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

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9/23/1992

OFFICE OF PESTICIDES AND TOXIC SUBSTANCES

MEMORANDUM:

Subject: Review of Developmental Toxicology Study with Oftanol, technical. (Barcode number: D180716, Rereg. Case 2345, PC Code 109401)

FROM: Steven L. Malish, Ph.D., Toxicologist S. L. Malish 9/14/92
Tox. Branch II, Review Section IV
HED (H7509C)

TO: Linda DeLuise Ph (2)/Ruby Whiters PM Team Reviewer
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HED (H7508W)

THRU : Elizabeth Doyle, Ph.D., Section Head E. A. Doyle 9/15/92
Tox. Branch II, Review Section IV
HED (H7509C)

and

Marcia van Gemert, Ph.D., Branch Chief Marcia van Gemert 9/15/92
Tox. Branch II
HED (H7509C)

ACTION REQUESTED: Review of developmental toxicology study submitted under Section 6(a)(2) of FIFRA.

Study Summarized:

MRID 42381201, Developmental Toxicity Study - rat (83-3)
Core - Guideline

The test compound, Oftanol, technical was administered to pregnant Charles River CD BR rats as a single daily dose by gavage at dose levels of 0 (Control), 0.05, 0.45 and 4.0 mg/kg/day from day 6 to 15 of gestation.

In the dams at 0.45 and 4.0 mg/kg, plasma, erythrocyte and brain cholinesterase were inhibited compared to the control values on day 16. At day 20, erythrocyte cholinesterase at 0.45 mg/kg showed virtually no inhibition while erythrocyte and brain cholinesterase showed some reversal of inhibition at 4.0 mg/kg. Plasma

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cholinesterase at 0.45 and 4.0 mg/kg showed a complete reversal of inhibition on day 20. The test compound showed no other maternal toxicity.

Maternal reproductive and fetal developmental toxicity were not observed.

NOEL (maternal)= 0.05 mg/kg, LOEL (maternal)= 0.45 mg/kg
NOEL (develop.) = 4.0 mg/kg, LOEL (develop.) = not established

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Primary Review by: Steven L. Malish, Ph.D. S. L. Malish 9/14/92
Tox. Branch II, Review Section IV (H7509C)
Secondary Review by: Elizabeth A. Doyle, Ph.D. E. A. Doyle 9/15/92
Tox. Branch II, Review Section IV (H7509C)

DATA EVALUATION RECORD

Study Type: (83-3) Developmental Toxicity Study - Rat

MRID No.: 423812-01

Test Material: Oftanol, technical

Synonyms: Isofenphos

Sponsor: Miles, Inc.
Agriculture Division
Kansas City, MO

Study Number: MTD0259

Testing Facility: Corporate Toxicology-Healthcare
Miles Inc.
P.O. Box 40
Elkhart, IN 46515

Title of Report: A Development Toxicity Study in Rats with Oftanol Technical

Author: G.R. Clemens, K.G. Hilbish, D.S. Grosso, and R. E. Hartnagal Jr.

Report Issued: June 16, 1992

Conclusions:

The test compound, Oftanol, technical was administered to pregnant Charles River CD BR rats as a single daily dose by gavage at dose levels of 0 (Control), 0.05, 0.45 and 4.0 mg/kg/day from day 6 to 15 of gestation.

In the dams at 0.45 and 4.0 mg/kg, plasma, erythrocyte and brain cholinesterase were inhibited compared to the control values on day 16. At day 20, erythrocyte cholinesterase at 0.45 mg/kg showed virtually no inhibition while erythrocyte and brain cholinesterase showed some reversal of inhibition at 4.0 mg/kg. Plasma cholinesterase at 0.45 and 4.0 mg/kg showed a complete reversal of inhibition on day 20. The test compound showed no other maternal toxicity.

Maternal reproductive and fetal developmental toxicity were not observed.

NOEL (maternal)=0.05 mg/kg, LOEL (maternal)= 0.45 mg/kg
NOEL (develop.)=4.0 mg/kg, LOEL (develop.)=not established

Classification: Core - guideline

This study satisfies the guideline requirement 83-3 for a developmental toxicity study.

Quality Assurance - A signed and dated quality assurance statement was provided.

Flagging Criteria:

A signed and dated flagging statement was included on p. 4 of the original report that noted that "this study neither meets or exceeds any of the applicable [flagging] criteria." The reviewer agrees with this conclusion.

A. Materials and Methods

Test Compound:

Chemical:	1-methylethyl 2-[[ethoxy[(1-methyl-ethyl)-amino]phosphinothioyl]oxy]benzoate
Synonym:	Oftanol, technical
Purity:	91.4% a.i.
Description:	clear, colorless liquid
Batch No.:	0-00-5281
Manufacturer:	Miles Inc, Agriculture Division, Kansas City, MO
Storage:	frozen, technical material; dosing emulsions stored at ≤5°C.
Stability:	stable at least 28 day

Vehicle:

Chemical:	0.5% w/v carboxymethylcellulose and 0.4% polyoxyethylene sorbitan mono-oleate (Tween 80) in distilled water
Batch No.	not specified
Storage:	vehicle stored in refrigerator
Stability:	stable at least 28 days

Dosing Solution Preparation

On each day of dosing, stock emulsions were stirred and aliquots removed to prepare the dosing emulsions. Stirring continued during the dosing procedure. The dosing emulsions were formulated to contain 100% active ingredient by weight.

