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

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MEMORANDUM

OFFICE OF
PESTICIDES AND TOXIC
SUBSTANCES

SUBJECT: 627. 041701. Fonofos. Review of Submitted Amendment to Mouse Developmental Toxicity Study

Tox. Chem. No. 454B
Project No. 2-0279

TO: Joanne Edwards, PM Team #72
Special Review and
Reregistration Division (H7508W)

FROM: Pamela M. Hurley, Toxicologist
Section I, Toxicology Branch I
Health Effects Division (H7509C)
Pamela M. Hurley 11/18/91

THRU: Roger L. Gardner, Section Head
Section I, Toxicology Branch I
Health Effects Division (H7509C)
Roger L. Gardner 11-19-91

Record No(s). S405971

Background and Request:

ICI Americas, Inc. has submitted an amendment to the developmental toxicity study in CD-1 mice with Dyfonate Technical (MRID 420576-01). This study was conducted by the Stauffer Chemical Company in 1982. The amendment consists of the reporting of a transcription error for fetotoxic effects in both the summary and conclusion sections of the original report. The Toxicology Branch (TB-I) has been asked to review and comment on the amendment.

Toxicology Branch Response:

The Toxicology Branch has reviewed the amendment and agrees with the Registrant that the NOEL for fetotoxicity should be set at 4 mg/kg/day and the LEL should be set at 6 mg/kg/day. The following paragraphs discuss our conclusions in detail.

The amendment states that the summary section (page iv) and the conclusions section (page 7) of the original report stated that there was an increase in litter incidence of a slight dilation of the 4th ventricle in the brain in both the 4 and 8 mg/kg/day dose groups. This conflicts with the results section of the report which shows that there was a significant increase

in the number of litters with fetuses having a slightly dilated 4th ventricle for groups receiving 6 and 8 mg/kg/day. The amendment further states that the reported effects at 4 mg/kg/day was a transcription error. Both the summary and conclusions sections should state that the slight dilation of the 4th ventricle in the brain was increased in litter incidence at the 6 and at the 8 mg/kg/day dose levels.

The Toxicology Branch (TB-I) has examined the amendment, the original report and our original reviews of the report. Our original review agreed with the amendment and reported the increased incidence of slightly dilated fourth ventricles in litters at the 6 and 8 mg/kg/day dose groups (memorandum from M. Sochard to W. Miller, dated 6/9/84). The original review had some questions as to whether or not these effects as well as the observed elevations in sternebrae malignment should be considered to be either embryotoxic or teratogenic effects. The Registrant responded to these questions and in our second review of this study, we accepted these lesions as being developmental variants or delayed developmental processes of minor morphologic significance (memorandum from E. Budd to W. Miller, dated 2/19/85). However, based on the data in the tables, the second review stated that the NOEL for fetotoxicity should be set at 2 mg/kg/day. We have reviewed the data again and agree with the Registrant that the NOEL should be set at 4 mg/kg/day for fetotoxicity. The NOEL for maternal toxicity will remain at 6 mg/kg/day and the LEL will remain at 8 mg/kg/day. The NOEL for developmental (teratogenic) effects will remain at > 8 mg/kg/day (HDT). The core grade of the study will remain at core minimum.

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MEMORANDUM

OFFICE OF
PESTICIDES AND TOXIC SUBSTANCES

TO: Mr. W.H. Miller, PM#16
Registration Division (TS-767C)

THRU: Mr. Edwin R. Budd, Section Head
Section II, Toxicology Branch
Hazard Evaluation Division (TS-769)

Rec'd 6/9/83

SUBJECT: 476-1994. Dyfonate - Mouse Teratology Study; EPA Accession No. 248893

Tox. Chem.#454B

Action Requested:

Stauffer Chemical Company has submitted a mouse teratology study for review under #476-1994 in response to 3c2b, a data call-in on the chemical Fonofos.

Recommendation:

1. Toxicology Branch defers making any conclusions relative to teratogenicity as well as assigning a core category for the mouse teratology study reviewed here for reasons discussed below in (3) and (4) and pending receipt of information requested from the registrant in (5).

2. The maternal toxicity NOEL is 6 mg/kg/day with the LEL at 8 mg/kg/day (some symptoms of neurotoxicity, including tremors, chromodacryorrhea, dacryorrhea).

3. Toxicology Branch is concerned about data presented in this study showing that statistically significant numbers of litters from dams treated with 6* and 8 mg/kg/day of test chemical had dilation of the fourth ventricles. It is not clear from the evidence given that these effects are embryotoxic rather than teratogenic.

4. Furthermore, Toxicology Branch finds that it is not clear that the statistically significant elevation in values for sternebral malalignment in fetuses of the 8 mg/kg/day treatment group ~~in due to~~ are embryotoxic rather than teratogenic effects.

