2-7-86

004928

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

MEMORANDUM

OFFICE OF
PESTICIDES AND TOXIC SUBSTANCES

SUBJECT: Experimental Use Permit (8340-EUP-RN) and Temporary Tolerance (4G3156) for HOE 39866. Caswell #580I; Accession #073916, Tox. PN #911/912.

TO: Richard Mountfort (23)
Registration Division (TS-767C)

FROM: D. Stephen Saunders Jr., Ph.D. DSS 2/5/86
Toxicologist, Section V
TOX/HED (TS-769C)

THRU: Laurence D. Chitlik, DABT W. Tieters for L. Chitlik 2-6-86
Head, Section V
TOX/HED (TS-769C)
and
Theodore M. Farber, Ph.D.
Chief, Toxicology Branch
TOX/HED (TS-769C)

Action Requested

Review the acute inhalation, mouse micronucleus, acute dermal toxicity, and revised study reports of two rat teratology studies submitted in support of the requested EUP/temporary tolerance.

Recommendations/Discussion

1. The acute inhalation toxicity study in rats (report #84.0889) was classified as Core-Minimum data. The 4-hour LC50 for males was 1.26 mg/L (Toxicity Category II), and for females was 2.60 mg/L (Toxicity Category III).

2. The acute dermal toxicity study in male rats (report #84.0515) was classified as Core-Minimum data when combined with the results of a previously submitted study conducted in females (see "Background"). The dermal LD50 for the water soluble formulation (19.5% a.i.) was determined to be 1380 mg/kg (Tox. Category II) in males. The dermal LD50 of this compound in females was previously determined to be 804 mg/kg (also Tox. Category II).

Recommendations/Discussion (con't)

3. The two submitted rat teratology studies, when considered together, are classified as Core-Minimum data. In the first study (#G2R0303, report #85.0748, revision of report #545/80), doses of 10, 50, and 250 mg/kg/day produced clinical signs of toxicity in dams, and an increased fetal and litter incidence of dilated renal pelvis with hydroureter. These findings were noted in all treatment groups, and a NOEL for maternal or developmental toxicity could not be established from this study alone.

In the second study (#G2R0342, report #85.0771, revision of report #570/82), lower doses of 0.5, 2.24, and 10 mg/kg/day were tested. No treatment-related maternal or developmental effects were noted in this study, even at the dose of 10 mg/kg/day which produced maternal and developmental toxicity in the first study.

Therefore, when the two studies are considered together, a NOEL for both maternal and developmental toxicity of 2.24 mg/kg/day is obtained. The LEL for maternal toxicity is 10 mg/kg/day, based on clinical signs of hyperactivity. The LEL for developmental toxicity is also 10 mg/kg/day, based on an increased fetal and litter incidence of dilated renal pelvis with hydroureter. The ratio of NOELs for maternal and developmental toxicity (A/D ratio) is therefore 1.0, indicating that developmental toxicity is only noted at doses that also produce maternal toxicity.

4. The revised report (report #85.0724, revision of report #83.0555) of the previously submitted mouse micronucleus test (study #83.0165) included data for a preliminary range-finding study which provided justification for the doses selected in the main study. The data demonstrated that doses of 250 or 300 mg/kg/day were lethal to male and female mice, and therefore it is concluded that the HDT of 200 mg/kg/day in the main study approximated an MTD in mice. It is recommended that the mouse micronucleus study be re-classified as Core-Minimum data.

Background

The data contained in this submission were submitted to the Agency in response to a previous Toxicology Branch review of these or related studies (memo Saunders to Mountfort, 4-18-85).

In our previous evaluation, the rat acute inhalation study was classified as Supplementary data because an insufficient number of doses was tested to determine the LC50 of the technical material. It was recommended that the study be repeated with the undiluted technical powder; this suggested study was included in the present submission.

The previously submitted rat dermal toxicity study with the 19.5% a.i. WSL (Water Soluble Liquid) was classified as Supplementary data because only female rats were studied. A repeat study in which males were tested was requested; this requested study was included in the present submission.

The previously submitted rat teratology studies were classified as Supplementary data because of numerous deficiencies in the study reports, and because individual animal and historical control data were required in order to complete the evaluation. The revised study reports and requested additional data were included in the present submission.

The previously submitted mouse micronucleus test was classified as Supplementary data because no justification for the selection of doses was provided. The present submission included a revised study report, and range-finding data to support the selection of doses in the main study.

DATA EVALUATION REPORT

STUDY TYPE: Acute inhalation study in rats.

ACCESSION NUMBER: 073916

TEST MATERIAL: Monoammonium-2-amino-4-(hydroxymethylphosphinyl)-butanoate.

SYNONYMS: HOE-39866

REPORT NUMBER: 84.0889

SPONSOR: American Hoechst Corporation
Agricultural Division
Somerville, NJ 08876

TESTING FACILITY: Abteilung fur Toxikologie
Gewerbetoxikologie
Hoechst Aktiengesellschaft
Frankfurt, W. Germany

TITLE OF REPORT: "Testing for Acute Dust Inhalation Toxicity in Male and Female SPF Wistar Rats."

AUTHORS: Hollander, H. and Weigand, W.

REPORT ISSUED: 3-26-85

Reviewer: D. Stephen Saunders Jr., Ph.D.
Toxicologist, Section V
TOX/HED (TS-769C)

Secondary Reviewer: Laurence D. Chitlik, DABT
Head, Section V
TOX/HED (TS-769C)

Conclusion: LC_{50} males = 1.26 mg/L
females = 2.60 mg/L
These values correspond to Toxicity Category II for males (0.2 to 2.0 mg/L) and Toxicity Category III for females (2 to 20 mg/L).