Formulation Attributes

Analysis of the various dosing emulsions resulted in -2.0% (low concentration) to +4.25% (high concentration) difference from the nominal concentration.

In the 28-day stability study, frozen test material was allowed to reach room temperature and then stirred for 15 minutes. Aliquots were taken from the top, middle and bottom of each container. The formulation was allowed to stand at room temperature until the assay was completed. Assay of dosing emulsions at various sampling times up to 28 day revealed differences from the nominal concentration of from -5.36 to +2.86%.

Test Animals:

Species:	rat
Strain:	Charles River Crl:CD®BR
Sex:	male/female
Groups:	Four (4) groups of 40 animals each
Age:	10 weeks of age
Weight:	292-343 gm (M), 207-303 (F)
Source:	Charles River Breeding Laboratories, Portage, MI

Acclimation and Housing

Animals were acclimated for a minimum of 5 days before being placed on test.

The animals were individually housed. Purina Certified Rabbit Chow #5002 and water were provided ad libitum throughout the study. After mating, all inseminated females were placed on ground diet throughout the gestation period in order to monitor food consumption.

MATERIALS AND METHODS

Study Design

This study was designed to assess the developmental toxicity potential of the test material, Oftanol, technical when administered by a single oral dose by gavage to rat on gestation days 6 to 15 inclusive (10 consecutive doses) at dose levels of 0 (vehicle control), 0.05, 0.45 or 4.0 mg/kg.

One hundred and sixty (160) virgin female rats were randomly assigned to control or treatment groups after acclimation. Dosing volume was based on the body weight on day 6 (Table 1).

Table 1

Group Treatments

Test Group	Dose (mg/kg)	Rats/Group
Control	0	40
Low	0.05	40
Mid	0.45	40
High	4.0	40

Mating

Natural insemination over 9 days was employed for breeding. One breeder male was housed with 2 females. The following morning, males were returned to their cages. Vaginal smears from all the females were obtained for evidence of copulation. The day of insemination was considered to be day 0 of gestation.

Observations:

All animals were observed twice a day for signs of toxicity.

Body Weight

Gravid animals were weighed on gestation days 0, 6-16 and 20.

Food Consumption

Food consumption of gravid animals were measured on days 1, 6, 7, 12, 16 and 20 of gestation.

Sacrifice

Day 16

Ten (10) females from each group were sacrificed on day 16 of gestation (1 day after the last dose) by CO_2 asphyxiation. Each dam was examined for confirmation of pregnancy and for gross pathology of the abdominal and thoracic viscera.

Blood and intact brain were collected for the measurement of plasma, blood and brain acetylcholinesterase activity.

Whole blood was obtained by open chest cardia puncture; EDTA was added as an anticoagulant. Brains were removed from the cranium, placed on dry ice and stored frozen.

Day 20

The remaining surviving dams were sacrificed by CO_2 asphyxiation on day 20 of gestation (5 days after the last dose). The first 10 dams confirmed to be gravid were subjected to blood and brain collection for assay of cholinesterase activity.

Material Reproductive Effects

The fertility and gestation index were calculated and the number of litters, number of deaths among dams, number of dams which aborted were tallied.

The uterine horns from all 30 animals were transected, removed and weighed. Each uterine horn was opened and the uterine walls inspected. The number of corpora lutea, implantation sites, resorptions and fetuses counted. The percentage pre-implanation and post-implanatation loss were calculate.

Gross pathology of the abdominal and thoracic viscera were noted.

Fetal Developmental Effects

Fetal and placental weights, viability and sex ratio were determined.

External/Visceral Examination

Each fetus was examined for external abnormalities.

Aqueous sodium pentobarbital administered by intracranial injection was used to sacrifice the fetuses. One half the fetuses from each dam was examined internally using a stereoscope.

Following the examination, fetuses were placed in Bouin's fixative. Sections were made "transversely through the mouth to the back of the head then frontally through the nasal septum, eyes and cerebrum."

Brains were collected from 10 fetuses per dose group for measurement of brain cholinesterase activity.

Skeletal Examination

The remaining fetuses were fixed in 70% ethanol and eviscerated using a variant of the KOH Alizarin Red S method for clearing and staining fetal bone tissue. Various portions of the skeleton were examined and compared to the control. Statistical analysis was performed on both the fetal and litter incidences.

Statistical Analysis

The following statistical methods were used: chi-square, Dunn, Dunnett's, Fisher's exact and Kruskal-Wallis. The details of each test can be found in the original report.

