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

076884

JAN 18 1990

MEMORANDUM

OFFICE OF
PESTICIDES AND TOXIC SUBSTANCES

Subject: Hexahydro-1,3,5,tris(2-hydroxyethyl)-s-triazine; Grotan; Triazine; EPA ID# C63272; Record # 248034; MRID # 411698-01

To: James Wilson/John Lee, PM#31
Disinfectants Branch
Registration Division (H7505C)

Tox. Chem. No. 481C
Proj. No. 9-1786

From: Joycelyn E. Stewart, Ph.D. 1/17/90
Section II, Toxicology Branch I
Health Effects Division (H7509C)

Thru: Marion Copley, D.V.M., Section Head
Section II, Toxicology Branch I
Health Effects Division (H7509C)

Registrannt: Triazine Joint Venture
Montvale, New Jersey

Action Requested: Review rat teratology study submitted in response to Antimicrobial Data Call-In notice.

Conclusion: The data submitted demonstrate that Triazine administered at doses of 0, 250, 500, and 750 mg/kg/day to Sprague-Dawley derived rats from day 6 through day 15 of gestation did not cause any developmental toxicity in the offspring thereof. The NOEL is 750 mg/kg/day.

The compound produced maternal toxicity at the high dose, demonstrated by decreased body weight gain and ulceration and/or scarring of the the stomach mucosa. The NOEL is 500 mg/kg and the LOEL is 750 mg/kg.

The study is classified core-Minimum for regulatory purposes.

1070

Reviewed by: Joycelyn Stewart JS 1/12/90
Section II, Toxicology Branch I/HED (H7509C)

Secondary Reviewer: Marion Copley MC 1/12/90
Section II, Toxicology Branch/HED (H7509C)

007684

DATA EVALUATION REPORT

STUDY TYPE: Developmental Toxicity
Guideline §83-3

TOX. CHEM. NO. 481C
PROJ. NO. 9-1786

MRID NO.: 411618-01

TEST MATERIAL: Hexahydro-1,3,5-tris(2-hydroxyethyl)s-triazime

SYNONYMS: Grotan; Triazine

STUDY NUMBER: LEF/8/89

SPONSOR: Triazine Joint Venture
Montvale, New Jersey

TESTING FACILITY: Toxicol Laboratories Ltd.
Herefordshire, England.

TITLE OF REPORT: Triazine Rat Teratology Study

AUTHOR(S): P. Ridgway

REPORT ISSUED: 7/8/1989

CONCLUSIONS: The data presented demonstrate that Triazine administered to Sprague-Dawley rats at doses of 0, 250, 500, and 750 mg/kg/day during gestation days 6 through 15, did not produce any developmental toxicity in the offspring. The NOEL was 750 mg/kg/day.

The maternal toxicity NOEL was 500 mg/kg/day, and the LOEL was 750 mg/kg/day based on decreased body weight gain and ulceration and/or scarring of the mucosal in the high dose females.

CLASSIFICATION: CORE Minimum

007684

A. MATERIALS

Test Compound: Hexahydro-1,3,5-tris(2-hydroxyethyl)s-triazine; 78.5% Purity; a clear yellow liquid; Lot No. not stated.

Vehicle(s): deionized distilled water was the vehicle.

Test Animals: Species: Rat
Strain: OFA-SD(IOPS-Caw)
Source: Iffa Credo, Belgium
Age: not stated
Weight: 200- 219 grams

B. STUDY DESIGN

This study was designed to assess the developmental toxicity potential of Triazine when administered by gavage from gestation days 6 through 15, inclusive.

Mating: Females were time mated with resident males of the same strain and source. One male was caged with two females. The presence of a vaginal plug was regarded as evidence of mating. The day on which the vaginal plug was seen was designated day zero of gestation. Pregnant females were randomly assigned to the following groups:

Group Arrangement:

Test Group	Dose Level (mg/kg)	Number assigned
Control	0	24
Low dose	250	24
Mid dose	500	24
High dose	750	24

Dosing:

All doses were in a volume of 10 ml/kg of body weight/day, prepared twice during the dosing period. The dosing solutions were analyzed for concentration. Stability of the dosing solutions had been determined in a previous study which was not submitted to the Agency. Dosing was based on daily body weight.