Classification: Core-Minimum

A. Materials

(1) Test material- Monoammonium-2-amino-4-(hydroxymethylphosphinyl)butanoate, code HOE 039866 OH ZC95 0001, 95.3% a.i.

(2) Test animals: Male and female (Hoe: WISKf[SPF71]) Wistar rats obtained from Hoechst breeding colony, Kastengrund. Rats were approximately 8-10 weeks old at initiation of treatment.

(3) Doses tested: 0.12, 0.19, 0.38 and 2.00 mg/L (analytical) in 5 rats/sex/dose.

B. Study Design

Rats were exposed for 4 hours by nose-only inhalation. The test material was ground into a fine powder with an "analytical grinding apparatus" to produce particles in the respirable range. Dust was introduced into the exposure apparatus with a "Wright Dust Feed" generator (L. Adams, Ltd., London), at an air flow rate of 1000 L/hour.

C. Methods and Results

(1) Analysis of particle size: The distribution of particle sizes was determined with an Anderson Cascade Impacter Mark III (Anderson Samplers, Inc., Atlanta). The device was operated at a vacuum throughput of 9.5 L/min, which yielded a flow rate of approximately 1.25 m/sec.

The submitted distribution of particle sizes (Appendix 6.2 of the study report) indicated that 6.4%, 7.4%, 12.8% and 29.9% of the particles were 1.5 microns or smaller for the generated concentrations of 0.12, 0.19, 0.38 and 2.00 mg/L, respectively. Similarly, 23.4%, 29.3%, 36.2% and 63.1% of these respective concentrations were particles of 3 microns or less.

(2) Animal observations: Rats were observed for 14 days after exposure, and "behavior checks were carried out" at unspecified intervals. Rats that survived the 14-day observation period were sacrificed and examined for gross changes at necropsy, as were animals that died on test.

The majority of deaths occurred between day 7 and 9 after exposure, with the exception of one male exposed to 0.19 mg/L (died day 4) and one female exposed to 0.38 mg/L (died day 5). Thus, the length of survival did not appear to be affected by dose. The effect of treatment on survival is shown below:

Concentration of HOE 39866	Mortality Males	Females	Total
0.12 mg/L	0/5	0/5	0/10
0.19	1/5	0/5	1/10
0.38	1/5	1/5	2/10
2.00	3/5	2/5	5/10

Based on these data, the LC_{50} for males was calculated as 1.26 mg/L, and for females as 2.60 mg/L.

Clinical signs reported for these animals included hyperactivity, "narrowed eye openings", "squatting position", and piloerection. No microscopic abnormalities were reported for the animals that were sacrificed at the end of the 14-day observation period. Animals that died on test were either autolyzed or cannibalized, thus necropsies of these animals were not performed.

004923

DATA EVALUATION REPORT

STUDY TYPE: Acute dermal toxicity study in rats.

ACCESSION NUMBER: 073916

TEST MATERIAL: Monoammonium-2-amino-4-(hydroxymethylphosphinyl)-butanoate.

SYNONYMS: HOE 39866

REPORT NUMBER: 84.0515

SPONSOR: American Hoechst Corporation
Agricultural Division
Somerville, NJ 08876

TESTING FACILITY: Pharma Forschung Toxikologie
Hoechst Aktiengesellschaft
Frankfurt, W. Germany

TITLE OF REPORT: "Testing for Acute Dermal Toxicity in Male Wistar Rats."

AUTHORS: Rupprich, and Weigand, W.

REPORT ISSUED: 8-9-84

Reviewer: D. Stephen Saunders Jr., Ph.D.
Toxicologist, Section V
TOX/HED (TS-769C)

Secondary Reviewer: Laurence D. Chitlik, DABT
Head, Section V
TOX/HED (TS-769C)

Conclusion: LD_{50} in males = 1380 mg/kg This value corresponds to Toxicity Category II (200 to 2000 mg/kg).

Classification: Core-Minimum

A. Materials

(1) Test material- Monoammonium-2-amino-4-(hydroxymethyl-phosphinyl)butanoate, code HOE 039866 OH SL19 A139, water-soluble concentrate, 19.5% a.i.

(2) Test animals: Male (Hoe: WISKf[SPF71]) Wistar rats obtained from Hoechst breeding colony, Kastengrund, 5 rats/dose. Rats were approximately 8-10 weeks old at initiation of treatment.

(3) Doses tested: 800, 1000, 1250, 1600, and 2000 mg/kg applied dermally in volumes of 0.719, 0.898, 1.123, 1.437, and 1.797 ml/kg, respectively.

B. Study Design

A 30 cm^2 area of the nape skin was shaved 1 hour before treatment. An appropriate volume of the undiluted test formulation was applied so as to achieve the desired dermal dose. The treated area was covered with foil and fixed with an elastic plaster bandage for 24 hours, at which time the treated skin was washed with warm water to remove unabsorbed test material.

C. Methods and Results

(1) Mortality: Rats were observed for 14 days after treatment, during which time animals were observed at unspecified intervals to determine "the course of intoxication and lethality". Rats that died on test, and those that survived the 14-day observation period, were necropsied and examined for gross changes.

Animals that died as a result of treatment died within 3-5 days after exposure. The effect of treatment on survival is shown below:

Dose (mg/kg)	Applied Volume (ml/kg)	Deaths
800	0.719	1/5
1000	0.898	1/5
1250	1.123	2/5
1600	1.437	3/5
2000	1.797	4/5

Based on these results, a dermal LD_{50} of 1380 mg/kg was calculated for male rats.

