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

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OCT 30, 1991

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OFFICE OF
PESTICIDES AND TOXIC
SUBSTANCES

MEMORANDUM

SUBJECT: 6(A) (2) Reregistration Special Review of a Developmental Toxicity Study in Rats (Terbuthylazine).

Tox.Chem No.: 125B
MRID No.: 419627-01
HED Project No.: 1-2229

To: Christine Rice
Special Review and Reregistration Division
Accelerated Registration Branch
Reregistration Section 2 (H7508)

From: Henry Spencer, Ph.D. 10/30/91
Acting, Section Head Section 3
Toxicology Branch 1
Health Effects Division (H7509C)

Thru: Karl Baetcke, Ph.D. 10/30/91
Chief
Toxicology Branch 1
Health Effects Division (H7509C)

ACTION:

The registrant has submitted a possible 6(A)(2) study for special review: Developmental Toxicity Study in Rats (Terbuthylazine) MRID No. 419627-01.

CONCLUSIONS:

Maternal NOEL equals 5 mg/kg/day by gavage, and Maternal LOEL equals 30 mg/kg/day. Maternal results are based on lack of weight gain in dosing and reduced food intake.

Developmental NOEL equals 5 mg/kg/day, and developmental LOEL equals 30 mg/kg/day. Developmental results are based on the defect of digit ossification (lack of ossification).

The maternal and fetal NOEL agrees with the conclusions by the

registrant.

Though the apparent increase in number of fetuses affected is noted at the top two dose levels, only one parameter suggests that a dose trend may exist for litters, This effect is noted as absent ossification of the posterior digit-2 in the proximal phalanx.

There is an increase from 12 in controls to 14 affected litters (not statistically significant) at the HDT, and an increase in affected fetuses from 16% in controls to 36% at the HDT. This is a mean increase per litter of from 2 per litter to 4.2 per litter. However, the data are so inconsistent that one could invoke a statistical effect rather than a biological effect. In addition, the HED standard evaluation procedure for developmental toxicity indicates that the litter is the unit of comparison for effects rather than the fetus. These apparent effects on fetuses were not significantly different from historical control data submitted by the registrant. It should be noted that affected litters were also within historical control range.

In support of the lack of a biological effect at doses below the HDT, this reviewer notes that no other type of growth retardation, such as reduced fetal birth weights, or ossification delays other the digits or other indications of fetal toxicity such as pre and post implantation loss, early or late resorption or significanges in numbers of skeletal anomalies are reported in this study.

Therefore, this reviewer concludes that this study should not be considered 6(a)(2) data; especially with the observation of a NOEL of 5 mg/kg (MDT) for developmental toxicity. In addition, terbuthylazine is used for water cooling towers, ornamental pools and fountains, thus with this use pattern and a NOEL of 5 mg/kg/day, the Agency does not consider regulatory action appropriate at this time. The Peer Review Committee for Reproductive Effects must determine the final NOEL for terbuthylazine when it considers this algicide.

This study is classified as Core minimum data.

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GUIDELINE: 83-3

Primary Review by: Henry Spencer, Ph.D. Acting Section Head, Review Section 3, Toxicology Branch I/HED 10/30/91

Secondary Review by: David G Anderson, Ph.D. David G Anderson 10/30/91
Section 3, Toxicology Branch I/HED

DATA EVALUATION RECORD

Study Type: Teratology - Developmental Toxicity
Species: Rat
Guideline: 83-3 (a)

EPA Identification No.s: EPA MRID (Accession) No. 419627-01
EPA ID No. 080814
EPA Record No. S401914
EPA Shaughnessy No. 080814
Caswell No. 125B
HED Project No. 1-2229
Document No. -

Test Material: Terbuthylazine: 2-(tert-butylamino)-4-chloro-6-(ethylamino)-s-triazine. Batch Sg 6925

Synonyms: GS- 13529

Sponsor: CIBA-Geigy Limited, Agricultural Div. Basle, Switz.

Study Number(s): 891220

Testing Facility: CIBA-Geigy Ltd. Experimental Toxicology 4332 Stein, Switz.

Title of Report: Developmental Toxicity (Teratogenicity) Study in Rats with Gs 13529 Technical (Oral Administration).

Author(s): R.E. Fitzgerald

Report Issued: Oct. 17, 1990

Bibliographic Citation: (for standards)

Conclusions: Maternal NOEL = 5 mg/kg/day by gavage
Maternal LOEL = 30 mg/kg/day
based on lack of weight gain in dosing(also reduced food intake).

Developmental Toxicity NOEL = 5 mg/kg/day
Developmental Toxicity LOEL = 30 mg/kg/day based on the defect of

digit ossification(lack of ossification).
The maternal and fetal NOEL agrees with the registrant's conclusions.