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5. Because of the question of embryotoxicity vs teratogenicity raised in (3) and (4) above, Toxicology Branch requests the following from the registrant:

a) Evidence to support the registrant's contention that the effects are embryotoxic rather than teratogenic.

b) Information on historical controls for the above effects taken from control animals one year prior to, concurrent controls and one year following the period of this study.

c) Based on the results of (a) and (b), Toxicology Branch may require additional testing.

Detailed Review of Study

A Teratology Study in CD®-1 Mice with Dyfonate Technical. Study#T10192, Stauffer Chemical Company Environmental Health Center, Farmington, Connecticut 06032. Dated April 2, 1982. Accession Number 248893. Pages 1-103 and i-vi.

Protocol:

CD®-1 mice were obtained from Charles River Laboratories, Kingston, N.Y. Following overnight cohabitation with males, mated females, aged 62-73 days, were assigned in groups of 30 to provide dosage groups of 0, 2, 4, 6 or 8 mg/kg/day. Animals were orally intubated on days 6-15 of gestation with Dyfonate technical (Lot#EHC-0258-45, 95.6% pure by weight), in corn oil (Mazola, Best Foods, Englewood Cliffs, N.J.) to provide a dosage volume per animal of 5.0 ml/kg. Samples of dosages administered were analytically examined to assure target concentrations. Selections of dosages were based on LD_{50} acute tests which showed 40% mortality at 7 days in females dosed at 8 mg/kg/day. (Pregnant females proved more resistant to this dosage regimen.)

Maternal Observations:

Dams were observed daily for pharmacological signs or symptoms, with body weights and food intake, respectively, measured on days 0, 6, 9, 12, 15, 18 and 0-6, 6-9, 9-12, 12-15 and 15-18. They were sacrificed by CO_2 inhalation on day 18, partially necropsied and examined for macroscopic changes in the oral cavity, oesophagus, trachea, thyroid, mandibular and parotid salivary gland, all organs of the thoracic and abdominal cavities and the liver removed and weighed. The ovaries and uteri with cervixes were immediately removed, trimmed, and weighed. Ovaries were examined and corpora lutea counted. The uterus was opened and examined for the number and distribution of fetuses and resorptions, with resorption sites categorized as early, mid or late. Placentas and associated fluids were visually inspected.

Fetal Observations:

Fully developed fetuses were considered dead if no reflexes occurred on pressing the neck at removal from the uterus. Fetuses were weighed, sexed and examined for external malformations, and live pups were sacrificed by intrathoracic injection of sodium pentobarbital. One-half of the fetuses were fixed in Bouin's solution for internal, soft tissue examination. The remainder were fixed in 70% alcohol following evisceration, then cleared and stained with Alizarin Red S for determination of skeletal anomalies.

Data Interpretation:

Anomalies were ranked according to severity; "four" was considered the most severe (malformations with lethal outcome); "three" was less severe (non-lethal outcome but with impaired physiological function); "two" represented variants which are increased in number by known teratogens or by unknown background data, and "one" corresponding to variants found in control populations or occurring as artifacts.

Statistics:

The Fischer exact test (significance at $p < 0.05$) was employed to determine significance of anomalies, with litters compared separately and collectively. A non-parametric statistical method (Mann-Whitney U rank test at $p < 0.05$) was used on continuous data including body weights, food intake and percent incidence data.

Results:

Maternal Observations

No significant changes were observed among groups treated with 0, 2, 4, or 6 mg/kg/day including the following parameters: Total body weights, food intake, weight changes throughout gestation, and net body weight changes. However, the high dose group (8 mg/kg/day) did show decreases in values for these parameters (refer to Table 1).

There were no significant differences between the control group and any treatment group as to: Uterine weights, liver total and relative weights, the number of corpora lutea, percent viable fetuses, early, mid or late resorptions, number of fetuses per dam, percent female to male fetuses, or fetal weight. It appears that some maternal toxicity was evident in the high dose group (5/6 non-gravid females had symptoms of neurotoxicity; refer to Table 1). Of the pregnant females, high dose (8 mg/kg/day) animals had symptoms of neurotoxicity, including chromodacryorrhea, (2 animals), dacryorrhea (2 animals) and tremors (1 animal); see Table 1).

Fetal Observations:

There were no statistically significant findings for external anomalies in the fetuses of any treatment group, although cranial hematomas at the category one rank were slightly increased in fetuses of the high dose (8 mg/kg/day) group. Statistically significant numbers of rank two for slightly dilated fourth ventricles were found in litters at 6- and 8 mg/kg/day. (Refer to Table 2.)

Examination for skeletal anomalies revealed statistically significant anomalies of rank one and two for, respectively, treatment groups at 4- and 8 mg/kg/day. (Refer to Table 2.) Elevation in numbers of sternebrae malignment at rank two were found in litters and fetuses of treatment groups 4-, 6- and 8 mg/kg/day. Statistical significance for these effects was found only in litters of the high dose group (8 mg/kg/day). No dose-response relationship was evident for these groups. The characteristic "sternebrae lobed" at rank one was found in greater numbers in fetuses of treatment groups 4- and 6 mg/kg/day, but the values were not statistically significant, and no dose-response relationship was found.