Clinical signs observed in treated animals included reduced activity, agitation, enhanced startle reflex, convulsions, piloerection, and other signs consistent with a "poor general condition". These signs were noted in all treatment groups, however were noted with greatest frequency at the highest doses. The signs were noted within one hour of treatment, and persisted through 5 days after treatment. The investigators noted that all surviving rats remained "timid and agitated" throughout the 14-day observation period.

Necropsy of rats which died on test revealed liver and spleen reduced in size, lungs congested with blood, reddening of the gastric mucosa, and dark discoloration of the adrenals. Rats which survived to 14 days revealed no gross abnormalities at necropsy.

DATA EVALUATION REPORT

STUDY TYPE: Teratology study in rats.

ACCESSION NUMBER: 073916

TEST MATERIAL: Monoammonium-2-amino-4-(hydroxymethylphosphinyl)-butanoate.

SYNONYMS: HOE 39866

STUDY NUMBER: G2R0303

REPORT NUMBER: 85.0748 (revision of report #545/80)

SPONSOR: American Hoechst Corporation
Agricultural Division
Somerville, NJ 08876

TESTING FACILITY: Pharma Forschung Toxikologie
Hoechst Aktiengesellschaft
Frankfurt, W. Germany

TITLE OF REPORT: "Testing for Embryotoxicity in Wistar Rats Following Oral Administration. Revised Version of Report No. 545/80 of 10-20-80."

AUTHORS: Baeder, Weigand, and Kramer.

REPORT ISSUED: 7-19-85

Reviewer: D. Stephen Saunders Jr., Ph.D.
Toxicologist, Section V
TOX/HED (TS-769C)

Secondary Reviewer: Laurence D. Chitlik, DABT
Head, Section V
TOX/HED (TS-769C)

Conclusion: NOELs for maternal and developmental toxicity were not established, and are both <10 mg/kg/day, the LDT. Dose-related incidences of hyperactivity in dams, and dilated renal pelvis with hydroureter in fetuses, were noted in all treatment groups.

Classification: Core-Minimum, when considered with the data from study #G2R0342, report #85.0771.

Background

The present submission is a revision of study #545/80, which was submitted to the Agency and has been reviewed (memo Saunders

to Mountfort, 4-18-85). In that review, the study report was found inadequate and a rewrite was requested, along with additional individual animal, range-finding, and historical control data. These data were included in the present submission. This DER will review only those portions of the submission that relate to the deficiencies identified in the original review, and will refer to that review where appropriate.

A. Materials

(1) Test material- Monoammonium-2-amino-4-(hydroxymethylphosphinyl)butanoate, code HOE 039866 OH AT201, operation no. 1938 m + n, 97.2% a.i.

(2) Test animals: Sexually mature, virgin female (Hoe:WISKf[SPF71]) Wistar rats obtained from Hoechst breeding colony, Kastengrund. Rats were approximately 70 days old at initiation of treatment.

(3) Doses tested: 0, 10, 50 and 250 mg/kg by gavage in 5 ml/kg body weight of redistilled water, 20 rats/dose. Doses were prepared daily before treatment. The test material was determined to be stable in water for a period of at least 5 days.

B. Study Design

Females in estrous were paired overnight with fertile males. Detection of sperm in a vaginal smear was considered as day 1 of gestation. Animals were assigned to treatment groups "in accordance with Randomization Scheme 42/80" and were treated by gavage from days 7-16 of presumed gestation with either vehicle (redistilled water) or the test material in a volume of 5 ml/kg. Food and water were available ad libitum.

C. Methods and Results

Range-Finding Study

Two or three pregnant dams were given doses of 32, 63, 125 or 250 mg/kg by gavage over days 7-16 of presumed gestation. These animals were evaluated for clinical signs of toxicity, effects on body weight and food consumption, reproductive effects, and fetal effects.

Results- One dam of the 32 mg/kg/day group (#1391) had blood around the eyes and mouth over days 17 or 18 to 21, clear lacrimation from days 16 to 18, and inflamed eyes and bristled fur from days 16 to 21. No signs were noted in the other 2 dams from this group, nor in dams from the 63 and 125 mg/kg/day groups. One dam given the high dose of 250 mg/kg/day had bristled fur on day 11, and was normal for the remainder of the observation period, as were the other animals in that group. No dams died on test.

Body weight gain in treated animals was similar among the 4 treatment groups, with the exception of one low dose dam (#1391) and one high dose dam (#1399) who weighed only 231 and 216 grams, respectively, on day 21 (compared to 256 to 355 grams in the other groups). The low dose dam was the animal with clinical signs in the low dose group, whereas the high dose dam had only resorptions and dead fetuses at necropsy. Food consumption was similar among treated animals with the exception of the low dose (#1391) and high dose (#1399) dams whose consumption was about 25% less than the other treated animals.

The only effect on reproductive parameters noted at necropsy was seen in dam #1399, who had only dead implantations. Reproductive parameters (fetal weights, number of resorptions, litter size, and sex ratio) did not appear to be affected in a dose-related manner, however dam #1391, who had clinical signs and reduced food consumption and weight gain, had 11 live fetuses that weighed an average of only 2.27 $\pm$ 0.23 grams, and had crown-rump lengths of 3.05 $\pm$ 0.14 cm. The other two dams in this low dose group had fetuses that weighed an average of 3.64 and 3.42 grams and had crown-rump lengths that averaged 3.78 and 3.67 cm. Fetuses were not evaluated for variations or malformations.

At necropsy of dams, distension of one or both renal pelvises was noted in one dam each from the 63, 125, and 250 mg/kg/day groups. In addition, one low dose dam (#1392) had "vagina filled with brown mucous", and high dose dam #1399 had "corpora lutea dark red".