Core Classification: minimum data

A. Materials

Test Compound: Purity: 96.4%
Description: White powder
Lot No.: S.G.6925
Contaminant: not supplied

Vehicle(s): Aqueous corn starch suspension (3%w/w)

Test Animal(s): Species: Rat-albino
Strain: Tif:RAI + (SPF) hybrids of RII/1xRII/2.
Source: CIBA-Geigy.,Stein, Switz.
Age: about 2 months
Weight: 190-210 g [illegible]ns(females).

B. Study Design

This study was designed to assess the developmental toxicity potential of GS 13529 technical when administered by gavage to pregnant rats on gestation days 6 through 15, inclusive.

Mating was by natural insemination using males of proven fertility at a ratio of 3 females to 1 male. Mating occurred from 3 AM to 7 PM at which time copulation was evaluated by the presence of vaginal sperm.

Group Arrangement:

Test Group	Dose Level (mg/kg)	Number Assigned
Control	0 (10ml/kg)	24
Low Dose	1	24
Mid Dose	5	24
High Dose	30	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 and stability based on previous homogeneity and stability analysis. Dosing was based on daily

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gestation day 6-15 body weights. Solutions were stored refrigerated at 3-6 C and protected from light. All solutions were brought to room temperature prior to use.

Observations

Food consumption was determined on days 6, 11, 16 and 21 of gestation. Tap water was provided ad libitum from bottles. The animals were checked for mortality or abnormal body weights condition from day 0 to day 21 . Dams were sacrificed by CO^2 inhalation on day 21 of gestation. Examinations at sacrifice consisted of: see pp 20 excerpted from the study.

The fetuses were examined in the following manner (as excerpted from the study): see pp 21, 22, 23, 24.

Historical control data were provided from 9 studies to allow comparison with concurrent controls in both reproductive parameters and fetal and skeletal and changes.

Statistical analysis

The following statistical analysis methods were employed as excerpted from the study (see pp 24).

Compliance

A signed Statement of Confidentiality Claim was provided.

A signed Statement of compliance with EPA GLP's was provided (prior to GLP's).

A signed Quality Assurance Statement was provided.

Results

Maternal Toxicity Cage side observations did not reveal significant maternal toxicity.

Mortality Only 1 female in the control group died prematurely due to intubation.

Clinical Observations Several observations were noted in control and high dose groups including crusting of head, neck or ears and chromodacryohemorrhea. These effects were not obviously chemically related.

Body Weight

The investigators supplied the following data:

Table I: Body Weight Gains (grams)[a]

Group:	Prior to Dosing Period	Dosing Period	Post Dosing Period	Entire Gestation Period	Corrected Body Weight Gains Dosing P.[1]	Entire[2]
Control (n=20)	30.9	68.5	77.4	176.8	39.4	70.3
LDT(n=24)	29.8	69.5	74.1	173.4	37.2	67.0
MDT(n=23)	32.6	74.3	74.1	181.6	36.3	60.9
HDT(n=18)	32.7	49.3[b]	77.4	158.1[c]	16.0[b]	47.8[c]

[1] = corrected body weight gain for dosing period = body weight gain for dosing period minus gravid uterus weight.
[2] = corrected body weight gain for entire gestation period = body weight gain for entire gestation period minus gravid uterus weight.
a = Data extracted from (study or report number and tables or appendices used)
b = $p < 0.01$
c = $p < 0.05$

Body weight gains in the study appear significantly depressed during the period of dosing (days 6-16). Body weight gains were essentially the same as the controls following the dosing period. The maternal weights were reduced but not the gravid uterine weights. Therefore toxicity at this level of evaluation appeared to be the maternal side. A NOEL can be established at 5 mg/kg for maternal toxicity. An LEL for weight reduction is 30 mg/kg (HDT). The gravid uterine weights in the top 2 doses were slightly greater than the control and LDT values.

When these values are subtracted, the slightly lower net body weight change, a lower than control value for corrected body weight gains is noted at the MDT and HDT. These values for the MDT are not considered to be chemically induced, particularly since greater food intake as noted in Table II early in the study could account for the slight body weight changes.

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Food Consumption

The investigators supplied the following data:

Table II: Food Consumption Data (mean grams/day/animal)

Group:	Prior to Dosing Period	Dosing Period	Post-Dosing Period	Entire Gestation Period
Control(n=20)	20	23.5	25	23
LDT(n=24)	20	23.5	25	23
MDT(n=24)	21	24.0	26	23.75
HDT(n=20)	20	19.5	25	21

Food consumption data in the dose period is effected by the chemical while the post dosing period food consumption was similar to the control group.