Observations

Animals were acclimated to the laboratory for 25 days prior to study start. After mating, the females were housed individually in animal rooms with constant temperature and humidity (T 22 ± 3°C; H 50 ± 20%). Food and water were available ad libitum. The animals were checked for mortality or abnormal condition daily throughout the study, and were weighed on days 0, 6, 15, and 20 of gestation. Food consumption was recorded over the following periods: gestation days 0-6, 6-11, 11-15, and 15-20. The dams were sacrificed on gestation day 20 by CO_2 asphyxiaton, and the fetuses were delivered by Caesarian section. The thoracic and abdominal cavities were opened and the dams examined for: (1) abnormalities of maternal organs;(2) uterine weights, number of corpora lutea (3) number and distribution of implantation sites, including early and late resorptions, and live and dead fetuses.

Animals dying during the study were examined grossly. Organs showing abnormalities were retained for possible microscopic examination.

The fetuses were examined in the following manner: (1) individual fetuses were weighed and sexed, (2) external abnormalities were recorded.

One half of the live fetuses from each litter were examined for skeletal abnormalities following light fixation, KOH clearing, and aliziran red S staining. The bones in each fetus were identified and examined for normality with respect to shape, size, and degree of ossification.

The remaining fetuses from each litter were fixed in Bouin's fluid, and examined by a combined sectioning/dissection technique for visceral abnormalities.

Statistical Analysis

The following statistical analysis methods were used:

ANOVA followed by Student's "t" test: maternal body weights, body weight gain and food consumption, number of corpora lutea, number of live fetuses, implantation sites, fetal body weights.

Kruskal-Wallis test: percent pre-implantation and post-implantation losses, fetal sex ratios, fetal malformations and fetal variations.

The percentage of fetuses in each litter showing each category of malformation and/or variation was calculated. The group means were calculated from the litter means and the results compared using the Kruskal-Wallis test.

The significance level was set at $p \leq 0.05$.

4

Compliance

- A signed Statement of No Confidentiality Claim dated 7/8/1989 was provided.
- A signed Statement of Compliance with EPA GLP's dated 6/16/1989 was provided.
- A signed Quality Assurance Statement dated 6/19/1989 was provided.

C. RESULTS

1. Dosing Solutions Analysis

The results presented indicate that the mean concentrations of the dosing solutions ranged between 99.2 and 105 percent of the target concentrations.

The compound was reported to be stable in solution for twenty eight days. Details of the stability study were not submitted.

2. Maternal Toxicity

Mortality

One high dose dam was sacrificed on gestation day 14. Prior to sacrifice on that day the animal exhibited labored breathing, rales, emaciation, piloerection, red staining around the mouth and nose and pale extremities. All other animals survived the study.

Clinical Observations

High dose females exhibited post dosing salivation. Rales, labored breathing, wheezing, and tachypnea were observed occasionally in the mid and high dose groups toward the end of the dosing period. No other clinical signs were reported.

Body Weight

Maternal body weight gain was reported to be significantly reduced in high dose females during the dosing period. Maternal body weight gains were statistically significantly reduced in the high dose females during days 6 to 9, 6 to 12, 6 to 20, and when adjusted for gravid uterine weights on day 20. Representative body weight gains are shown in Table 1.

5

Table I: Body Weight Gains (grams) in Pregnant Female Rats Administered Triazine [a]

	Prior to Dosing Period	Dosing Period	Post-Dosing Period	Entire Gestation Period
Group:				
Control	25	48	68	140
LDT	24	51	70	145
MDT	25	47	73	145
HDT	24	36**	66	126

a = Data extracted from Table 2.
** significantly different from control $p < 0.01$

Food Consumption

As shown in Table II, food consumption was significantly lower in the high dose females during the dosing period than in the controls.

Table II: Food Consumption Data(g/rat/day)± S.D.

	Prior to Dosing Period	Dosing Period	Post-Dosing Period
Group:			
Control	23.0± 2.0	27.7± 2.0	27.7± 2.8
LDT	23.0± 1.8	27.6± 2.6	27.7± 2.5
MDT	23.7± 2.2	26.7± 2.9	27.6± 2.9
HDT	23.1± 1.5	23.5± 4.3***	27.0± 4.1

a = Data extracted from Table 3, Appendix 3.
*** significantly different from control $p < 0.001$

Gross Pathological Observations

Stomach lesions, characterized by ulceration and/or scarring of the mucosa were observed in 14 of 20 high dose females, including the dam sacrificed on day 14. No gross abnormalities were reported in the other dosage groups.