Based on these data, the investigators concluded that the observed findings "were not definitely attributable to administration of the test substance" since the findings did not appear to be dose-related. Doses of 10, 50, and 250 mg/kg/day were selected for the main study.

Main Study

(1) Clinical signs: Animals were observed daily for changes in behavior or general appearance.

Results- An apparent dose-related increase in the incidences of hyperactivity, bristled fur, "flabbiness", "arching of the spine", squatting, blood around the mouth, and vaginal hemorrhage was noted, with most signs observed in the mid (50 mg/kg/day) and high (250 mg/kg/day) dose groups (Enclosure 24, photocopied from the study report). No significant findings were noted in control dams. Most of the dams sacrificed due to vaginal bleeding also exhibited other clinical signs, principally hyperactivity/"motorial unrest", bristled fur, and/or arching of the spine. Thus, the finding of vaginal bleeding (which was likely due to abortions) appeared to be related to maternal toxicity. Interestingly, 11 dams from the mid dose group and 4 dams from the high dose group were considered to have "no abnormal deviations" throughout the observation period.

ENCLO E 24
HOECHST AG

PHARMA RESEARCH TOXICOLOGY

STUDY: EMBRYOTOXICITY PRELIMINARY PREPARATION: Ibe 39866 0 H AT201
SEX: FEMALE CESAREAN SECTION ON DAY 21 DOSING FROM DAY 7 - 16 POST COPULATIONEM
ROUTE: ORAL VEHICLE: REDISTILLED WATER
START OF STUDY: 03/05/80 STUDY NO: G2R0303

ANIMAL: RAT WISTAR

SURVEY OF CLINICAL FINDINGS IN DAMS

Findings	Control	10 mg/kg	50 mg/kg	250 mg/kg
	Number of dams affected/Number of dams (% in brackets)			
Hyperactivity	0/20	2/20 (10)	7/20 (35)	5/20 (25)
Bristled fur	0/20	0/20	3/20 (15)	9/20 (45)
Flabbiness	0/20	0/20	2/20 (10)	7/20 (35)
Arching of the spine	0/20	0/20	1/20 (5)	5/20 (25)
Squatting	0/20	0/20	2/20 (10)	1/20 (5)
Purulent lacrimation	0/20	0/20	0/20	1/20 (5)
Blood around the mouth	0/20	0/20	0/20	3/20 (15)
Vaginal haemorrhage	0/20	0/20	4/20 (20)	8/20 (40)
Local alopecia	2/20 (10)	1/20 (5)	0/20	4/20 (20)
Dam died after previously lying in a lateral position	0/20	0/20	0/20	1/20 (5)

(2) Body weights and Food consumption: The submitted methods indicated that body weights were determined weekly; however the submitted data indicated that dams were weighed on days 0, 7, 14, 17 and 21 of presumed gestation. The submitted summary tabulation bore a legend stating that "The area under the body weight curve was used for the evaluation of days 7-17". The meaning of this statement is unclear to this reviewer. The methods also stated that food consumption was observed "continuously", however data were reported for the same intervals as for body weights. Data were evaluated statistically by the Dunnett's T-test and/or the "Nemenyi many-one version of the H-test".

Results- As was noted in the original review of this study, a slight reduction in body weight gain (<10%) during gestation was noted in those high dose dams that survived to terminal sacrifice (day 21). This finding was apparently related to slightly decreased food intake in these animals, which was also reduced by about 10% over days 7-17, but was statistically significant ($p < 0.05$) only over days 14-17.

In contrast, the mid and high dose dams that died or were sacrificed due to vaginal bleeding, in most cases, actually lost body weight over the treatment period. The loss of body weight appeared to be related to a rapid decline in food intake in these animals with the initiation of treatment.

(3) Necropsy Data: (a) Reproductive parameters- Dams were sacrificed on day 21 by stunning and exsanguination. The uterus was opened, and live and dead fetuses, resorption sites, placentas, and corpora lutea were counted and examined macroscopically. Uteri were stained with ammonium sulphide for visualization of implantation sites. Fetal body weight, crown-rump length, and sex were determined at autopsy. Fetal data were evaluated statistically by the Dunnett's T-test and/or the "Nemenyi many-one version of the H-test". Caesarean section results were evaluated for statistical significance using the Goodman's Simultaneous Comparison.

Results- As was noted in the original review of this study, the major effect of treatment on reproductive indices was a dose-related increase in the incidence of post-implantation loss. This effect was seen only in those dams exhibiting weight loss, vaginal bleeding and other clinical signs. At necropsy, most of the dams sacrificed due to bleeding, and the dam that died on test, had either resorptions or implantation sites in the uterus. One mid dose dam (#55), sacrificed day 20, had 12 stunted (0.95 - 1.15 g), live fetuses and an implantation site; one high dose dam (#79), sacrificed day 19, had 12 stunted fetuses (0.35 - 0.62 g) of which 4 were alive and 8 dead at necropsy. The vaginal bleeding was likely due to abortion of fetuses; mid dose dam #42 and high dose dam #61 both were observed to have fetuses (early implants) in the birth canal at necropsy after sacrifice for vaginal bleeding.

For those mid and high dose dams that survived to day 21 sacrifice, litter size, fetal body weights and crown-rump lengths were comparable to control (see Table 1, photocopied from the original review). A statistically-significant increase in the number of dead fetuses was noted in the high dose group, however this finding was due to a single dam (#73) with 12 dead fetuses. This dam also lost body weight over the treatment period and had clinical signs of bristled fur and "motorial unrest".

