increased fetal resorptions at 75 mg/kg-d (Hoberman, 1980).
    - Supplements provided re-analyses
  - Multiple effects (Alvarez, 1991a)
    - Fetal malformations, minor fetal skeletal defects (400 mg/kg-d)
    - Reduced fetal body weights (400 mg/kg-d)
    - Decreased sternebrae ossification (1000 mg/kg-d)
    - Supplements provided additional analyses
- Rat
  - Reduced fetal body weights at 1500 mg/kg-d (Alvarez, 1991b).

Rat Reproduction Studies:

- Three-generation Study (Wood, 1981)
  - Reduction of fertility index at 2500 ppm (3rd generation, both matings)
    - Supplements provided re-analyses.
- Two-generation Study (Mylchreest, 2005b)
  - No effects on fertility at dose-levels as high as 7500 ppm