Table III: Cesarean Section Observations[a]

Dose:	Control	LDT	MDT	HDT
#Animals Assigned	24	24	24	24
#Animal Mated/Inseminated	21	22	24	22
Pregnancy Rate (%)	87	91	100	91
Maternal Wastage				
#Died	0	0	0	1
#Died/pregnant	0	0	0	1
#Non pregnant	3	2	0	0
#Aborted	0	0	0	0
#Premature Delivery	0	0	0	0
Total Corpora Lutea	384	413	443	401
Corpora Lutea/Dam	18.3	18.8	18.5	18.2
Total Implantations	303	332	378	352
Implantations/Dam	14.4	15.1	15.8	14.5
Total Live Fetuses	288	322	364	320
Live Fetuses/Dam	13.7	14.6	15.2	14.5
Total Resorptions	14	8	13	12
Early	14	8	13	12
Late	0	0	0	0
Resorptions/Dam	0.66	0.36	0.54	0.54
Total Dead Fetuses	0	0	0	0
Dead Fetuses/Dam	0	0	0	0
Mean Fetal Weight (gm)	3.78± 0.35	3.79± 0.34	3.78± 0.23	3.72±0.29
Preimplantation Loss(%)	21.3	20.2	14.2	18.1
Postimplantation Loss(%)	4.8	2.8	3.6	3.0
Sex Ratio (% Male)	54	50	49	53

[a]=Data extracted from Tables 4, 5; appendices 5, 6, 7.

There were no significant differences between the control and treated groups with respect to pregnancy rates, numbers of corpora lutea, implantations, or fetuses per dam. There were no abortions and no premature deliveries.

1

3. Developmental Toxicity

The incidence of fetuses with major external and visceral abnormalities was not compound related. The following were reported: one control fetus and one high dose fetus with umbilical hernias, and the same high dose fetus with exencephalia, microphthalmia, protruding tongue, and a cleft palate.

The incidence of minor visceral fetal abnormalities was similar among the control, low dose and mid dose groups. The visceral abnormalities reported were unilateral/bilateral pelvic cavitation and unilateral/bilateral urethral dilation. The high dose group had a lower incidence of these abnormalities than the controls.

The incidence of minor skeletal abnormalities was increased in fetuses from the treated dams, primarily due to increased incidences of retarded ossification of vertebral thoracic centra and the presence of vestigial 14th ribs. The investigators reported that these increased incidences were not statistically significant when the data were analysed by the Kruskal-Wallis test, and so they did not consider them treatment related. Table 4 shows the incidences of these observed abnormalities.

Table IV:

External Examinations

Observations†	Control	Low Dose	Mid dose	High dose
# pups (litters) examined	288(21)	322(22)	364(24)	320(22)
Exencephaly	0	0	0	1 (1)[ab]
Subarachnoid space hemmorrhage	0	0	1 (1)	0
Hematoma	2 (2)	0	2 (2)	1 (1)
Runting	1 (1)	3 (3)	1 (1)	2 (2)
Visceral Examinations				
Umbilical hernia	1 (1)	0	0	1 (1)[b]
Unilateral/bilateral increased pelvic cavitation	23 (13)	23 (12)	26 (13)	16 (7)
Unilateral/bilateral uretheral dilation	27 (14)	28 (14)	27 (11)	17 (8)
Skeletal Examinations				
# pups (litters) examined	144(21)	162(22)	182(24)	161(21)
Ribs				
Vestigial 14th rib	13 (4) 8.1%	17(7) 11%	23(12) 13%	23(13) 15%
Extra 14th rib	2 (1) 1.2%	0 0	2 (1) 1.2%	1 (1) 1.5%
Vertebrae				
Retarded ossification of thoracic vertebrae	3 (3) 2.1%	3 (3) 2.2%	8 (5) 4.9%	10(5) 8.5%

(a) Fetal (litter) incidence
(b) This fetus also had microphthalmia, protruding tongue, and cleft palate.
Data abstracted from Table 8 and Appendix 10.

D. DISCUSSION/CONCLUSION

The data presented demonstrate that administration of Triazine at dosage levels of 0, 250, 500, and 750 mg/kg/day during the period of organogenesis produced maternal toxicity in the form of decreased body weight gain and stomach lesions in the high dose females.

The data presented did not demonstrate any differences between the control and treated dams with respect to number of corpora lutea, implantation sites, number of live fetuses, or early and late resorptions.

In addition, those dosage levels did not produce any developmental toxicity as measured by fetal pup weight, external, or visceral, abnormalities.

There were increased incidences of vestigial 14th ribs and retarded ossification of the vertebral thoracic centra which appeared to be dose related. However, since they were not statistically significant, and the incidence of these abnormalities is highly variable in rats, Toxicology Branch does not consider them to be treatment related.

The maternal toxicity NOEL is 500 mg/kg/day and the LOEL is 750 mg/kg/day

The Developmental Toxicity NOEL is 750 mg/kg/day

E. CLASSIFICATION: CORE Minimum Data.