(b) Necropsy findings in dams- After caesarean section, dams were dissected and organs were examined for gross changes. The weights of heart, liver, kidneys, spleen and adrenals were determined. Data were evaluated statistically by the Dunnett's T-test and/or the "Nemenyi many-one version of the H-test".

Results- The original review of this study identified the major findings in dams at necropsy as dilation of one or both renal pelvises, which was present at a rate of about 10-20% in all treatment groups. The incidence of this finding did not appear to be related to treatment with the test compound. Historical control data, included with the present submission, indicate that this finding exists at an average background rate of about 10%, and ranged as high as 25%, in this strain of rat. Therefore, the incidences of this finding in the present study are considered normal for this strain of rat.

Enlarged adrenals were noted only in 1/4 mid dose and 5/8 high dose dams sacrificed due to bleeding, but were not observed in rats surviving to day 21 termination. No effect on organ weights was apparent in dams surviving to termination, however organ weights for rats sacrificed due to bleeding were not included in the individual animal organ weight data.

(4) Fetal Developmental Toxicity Indices: (a) External- After removal from the uterus, fetuses were examined for external appearance and the presence of anomalies. Data were evaluated for statistical significance with the Fisher's Exact Test and the Exact Simultaneous Comparison with Control in a contingency table.

Results- As was noted in the original review, an increase in the number of litters with stunted fetuses was noted in the mid dose (1 litter of 12 fetuses from dam #55, sacrificed day 20 due to vaginal bleeding) and high dose (1 litter of 12 fetuses from dam #73, sacrificed at termination, and 1 litter of 12 fetuses from dam #79, sacrificed on day 19 due to bleeding) groups. This finding was not observed in the control or low dose groups, and appears to be related to treatment with the test compound, although a statistical assessment of this finding was not provided. Submitted historical control data indicate that stunting of fetuses was observed in 1 fetus from each of 2 litters (in 2 separate studies) out of a total of 17 studies and 333 litters between the years 1978 and 1981. Thus, the observed incidences in

the present study clearly lie outside of the range of historical controls.

(b) Soft tissue- About half of the fetuses from each litter were fixed in Bouin's fluid and examined in body cross-sections under a microscope for organ anomalies by the method of Wilson. For skeletal and soft tissue examinations, fetuses were selected alternately according to their location within the uterus. Data were evaluated for statistical significance with the Fisher's Exact Test and the Exact Simultaneous Comparison with Control in a contingency table.

Results- As was noted in the original review, a dose-related increase in the incidence of dilated renal pelvis with hydroureter was noted in all treatment groups (see Table 2, photocopied from the original review). Although statistically significant ($p<0.05$) only in the high dose group, the increases in the low and mid dose groups clearly appear to be related to treatment with the test compound. Historical control data for this finding indicate that the average fetal incidence of distended renal pelvis with hydroureter was 1.13 $\pm$ 1.15%, with a range of 0-3.5%, and the historical litter incidence of this finding was 6.53 $\pm$ 6.37%, with a range of 0-21.1%. Although the findings in the low and mid dose groups of the present study lie at the upper end of the historical control range, they must be considered as treatment-related in the present study in consideration of the relatively low concurrent control incidence, the apparent dose-dependency of the effect in all treatment groups, and the significantly higher incidence in the high dose group.

No other toxicologically-significant soft tissue findings were noted.

(c) Skeletal- About half of the fetuses from each litter (selected alternately according to location within the uterus) were fixed in alcohol, dissected under a magnifying glass, eviscerated and bleached in aqueous potassium hydroxide. Skeletons were stained with Alizarin Red-S and examined. Data were evaluated for statistical significance with the Fisher's Exact Test and the Exact Simultaneous Comparison with Control in a contingency table.

Results- A statistically significant ($p<0.05$) increase in the fetal incidence of non-ossification of metacarpal 5 was noted in the high dose group only. This finding was noted in 51.6% of high dose fetuses in comparison to 35.0% of control fetuses. However, the litter incidences were similar- 75% for control and 80% in high dose litters. The toxicological significance of this finding is questionable in consideration of the fact that other portions of the skeleton were not similarly affected by ossification delay or any other effect on skeletal development.

Table 1. Reproductive Data[a]

	DOSE (mg/kg) 0	10	50	250
Number of dams:				
-Pregnant	20	20	20	20
-deaths	0	0	0	1
-sacrificed	0	0	4	8
Dams on Day 21:				
-alive	20	20	16	11
-with fetal death only	0	0	0	1
-with live fetuses	20	20	16	10
-mean weight gain day 0-21 (g)	123	121	120	112
Mean Number/Dam:				
-corpora lutea	12.6	13.0	13.3	13.4
-implantations	11.9	11.9	12.6	12.8
-resorptions	0.60	0.55	0.81	0.91
Mean Fetal Data:				
-no. dead/litter	0	0	0	1.09*
-no. live/litter	11.3	11.3	11.8	10.8
-% male	54	53	47	53
-body weight (g)	3.29±0.36	3.41±0.41	3.20±0.28	3.19±0.28
-crown-rump (cm)	3.57±0.17	3.66±0.18	3.53±0.16	3.49±0.18
-placental weight (g)	0.53±0.07	0.51±0.06	0.47±0.05*	0.48±0.09

[a]data excerpted from submitted study. *$p < 0.05$

Table 2. Incidences of Distended Renal Pelvis and Ureter[a]

	DOSE (mg/kg) 0	10	50	250
No. fetuses examined	109	108	91	57
No. litters examined	19	19	16	10
Fetuses With:				
Dilated renal pelvis, one or both sides	10/6[b] (9.2/31.6)[c]	17/10 (15.7/52.6)	19/10 (20.9/62.5)	8/5 (14.0/50.0)
Hydroureter only	0	0	0	1/1 (1.8/10.0)
Dilated renal pelvis and hydroureter	1/1 (0.9/5.3)	3/3 (2.8/15.8)	4/3 (4.4/18.8)	9/5 (15.8/50.0)
Dilated renal pelvis and/or hydroureter	11/6 (10.1/31.6)	20/11 (18.5/57.9)	23/10 (25.3/62.5)	18/7 (31.6/70.0)

[a]data excerpted from submitted study.

