6-8-84

UNITED STATES ENVIRONMENTAL PROTECTION AGENCY
WASHINGTON, D.C. 20460

003876

JUN 8, 1984

OFFICE OF
PESTICIDES AND TOXIC SUBSTANCES

MEMORANDUM

SUBJECT: Antifoulant C-9211M - EPA Symbol 707-RTL
CASWELL Nos. 314B and 202C

TO: Mr. Richard F. Mountfort, PM #23
Fungicide Herbicide/RD (TS-767)

THRU: Robert B. Jaeger, Section Head 6/8/84
Review Section #1
Toxicology Branch/HED (TS-769)

FROM: Carlos A. Rodriguez
Review Section #1
Toxicology Branch/HED (TS-769)

Action Type:

Review of teratology study to support the registration of Antifoulant C-9211M product.

Evaluation:

Although no terata related to compound administration were observed at any dose test levels, a NOEL for systemic toxicity could not be determined at this time until the information requested under "Conclusions", items 4 and 5, is submitted and evaluated.

A. Compound Identification

C-9211M (48.9% total active ingredient) is a mixture of:

3-4B — 4,5-dichloro-2-n-octyl-3(2H) isothiazolone----------40.30%
202C — 4-chloro-2-n-octyl -3(2H)-isothiazolone----------8.60%

1/8

Review: Oral Range Finding Study

C-9211M-Oral Range-Finding Study in Pregnant Rats, (Rohm and Haas Co., Toxicology Dept., Report No. 81R-254, October 24, 1983).

Sexually mature Sprague-Dawley pregnant female rats with initial body weight ranging from 177 to 237g were used in this study. The test material (48.9% a.i: in [redacted] was administered orally by gavage in 0.5% methylcellulose from day 6 through 15 of gestation and both vehicle (0.5% methylcellulose) and solvent [redacted] control groups were included. Animals were housed individually, except during mating. Doses administered on Day 6 through 10 were based on body weights recorded on Day 6 of gestation. Doses administered on Day 11 through 15 of gestation were based on body weight on Day 11 of gestation.

There were 8 animals in each group examined which included the vehicle and solvent controls, 5.62, 28.1, 56.2, 112.4 and 22.4 mg/kg of C-921101 per day.

Observations for toxic signs were made twice daily during Day 6 through 15 of gestation. Body weights were recorded on Day 0 and daily on Day 6 through 20 of gestation.

All surviving animals were sacrificed by carbon dioxide asphyxiation. All fetuses were removed by cesarean section and the number of corpora lutea, implantation sites, resorptions, live and dead fetuses were recorded. The abdominal and thoracic cavities were examined, and the carcasses were discarded.

Results: (Laboratory Report)

Maternal Observations

Mortality: The few deaths which occurred appeared to be unrelated to treatment, except at the high dose (6/8 died).

Pregnancy: One rat in the solvent control, and one rat in each of the 112.4 and 224.8 mg/kg groups were not pregnant.

Toxic signs: Essentially unremarkable except at the 2 high doses where excess salivation, red tearing, red nasal discharge, stained urogenital area, alopecia and gasping were more frequent.

There were no difference between mean body weight of controls and the treated groups at 5.62 or 28.1 mg/kg. At 56.2 mg/kg mean body weight were decreased, but not subtantially different from controls. At 112.4 and 224.8 mg/kg maternal mean body weight gains were reduced. The effects on maternal body weight at 56.2, 112.4 and 224.8 mg/kg were treatment related. The mean values are based on those Does that were pregnant and which did not die in the study.

Cesarean Data (Table 2-Appendix 3)

The mean number of corpora lutea, total implantations, early resorptions, or late resorptions were unaffected by treatment, except at the 224.8 mg/kg when sample size is considered too small for comparative purposes.

Dose	Corpora lutea	Implantations	Resorptions		Fetuses	
			Early	Late	Viable	Non Viable
Vehicle	14.5	13.9	1.0	0.0	12.9	0.0
Solvent	14.7	14.4	0.7	0.0	13.7	0.0
5.62	16.1	15.5	1.3	0.0	14.3	0.0
28 .1	15.4	14.4	0.9	0.0	13.5	0.0
56.2	15.4	14.6	0.9	0.0	13.8	0.0
112.4	15.2	14.6	1.4	0.0	13.2	0.0
224.8*	13.0	12.0	0.0	0.0	12.0	0.0

*Includes only a sample size of 1, due to death of dams caused by treatment.

Conclusion:

No signs of embryotoxicity were observed at any dose.

Body weight loss was observed at 56.2, 112.4 and 224.8 mg/kg/day.

The NOEL (no-observed effect level) is considered to be 28.1 mg/kg/day.