Gross Pathological Observations

The investigators supplied the following data:

The only pathological observations in the dams was that the presence of fused placentas were observed in 0, 1, 5, 30 mg/kg dosed groups in 1, 1, 0 and 2 animals respectively. This is an incidental occurrence. No treatment related effects were noted.

Only the HDT (30 mg/kg) appeared to exhibit any gross toxicity to the dams or fetuses which took the form of abortions and/or premature delivery. However, excessive preimplantation loss at this dose was not evident, thus suggesting that the gross toxicity seen was only to the dams. Further, evaluation of the pups would be required to determine if subtle effects were noted from intrauterine exposure (evaluation of fetuses follow in Table IV).

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Cesarean section Observations

Table III: Cesarean Section observations[a]

Dose:	Control	LDT	MDT	HDT
#Animals Assigned	24	24	24	24
#Animals Mated/Inseminated	20	24	24	20
Pregnancy Rate (%)	83	100	100	83
Maternal Wastage				
#Died	1	0	0	0
#Died/pregnant	0	0	0	0
#Non pregnant	3	0	0	0
#Aborted	0	0	0	4
#Premature Delivery	0	0	1	2
Total Corpora Lutea	360	421	449	360
Corpora Lutea/dam	18±3.7	17.5±3.2	18.7±3.3	18.0±3.1
Total Implantation	310	368	387	325
Implantations/Dam	15.5±3.9	15.3±2.6	16.1±2.6	16.3±2.1
Total Live Fetuses	300	357	378	317
Live Fetuses/Dam	15±4.1	14.9±2.5	15.8±2.6	15.9±2.3
Total Resorptions	10	11	9	7
Early	.5±.6	.5±.7	.4±.6	.3±.5
Late	0	0	0	.1±.2
Resorptions/Dam				
Total Dead Fetuses	0	0	0	1
Dead Fetuses/Dam	0	0	0	.1±.2
Mean Fetal Weight (gm)	5.3±.3	5.3±.3	5.2±.4	5.4±.4
Preimplantation Loss(%)	14.7±12.4	12.2±8.2	13.3±8.9	8.9±7.5
Postimplantation Loss(%)	.5±.6	.5±.7	.4±.6	.4±.5
Sex Ratio (% Male)	45.7	47.6	49.5	53.0

[a] = Data extracted from (study or report number and tables or appendices used)

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2. Developmental Toxicity

Table IV: External Examinations

Observations*	Control	Low Dose	Mid Dose	High Dose
#pups(litters) examined	300	357	378	318
#pups(litters) affected	0/0	0/0	1/1	0/0

Table IV: Visceral Examinations

Observations*	Control	Low Dose	Mid Dose	High Dose
#pups(litters) examined	145/20	172/24	184/24	152/20
#pups(litters) affected	0/0	0/0	0/0	0/0
(individual observation with both fetal and litter incidence)	0 (0)[a]	0 (0)	0 (0)	0 (0)

(*) some observation may be grouped together
(a) fetal [litter] incidence

Table IV: Skeletal Examinations

Observations*	Control	Low Dose	Mid Dose	High Dose
Malformations				
#pups(litters) examined	155/20	185/24	194/24	165/20
#pups(litters) affected	0/0	0/0	0/0	0/0
Anomalies				
(individual observation with both fetal and litter incidence)	5 (5)[a]	9 (7)	11 (8)	6 (6)
Variations				
affected	155/20	185/24	189/23	153/18
percent affected	100/100	100/100	97.4/95.8	91.5/90.0

(*) some observation may be grouped together
(a) fetal [litter] incidence

Skeletal Variations have been extracted from various tables in the study report and are presented below.

Observations		Control	Low Dose	Mid Dose	High Dose
Metatarsal-1 absent ossification					
fetal incidence	N	9	13	25*	26**
	%	5.8	7.0	12.9	15.8
litter incidence	N	6	4	13	9
	%	30.0	16.7	54.2	45.0
Anterior digit-2 Proximal phalanx: absent ossification					
fetal incidence	N	2	0	4	11*
	%	1.3	0.0	2.1	6.7
litter incidence	N	2	0	4	6
	%	10	0	16.7	30
Anterior Digit-5 Proximal phalanx: absent ossification					
fetal incidence	N	7	9	7	18*
	%	4.5	4.9	3.6	10.9
litter incidence	N	5	4	4	8
	%	25.0	16.7	16.7	40.0
Poster. Digit-2 Proximal phalanx: absent ossification					
fetal incidence	N	24	37	67**	59**
	%	15.5	20.0	34.5	35.8
litter incidence	N	12	14	19	14
	%	60.0	58.3	79.2	70.0
Poster. Digit-3 Proximal phalanx: absent ossification					
fetal incidence	N	20	21	43*	38*
	%	12.9	11.4	22.2	23.0
litter incidence	N	11	8	16	13
	%	55.0	33.3	66.7	65.0
Proximal phalanx: poor ossification					
fetal incidence	N	8	12	17	12
	%	5.2	6.5	8.8	7.9
litter incidence	N	8	9	11	8
	%	40.0	37.5	45.8	40.0
Poster. Digit-4 Proximal phalanx: absent ossification					
fetal incidence	N	22	24	43	39*
	%	14.2	13.0	22.2	23.6
litter incidence	N	13	10	16	13
	%	65.0	41.7	66.7	65.0