[b]No. affected fetuses/litters (litter incidence calculated by reviewer).

[c]percent affected fetuses/litters (calculated by reviewer).

Discussion

The major effect of treatment with the test compound on fetal development was an apparently dose-related increase in the fetal and litter incidences of dilated renal pelvis with hydroureter. This finding was noted in all treatment groups, although the increased incidence was statistically significant only in the high dose (250 mg/kg/day) group. In addition, 1 mid dose litter and 2 high dose litters had all stunted fetuses, as compared to none in the control and low dose groups, and a historical control incidence of 2 fetuses from 2/333 litters.

The observed effects on fetal development appear to have occurred at doses that were maternally toxic. Clinical signs of hyperactivity/"motorial unrest" were noted in all treatment groups, and were clearly dose-related in incidence and duration. In the mid (50 mg/kg/day) and high (250 mg/kg/day) dose groups, additional signs of bristled fur, "clumsiness", arching of the spine, and squatting were noted. Although not all signs were noted in all animals, at least one of the above signs was noted in 2/20 low dose, 9/20 mid dose, and 15/20 high dose dams, as compared to 0/20 control dams. In addition, 4 mid dose and 8 high dose dams were sacrificed before the scheduled day 21 termination due to vaginal bleeding. This bleeding was likely due to abortions, as vacant implantation sites were noted in several animals, as well as concepta in the birth canals of two other animals.

Reproductive parameters, such as fetal weight and length, litter size, and sex ratio did not appear to be affected in rats that survived to scheduled termination. If one considers only the animals surviving to day 21, no effect on the number of resorptions was apparent. As noted above, a large number of resorptions, vacant implantation sites and/or stunted fetuses was noted in dams sacrificed early due to bleeding.

Maternal NOEL = Not established, <10 mg/kg/day.
Maternal LEL = 10 mg/kg/day (LDT) Hyperactivity

Developmental NOEL = Not established, <10 mg/kg/day.
Developmental LEL = 10 mg/kg/day Increased incidence of dilated renal pelvis with hydroureter.

Classification: Core-Minimum, when considered with the results of study #85.0771.

DATA EVALUATION REPORT

STUDY TYPE: Teratology study in rats.

ACCESSION NUMBER: 073916

TEST MATERIAL: Monoammonium-2-amino-4-(hydroxymethylphosphinyl)-butanoate.

SYNONYMS: HOE 39866

STUDY NUMBER: G2R0342

REPORT NUMBER: 85.0771 (revision of report #570/82)

SPONSOR: American Hoechst Corporation
Agricultural Division
Somerville, NJ 08876

TESTING FACILITY: Pharma Forschung Toxikologie
Hoechst Aktiengesellschaft
Frankfurt, W. Germany

TITLE OF REPORT: "Testing for Embryotoxicity in Wistar Rats Following Oral Administration. Revised version of Report No. 570/82 of 9-22-82."

AUTHORS: Baeder, Weigand, and Kramer.

REPORT ISSUED: 7-26-85

Reviewer: D. Stephen Saunders Jr., Ph.D.
Toxicologist, Section V
TOX/HED (TS-769C)

Secondary Reviewer: Laurence D. Chitlik, DABT
Head, Section V
TOX/HED (TS-769C)

Conclusion: (Based on consideration of the results of this study with those of the previous high dose study, #G2R0303).
Maternal NOEL = 2.24 mg/kg/day
Maternal LEL = 10 mg/kg/day Hyperactivity

Developmental NOEL = 2.24 mg/kg/day
Developmental LEL = 10 mg/kg/day Dilated renal pelvis with hydroureter.
A/D Ratio = 1.0

Classification: Core-Minimum When considered with the data from study #G2R0303, report #85.0748.

Background

The present submission is a revision of study report #570/82, which was submitted to the Agency and has been reviewed (memo Saunders to Mountfort, 4-18-85). In that review, the study report was found inadequate and a rewrite was requested, along with additional individual animal, range-finding, and historical control data. These data were included in the present submission. This DER will review only those portions of the submission that relate to the deficiencies identified in the original review, and will refer to that review where appropriate.

This study is the second of two teratology studies conducted in the rat with HOE 39866. In the first high dose study (#G2R0303, see DER for report nos. 85.0748 and 545/800) a dose-related incidence of dilated renal pelvis with hydroureter in fetuses was noted in all treatment groups, at doses that also produced maternal toxicity. Therefore, the present study was conducted at lower doses in order to determine the NOELs for maternal and developmental toxicity.

A. Materials

(1) Test material- Monoammonium-2-amino-4-(hydroxymethylphosphinyl)butanoate, code HOE 039866 OH AT201, operation no. 1938, 97.2% a.i.

(2) Test animals: Sexually mature, virgin female (Hoe: WISKf[SPF71]) Wistar rats obtained from Hoechst breeding colony, Kastengrund. Rats were approximately 70 days old at initiation of treatment.

(3) Doses tested: 0, 0.50, 2.24 and 10 mg/kg by gavage in 5 ml/kg body weight of redistilled water, 20 rats/dose. Doses were prepared daily before treatment. The test material was determined to be stable in water for a period of at least 5 days.