Rat Oral Teratology Study of C-9211 M, (Rohm and Haas, Toxicology Dept., Report No. 82 R-221, October 24, 1983).

Sexually mature Sprague Dawley virgin female rats, weighing approximately 190 to 263 grams were mated 1:1 with adult males from the same strain. A vaginal smear was taken daily from the female of each mating and examined for the presence of sperm. The day sperm were evident in a vaginal smear was considered as Day 0 of pregnancy. The test material was dissolved in 0.5% aqueous methyl cellulose and administered orally by gavage in a volume of 5 ml/kg body weight on Day 6 through 15 of gestation to 5 groups of 25 pregnant female rats at levels of 0 (vehicle control), 0 (solvent control), 11.2, 33.7 and 112.4 mg/kg/day. The vehicle control received aqueous 0.5% methylcellulose and the solvent control received [redacted] in aqueous 0.5% methyl cellulose. Dosages were calculated from individual body weights recorded on the most recent body weight of the animal.

Observations for survival and toxic signs were made daily. Body weights were recorded on Day 0, 6, 10, 13, 16, 18, and 20 of gestation.

On Day 20 of gestation all surviving animals were sacrificed by carbon dioxide. All fetuses were removed by cesarean section and the number of corpora lutea, implantation sites, resorptions, live and dead fetuses were determined and abdominal and thoracic cavities of all dams were examined for gross lesions, the stomach was also examined for gastric irritation. Gross lesions were preserved in 10% formalin.

Fetuses were individually identified, sex was determined, weighed and externally examined. One third of the fetuses were placed in Bouin's fixative and two thirds in 95% ethyl alcohol. Fetuses fixed in Bouin's were examined for soft tissue anomalies (Wilson 1965), while fetuses fixed in 95% ethyl alcohol were examined for skeletal anomalies.

Various parameters were analyzed statistically, including mean fetal weights for each litter, mean male/female pup weights, litter size, number of implantations/corpora lutea and live pup/implantations.

Results

Mortality and Observations

No deaths occurred in the vehicle control, solvent control or in the 33.7 mg/kg dose group. One death in the 11.2 mg/kg group was due to an intubation error. Six deaths occurred in the 112.4 mg/kg group (HDT) preceded by compound related toxic reactions.

Clinical signs including wheezing, lethargy, salivation, red exudate from nose or eyes and labored breathing were noted in 33.7 and 112.4 mg/kg groups.

Cesarean Data (Table 19)

All measured parameters (fertility index, corpora lutea, implantation sites per litter, implantation efficiency, live fetuses per litter, viability index, number of resorptions per litter) were similar in groups tested.

The following table has been reproduced from Registrant Report (Teratogenicity Study, of C-9211 in Rats, Table 3, Page 19, Acc. #252027).

	Vehicle Control	Solvent Control	11.2 mg/kg	33.7 mg/kg	112.4 mg/kg
No. Presumed Pregnant	25	25	25	25	25
No. Pregnant	25	25	23	23	25
Fertility Index	1.00	1.00	0.92	0.92	1.00
Corpora lutea/ Litter	15.6 (0.3)	15.7 (0.4)	15.9 (0.4)	15.5 (0.5)	15.4 (0.4)
Implantation/ Litter	14.2 (0.3)	14.3 (0.5)	15.2 (0.4)	14.2 (0.8)	14.8 (0.3)
Implantation/ Efficiency	0.91	0.95	0.96	0.92	0.96
Live Fetuses/ Litter	12.8 (0.6)	13.7 (0.5)	14.4 (0.3)	13.6 (0.8)	13.8 (0.4)
Viability Index	0.90	0.96	0.96	0.96	0.92
Resorptions/ Litter	1.4 (0.4)	0.6 (0.2)	0.7 (0.2)	0.6 (0.2)	1.2 (0.4)
Litters w/ Resorptions	15(67)	11(44)	12(52)	7(30)	11(44)
Litters > 2 Resorptions	6	0	2	1	4
Litters Resorbed	0	0	0	0	0

Maternal Body Weight

Females in the high dose group had statistically reduced body weights throughout the study. All other groups were comparable to controls.

Necropsy (Maternal)

Lung alterations described as red patches occurred in few instances in the controls and dosed groups. Sporadic incidence of discolored, fluid filled, pitted or dilated kidneys occurred in control and dosed groups. Red exudate around the nose, mouth, eye and vaginal area were noted at the highest dose.

Fetal Sex Ratio

Sex ratios were similar in all groups tested.

Fetal Morphology

Four litters with externally visible malformations were reported. One fetus in one litter in the 112.4 mg/kg group had a curly tail. One fetus in one litter of the solvent and vehicle control groups had omphaloceles. The report stated that one litter in the 11.2 mg/kg group had 4/15 fetuses with anomalies which included fetal anasacra, fused sternebrae, short jaws and shortened limbs. This statement was not supported with individual litter data.