Proximal phalanx: poor ossification					
fetal incidence	N	8	13	14	15
	%	5.2	7.0	7.2	9.1
litter incidence	N	8	10	9	9
	%	40.0	41.7	37.5	45.0
Poster. Digit-5 Proximal phalanx: absent ossification					
fetal incidence	N	55	71	100**	88**
	%	35.5	38.4	51.5	53.3
litter incidence	N	16	21	20	15
	%	80.0	87.5	83.3	75.0

Significantly Different From Control: *=P<0.05; **=P<0.01

D. Discussion/Conclusions

a. Maternal Toxicity: Maternal toxicity is manifested as a reduced weight gain in the dams treated with 30 mg/kg by gavage. A concurrent reduction in food intake is noted with the loss in weight gain. Rates of weight gain returned to normal following dosing period.

b. Developmental Toxicity: Toxicity is minimal throughout the study. No toxicity in utero was evident by the lack of uterine weight changes.

i. Deaths/Resorptions: Numbers of early or late deaths of fetuses were not significantly increased over controls in treated dams.

ii. Altered Growth: was not seen in newborn fetuses as indicated by fetal weights at birth.

iii. Developmental Anomalies:
1.External examination revealed only 1 fetus with a cleft palate.
2. Visceral examination did not reveal any reported anomalies, malformations, or variations.
3.Skeletal examination revealed a number of fetuses with increasing numbers of litters effected with unossified proximal phalanges of posterior digits.
a. Additionally, metatarsal-1 also exhibited absent ossification in fetuses at dosages of 5 and 30 mg/kg/day.
b. Anterior digits-2 and 5 showed absent ossification in fetuses in the proximal phalanges at 30 mg/kg.
c. Posterior digit-2, 3, 4 and 5 also exhibited absent ossification in fetuses in the proximal phalanges at 5 and 30 mg/kg; 5 and 30 mg/kg; 5 and 30 mg/kg; and 5 and 30 mg/kg respectively.
d. These skeletal anomalies were statistically

significant in fetuses at 5 and 30 mg/kg/day, but not in litters.

Though the apparent increase in number of fetuses affected is noted at the top two dose levels, only one parameter suggests that a dose trend may exist for litters, This effect is noted as absent ossification of the posterior digit-2 in the proximal phalanx.

There is an increase from 12 in controls to 14 affected litters (not statistically significant) at the HDT, and an increase in affected fetuses from 16% in controls to 36% at the HDT. This is a mean increase per litter of from 2 per litter to 4.2 per litter. However, the data are so inconsistent that one could invoke a statistical effect rather than a biological effect. In addition, the standard evaluation procedure for developmental toxicity indicates that the litter is the unit of comparison for effects rather than the fetus. These apparent effects on fetuses were not significantly different from historical control data submitted by the registrant. It should be noted that affected litters were also within the historical control range.

In support of the lack of a biological effect at doses below the HDT, this reviewer notes that no other type of growth retardation, such as reduced fetal birth weights, or ossification delays other the digits or other indications of fetal toxicity such as pre and post implantation loss, early or late resorption or significant changes in numbers of skeletal anomalies are reported in this study.

Therefore, this reviewer concludes that this study should not be considered 6(a)(2) data; especially with the observation of a NOEL of 5 mg/kg (MDT) for developmental toxicity. In addition, terbuthylazine is used for water cooling towers, ornamental pools and fountains, thus with this use pattern and a NOEL of 5 mg/kg/day, the Agency does not consider regulatory action appropriate at this time. The Peer Review Committee for Reproductive Effects must determine the final NOEL for terbuthylazine when it considers this algicide.

iv. Malformations: One fetus in the 5 mg/kg dosage level exhibited a cleft palate. No other malformations were reported.

D. Study Deficiencies: None significant.

E. Core Classification: Core Minimum Data.

Maternal NOEL = 5 mg/kg/day by gavage
Maternal LOEL = 30 mg/kg/day
based on lack of weight gain in dosing (also reduced food intake).

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Developmental Toxicity NOEL = 5 mg/kg/day
Developmental Toxicity LOEL = 30 mg/kg/day
based on the defect of digit ossification(lack of ossification).

The maternal and fetal NOEL agrees with the conclusions by the registrant.