B. Study Design

Females in estrous were paired overnight with fertile males. Detection of sperm in a vaginal smear was considered as day 1 of gestation. Animals were assigned to treatment groups "in accordance with Randomization Scheme 42/80" and were treated by gavage from days 7-16 of presumed gestation with either vehicle (redistilled water) or the test material in a volume of 5 ml/kg. Food and water were available ad libitum.

C. Methods and Results

(1) Clinical signs: Animals were observed daily for changes in behavior or general appearance.

Results- No effect of treatment on clinical signs was apparent. Hyperactivity or "motorial unrest" were not apparent even

at the HDT of .0 mg/kg. This dose, in the previous high dose study, produced hyperactivity in 2/20 dams. The only findings reported for any rats were alopecia, and scabbing on the head or neck. Neither finding occurred in a dose-related manner. All dams survived to scheduled sacrifice on day 21 of presumed gestation.

(2) Body weights and Food consumption: The submitted methods indicated that body weights were determined weekly, however the submitted data indicated that dams were weighed on days 0, 7, 14, 17 and 21 of presumed gestation. The submitted summary tabulation bore a legend stating that "The area under the body weight curve was used for the evaluation of days 7-17". The meaning of this statement is unclear to this reviewer. The methods also stated that food consumption was observed "continuously", however data were reported for the same intervals as for body weights.

Results- As was noted in the original review of this study, no effect of treatment on weight gain or food consumption was apparent at any of the intervals measured. The submitted individual animal data do not alter this assessment.

(3) Necropsy Data: (a) Reproductive parameters- Dams were sacrificed on day 21 by stunning and exsanguination. The uterus was opened, and live and dead fetuses, resorption sites, placentas, and corpora lutea were counted and examined macroscopically. Uteri were stained with ammonium sulphide for visualization of implantation sites. Fetal body weight, crown-rump length, and sex were determined at autopsy. Caesarean section data were evaluated for statistical significance using the Goodman's Simultaneous Comparison. Fetal data were evaluated by the Dunnett's T-test and/or the "Nemenyi many-one version of the H-test".

Results- Treatment with the test compound had no apparent effect on reproductive indices in the present study (see Table 1, photocopied from the original review of report #570/82). Litter size, fetal body weights and crown-rump lengths were comparable to control. A slight increase in the number of resorptions was noted in the mid and high dose groups, however this finding was not statistically significant. The submitted individual animal data do not alter this assessment.

(b) Necropsy findings in dams- After caesarean section, dams were dissected and organs were examined for gross changes. The weights of heart, liver, kidneys, spleen and adrenals were determined. Data were evaluated statistically by the Dunnett's T-test and/or the "Nemenyi many-one version of the H-test".

Results- The original review of this study identified increases in the absolute organ weights of kidney and spleen in high dose dams as potential treatment-related effects. Both values were significantly increased by about 10% ($p<0.05$). Dilation of one or both renal pelvises, which was present at a rate of about 10-20% in dams from all treatment groups in the high dose

study, was noted in the present study at rates of 5-10%, which was comparable to the high dose study and to historical control values. Enlarged adrenals were not noted in any of the control or treated dams in the present study.

(4) Fetal Developmental Toxicity Indices: (a) External- After removal from the uterus, fetuses were examined for external appearance and the presence of anomalies. Data were evaluated for statistical significance with the Fisher's Exact Test and the Exact Simultaneous Comparison with Control in a contingency table.

Results- As was noted in the original review, no effect of treatment on the incidence of external malformations/variations was apparent. No stunted fetuses were reported. The submitted individual animal data do not alter this assessment.

(b) Soft tissue- About half of the fetuses were fixed in Bouin's fluid and examined in body cross-sections under a microscope for organ anomalies by the method of Wilson. For skeletal and soft tissue examinations, fetuses were selected alternately according to their location within the uterus. Data were evaluated for statistical significance with the Fisher's Exact Test and the Exact Simultaneous Comparison with Control in a contingency table.

Results- As was noted in the original review of this study, no effect of treatment on the incidence of soft tissue malformations/variations was apparent. Specifically, dilated renal pelvis with hydroureter, which was noted in all treatment groups of the high dose study, was not noted in any control or treated fetuses in the present low dose study. Dilated renal pelvis or hydroureter were noted (separately) in several fetuses and litters of the present low dose study (see Table 2, photocopied from the original review), however the incidences did not appear to be treatment-related and were within the range of historical control values.

(c) Skeletal- About half of the fetuses from each litter (selected alternately according to location within the uterus) were fixed in alcohol, dissected under a magnifying glass, eviscerated and bleached in aqueous potassium hydroxide. Skeletons were stained with Alizarin Red-S and examined. Data were evaluated for statistical significance with the Fisher's Exact Test and the Exact Simultaneous Comparison with Control in a contingency table.

Results- No effect of treatment on skeletal development was apparent. Common findings in all groups without relation to treatment included poor ossification of one or more headbones and non-ossification of metacarpal 5.