Skeletal Variations:

The most common observed skeletal variations observed were: reduced ossification of the skull bones, hyoid unossified, 7th cervical ribs(s), reduced ossification of vertabrae, unossified sternebrae 5 and/or 6, unossified sternebrae 1 to 4, and metatarsal unossified.

Incidence of Variations

	Control Vehicle			Control Solvent			11.2 mg/kg			33.7 mg/kg			112.4 mg/kg		
No. of Fetuses examined	209			223			206			204			172		
	% of Fet.	No. of Fetuses	No. of Litters	% of Fet.	No. of Fetuses	No. of Litters	% of Fet.	No. of Fetuses	No. of Litters	% of Fet.	No. of Fetuses	No. of Litters	% of Fet.	No. of Fetuses	No. of Litters
Skull bones reduced ossification	9.1	(19)	[7]	13.5	(30)	[10]	13.1	(27)	[12]	5.9	(12)	[7]	6.4	(11)	[4]
Hyoid unossified	8.6	(18)	[8]	12.1	(27)	[12]	8.7	(18)	[7]	5.9	(12)	[8]	3.5	(6)	[4]
7th Cervical Rib(s)	2.4	(5)	[2]	5.4	(12)	[6]	1.0	(2)	[2]	2.0	(4)	[3]	0.0	(0)	[0]
Vertebrae reduced ossification	0.5	(1)	[1]	1.3	(3)	[2]	0.5	(1)	[1]	0.0	(0)	[0]	5.8	(10)	[2]
Sternebrae 5 and/or 6 unossified	32.1	(67)	[23]	51.6	(115)	[24]	46.6	(96)	[18]	46.1	(94)	[21]	55.8	(96)	[17]
Sternebrae 1 to 4 unossified	0.5	(1)	[1]	0.0	(0)	[0]	0.5	(1)	[1]	0.5	(1)	[1]	7.0	(12)	[3]
Metatarsal unossified	1.0	(2)	[2]	0.4	(1)	[1]	0.5	(1)	[1]	0.5	(1)	[1]	5.8	(10)	[4]
Bent ribs	1.9	(4)	[2]	3.6	(8)	[6]	1.9	(4)	[4]	7.4	(15)	[8]*	10.5	(18)	[6]*
Bent limbs	0.0	(0)	[0]	0.0	(0)	[0]	0.0	(0)	[0]	0.5	(1)	[1]**	4.7	(8)	[1]**

* Increase ($p = 0.058$) at 112.4 mg/kg and ($p = 0.34$) at 33.7 mg/kg.

** The incidence of bent limbs occurred in one litter in the 112.4 mg/kg group and in one fetus in the 33.7 mg/kg group.

Soft Tissue Variations:

There were no soft tissue compound related anomalies reported.

Conclusions:

1) Maternal toxicity was demonstrated by reduced body weight gain in the rats at 33.7 and 112.4 mg/kg and reduced maternal survival in the rats at 112.4 mg/kg group. No other toxic effects were seen. A maternally toxic effect level (LEL) is considered to be 33.7 mg/kg.

2) Fetal toxicity included decreased body weights at 112.4 mg/kg and skeletal variations at 33.7 mg/kg and 112.4 mg/kg dose groups.

3) The combined fetal weights of litter (80-07279) at 112.4 mg/kg group in which 8/8 fetuses examined show a skeletal malformation (bent limb bones) and the combined fetal weights of litter (82-07246) at 33.7 mg/kg group in which 1/10 fetuses examined show a skeletal malformation (bent limb bones) were significantly below the group mean body weight for each respective dose. Furthermore, examination of maternal body weights for each of these dams showed definite compound related effects at the high dose. However, the dam in the 33.7 mg/kg dose showed a decreased body weight prior to dosing and never fully recovered during compound administration. There was no reason given for this latter result. It appears that the skeletal malformation identified (bent limb bones) is the result of maternal toxicity rather than frank teratogenic effect. In view of these considerations it is unlikely the test substance produced any teratogenic effects at the dose levels tested.

4) Information contained in Appendix 7 and 8 indicates the doses as 0.0, 0.0, 10, 30 and 100 mg/kg which are different from the report. No explanation of change given. Request clarification of dosing be provided; need a signed copy of the protocol change performed by the laboratory.

5) Need clarification (individual litter data) of the multiple anomalies (external) in 4 pups in litter #8207236, 11.2 mg/kg dose group, indicated on pg 10 and pg 74 of report.

6) A NOEL for teratogenic effects was determined to be 112.4 mg/kg/day.

Classification: Supplementary. This study classification may be upgraded to a higher acceptable classification when the information request above is submitted and evaluated.