From the data presented, it is not possible to determine if the statistically significant effects described as "4th ventricles slightly dilated" for 6- and 8 mg/kg/day fetuses and "sternebrae malaligned" for 8 mg/kg/day fetuses are embryotoxic or teratogenic effects. Therefore, assignment of a core category as well as conclusions as to teratogenicity will be deferred until more information from which to derive these judgements is received from the registrant (see recommendations).

Core Category - Assignment deferred.

Teratogenicity NOEL - Conclusions deferred.

Maternal Toxicity NOEL = 6 mg/kg/day

Maternal LEL = 8 mg/kg/day (some symptoms of neurotoxicity, including tremors, chromodacryorrhea, dacryorrhea)

Minnie R. Sochard, Ph.D. M. Sochard 6/1/83
Toxicology Branch/HED (TS-769)

TS-769:th:TOX/HED:MSochard:6-1-83:card misc. #28

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Table 1. Maternal Findings of Interest in Animals Treated with Dyfonate on Days 6-15 of Gestation

	Dosage Groups - mg/kg/day				
Characteristic	0	2	4	6	8
Number	29	30	30	30	30
Pregnant (percent)	24(83)	26(87)	26(87)	22(73)	24(80)
Not pregnant	5	4	4	8	6**
Physical Symptoms					
Chromodacryorrhea	-	-	-	-	2
Dacryorrhea	-	-	-	-	2
Tremors	-	-	-	-	1
Differences in body weights	-	-	-	-	day 15 ↓
Differences in food consumptions	-	-	-	-	days 6-9 ↓ 9-12, ↓ 15-18 ↓
Net body weight changes	-	-	-	-	decreased
Unscheduled deaths	-	-	-	2*	-

*Two deaths of non-gravid animals which had tremors prior to death. One had no abnormal findings. The other had a "few" dark red flecks around both eyes and nares.

**Five of six non-pregnant females had symptoms of neurotoxicity ~~prior to death.~~

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Table 2. Effects of Interest in Fetuses of Dams Treated with Dyfonate

Characteristic	Maternal Dosage Groups - mg/kg/day 0		2		4		6		8	
Litters/Fetuses	L	F	L	F	L	F	L	F	L	F
Total Numbers	24	287	26	289	26	305	22	232	24	267
Soft Tissue Anomalies										
No. Examined	24	143	26	144	26	154	22	114	24	135
4th Ventricle slightly dilated	0	0	1	1	4	7	5*	5	5*	11
Lateral ventricles dilated	0	0	1	2	0	0	1	1	0	0
Skeletal Anomalies										
No. Examined	24	144	26	145	26	150	21	118	24	132
Sternebrae malaligned	9	12	12	25	16	31	13	23	17*	32
Sternebrae lobed	4	7	5	11	9	14	8	19	8	12

*Statistically significant, $p < 0.05$

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1. Mouse Teratology Study

Based, in part, on the additional information and comments from Stauffer Chemical Company on the mouse teratology study, Toxicology Branch (TB) has concluded the following.

(a) The sternebral malalignment and slight dilation of the 4th cerebral ventricles observed in this study are, most likely, not frank malformations (i.e., are not terata) but, rather, are manifestations of fetotoxicity. These lesions most probably indicate developmental variants or delayed developmental processes of minor morphologic significance.

(b) Most of these lesions occurred at or very near maternally toxic dosage levels. Due to the incidence patterns, it is most difficult to assign a definitive NOEL to these effects. The NOEL is probably about 2 mg/kg/day (the lowest dosage level).

(c) This study is classified as Core Minimum. Although some slight uncertainty exists regarding the NOEL for fetotoxicity, this is considered to be of insufficient concern at this time to warrant requiring the study to be repeated. No teratogenic effect due to the test material was observed in this study at dosage levels up to and including 8 mg/kg/day (the highest dosage level). The maternally toxic NOEL is 6 mg/kg/day and the maternally toxic LOEL is 8 mg/kg/day.

2. Neither TB nor PM Team #16 in Registration Division (RD) is aware of or has been able to locate the December 9, 1981 letter referred to by Stauffer. Stauffer should be asked to provide EPA with a copy of the letter. Also, the 3-generation reproduction study in rats (Woodard Research Corp., dated January 10, 1969; MRID #00082234) has been misplaced in TB and can not be found at this time. TB requests RD to obtain another copy of this study and submit it to TB for review for the purpose of determining whether or not it contains an acceptable teratology study incorporated into it. In the interim, the data gap for a second species teratology study should remain.

3. Change in Submission Date

TB has no objection to changing the submission date for the final report of the new chronic feeding/oncogenicity study on rats from March 31, 1987 to June, 1989.

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