RESULTS:

Observations

At the 4.0 mg/kg dose group, tremor and ear twitchings occurred in a single animal on day 14. Ear twitching was observed in 2 other animals, one animal on day 13 and the other on day 14. [The authors note that 4 animals were affected]. No other abnormal signs were noted.

Weight and Food Consumption

No change in weight or food consumption was noted throughout the gestation period.

Gross Pathology

Gross pathology of the abdominal and thoracic viscera of the dams was considered to be unremarkable versus the controls.

Maternal Reproductive Effects

The test compound showed no effect on maternal reproductive efficiency versus the controls. The only parameter found significant ($p \leq 0.05\%$) was a reduction in the mean pre-implantation loss of 4.5% and 3.1%, respectively, in the 0.05 and 4.0 mg/kg groups compared to the control value of 20.9%. This effect was not considered of any toxicological importance.

Fetal Development Effects

No change in the fetus viability, weight or the incidence of external abnormalities were noted between the control and treated animals. At the 5.0 mg/kg dose level, 2 non-viable fetus occurred in 2 different litters; this difference was not statistically significant.

No changes were noted in the viscera of the treated versus the control animals. Skeletal abnormalities or variations, as analyzed by both the fetal and litter incidences were considered to be unremarkable when compared to the control group.

Cholinesterase Determinations

At 0.05 mg/kg a slight but non-significant depression of plasma cholinesterase was noted at day 16 when compared to the respective

009740

control. At the 0.45 and 4.0 mg/kg dose levels, inhibition of 23.2% and 78.5%, respectively, occurred. By day 20, no inhibition of plasma cholinesterase was seen at any dose level (Table 2).

At day 16, erythrocyte cholinesterase was inhibited 20.2% and 72.5%, respectively, at 0.45 mg/kg and 4.0 mg/kg. By day 20, the inhibition had decreased to a non-statistically significant 11.1% at 0.45 mg/kg and 58.7% at 4.0 mg/kg (Table 2).

Brain cholinesterase at 0.45 mg/kg on days 16 and 20 showed a decrease of 15.6% and 9.6%, respectively, when compared to the corresponding control. At 4.0 mg/kg, decreases of 70.6 and 39.2% were seen on days 16 and 20, respectively (Table 2).

In the plasma, erythrocyte and brain, a reversal of cholinesterase inhibition was noted from day 16 to day 20 (Table 2).

No inhibition of the fetal brain cholinesterase was seen on day 20 when compared to the corresponding control (Table 2).

Table 2

Mean Cholinesterase Activity in Animals Administered Various Doses of Oftanol technical on Day 16 and 20[1,2]

	Dose (mg/kg)						
Time (days)	0.0 Mean	0.05 Mean	(% Inh)	0.45 Mean	(% Inh)	4.0 Mean	(% Inh)
			Plasma (U/ml)				
16	1.97	1.73	12.2	1.35[a]	31.5	0.75[a]	61.9
20	1.43	1.71	0	1.49	0	1.54	0
			Erythrocyte (U/ml)				
16	1.09	1.10	0	0.87[a]	20.2	0.30[a]	72.5
20	1.26	1.50	0	1.12	11.1	0.52[a]	58.7
			Brain (mU/gm)				
16	1652	1552	6.0	1395[a]	15.6	485[a]	70.6
20	1738	1692	2.6	1572[a]	9.6	1056[a]	39.2
			Fetal Brain (mU/gm)				
20	1156	1111	3.9	1147	0.8	1186	0

[1]Adapted from original report, p. 23.
[2]9 to 10 gravid animals with live progeny at each sampling period per dose group.
[a]$p<0.05$ (Dunnett's test).

DISCUSSION:

The test compound at the tested doses showed no evidence of toxicity or any maternal reproductive effect, i.e., embryo-lethality, nor was there evidence of fetal weight change or increased mortality. The test article, moreover, did not effect the fetal external, soft tissue or skeletal development either in the number of variations and or malformations. No inhibition of fetal brain cholinesterase was noted.

On day 13 or 14, at the 4.0 mg/kg dose group, tremor and ear twitchings occurred in a single dam and on a single occasion; ear twitching was observed in 2 other dams from this group. No other abnormal signs were noted.

These signs were suggestive of cholinesterase inhibition seen in the analyses of plasma, blood and brain samples. The inhibition was either wholly or partially reversible by day 20.

Conclusions:

The test compound, Oftanol, technical was administered to pregnant Charles River CD BR rats as a single daily dose by gavage at dose levels of 0 (Control), 0.05, 0.45 and 4.0 mg/kg/day from day 6 to 15 of gestation.

In the dams at 0.45 and 4.0 mg/kg, plasma, erythrocyte and brain cholinesterase were inhibited compared to the control values on day 16. At day 20, erythrocyte cholinesterase at 0.45 mg/kg showed virtually no inhibition while erythrocyte and brain cholinesterase showed some reversal of inhibition at 4.0 mg/kg. Plasma cholinesterase at 0.45 and 4.0 mg/kg showed a complete reversal of inhibition on day 20. The test compound showed no other maternal toxicity.

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