Table 1. Reproductive Data[a]

	DOSE (mg/kg) 0	0.50	2.24	10.0
Number of dams:				
-pregnant/inseminated	20/21	20/21	20/24	20/21
-deaths	0	0	0	0
-sacrificed	0	0	0	0
Dams on Day 21:				
-alive	20	20	20	20
-with live fetuses	20	20	20	20
-weight gain days 0-21 (g ± s.d.)	123±19	135±14	130±12	125±10
Mean Number/Dam:				
-corpora lutea	13.1	12.8	14.3	13.8
-implantations	12.3	12.6	12.6	12.2
-resorptions	0.25	0.35	0.70	0.80
Mean Fetal Data:				
-no. dead/litter	0	0.05	0	0
-no. live/litter	12.0	12.3	11.9	11.4
-% male	51	52	53	51
-body weight (g)	3.27±0.21	3.35±0.19	3.32±0.17	3.42±0.27
-crown-rump (cm)	3.64±0.12	3.61±0.07	3.58±0.10	3.65±0.12
-placental weight (g)	0.49±0.05	0.48±0.05	0.49±0.03	0.51±0.06

[a]data excerpted from submitted study. Fetal weight and length data do not include values of dead fetus from 0.50 mg/kg group.

[b]calculated by reviewer.

Table 2. Incidences of Fetal Malformations[a]

	DOSE (mg/kg) 0	0.5	2.24	10.0
No. fetuses examined	118	118	112	110
No. litters examined	20	20	20	20
Fetuses With:				
Dilated renal pelvis, one or both sides	2/2[b] (1.7/10.0)[c]	1/1 (0.8/5.0)	1/1 (0.9/5.0)	0
Hydroureter only	0	1/1 (0.8/5.0)	0	0
Dilated renal pelvis and hydroureter	0	0	0	0
Dilated renal pelvis and/or hydroureter	2/2 (1.7/10.0)	2/2 (1.7/10.0)	1/1 (0.9/5.0)	0

[a]data excerpted from submitted study.

[b]no. affected fetuses/litters (litter incidences calculated by reviewer).

[c]percent affected fetuses/litters (calculated by reviewer).

Discussion

No effect of treatment on fetal development was apparent at the tested doses of 0.50, 2.24, or 10 mg/kg/day in the present study. This finding is in contrast to the dose-related increase in the fetal and litter incidences of dilated renal pelvis with hydroureter that was noted in all treatment groups from the previous study, which tested higher doses of 10, 50, and 250 mg/kg/day.

Similarly, little effect of treatment on maternal health was apparent. Slight (10%) increases in absolute kidney and spleen weights were noted in high dose dams. Although significantly different from control ($p<0.05$), the values in high dose dams were reportedly within the normal range, and were similar to control values from the previous study. This apparent finding is of doubtful toxicological significance. Other parameters, such as body weight, food consumption, and physical appearance were unaffected by treatment. Specifically, hyperactivity, which was noted in 2/20 dams treated with 10 mg/kg/day in the previous study, was not noted in any high dose dams in the present study treated at the same dose level.

Therefore, although no effects of treatment were apparent in the present study, when these data are considered with the results from the previous study (#G2R0303, report no. 85.0748), the dose of 10 mg/kg/day appears to be the Lowest Observed Effect Level for both maternal and developmental toxicity, and 2.24 mg/kg/day appears to be the No Observed Effect Level for these endpoints. The ratio of maternal to developmental NOEL, known as the A/D ratio, is therefore 1.0, suggesting that fetal effects only occur at doses that are maternally toxic.

Based on the joint consideration of high dose study #G2R0303 with low dose study #G2R0342:

Maternal NOEL = 2.24 mg/kg/day
Maternal LEL = 10 mg/kg/day Hyperactivity

Developmental NOEL = 2.24 mg/kg/day
Developmental LEL = 10 mg/kg/day Increased incidence of dilated renal pelvis with hydroureter.

Classification: Core-Minimum When considered with the results of study #G2R0303, report #85.0771.

DATA EVALUATION REPORT

STUDY TYPE: Mouse micronucleus test.

ACCESSION NUMBER: 073916

TEST MATERIAL: Monoammonium-2-amino-4-(hydroxymethylphosphinyl)-butanoate.

SYNONYMS: HOE-39866

STUDY NUMBER: 83.0165

REPORT NUMBER: 85.0724 (revision of report #83.0555)

SPONSOR: American Hoechst Corporation
Agricultural Division
Somerville, NJ 08876

TESTING FACILITY: Pharma Research Toxicology
Hoechst AG
Frankfurt, W. Germany

TITLE OF REPORT: "Micronucleus Test in Male and Female NMRI Mice After Oral Administration."

AUTHORS: Jung, Weigand, and Kramer.

REPORT ISSUED: 7-12-85

Reviewer: D. Stephen Saunders Jr., Ph.D.
Toxicologist, Section V
TOX/HED (TS-769C)

Secondary Reviewer: Laurence D. Chitlik, DABT
Head, Section V
TOX/HED (TS-769C)

Conclusion: The data demonstrated that the highest dose tested of 200 mg/kg in the mouse micronucleus study was adequate. Higher doses of 250 or 300 mg/kg produced clinical signs of toxicity and death in test animals.

Classification: Acceptable when considered with the data from the main study (report #83.0555).

Background

This study report is a revision of report #83.0555, which was previously reviewed in Toxicology Branch (memo Saunders to Mountfort, 4-18-85). In that review, this study was found unacceptable because no justification of the doses tested was presented. Included in the present submission was a rewrite of the original report, and the range-finding data which were the basis for the selection of doses in the main study. The revised study report does not present any data additional to that in the main study, therefore only the range-finding data are reviewed in this DER.

A. Materials

(1) Test material- Monoammonium-2-amino-4-(hydroxymethylphosphinyl)butanoate, code HOE 039866 OH ZC95 0001, 95.3% a.i. Positive control- cyclophosphamide (Endoxan).

(2) Test animals: Male and female (Hoe: NMRKf[SPF71]) NMRI mice obtained from Hoechst breeding colony, Kastengrund. Mice were approximately 7-12 weeks old at initiation of treatment.

(3) Doses tested: 0, 8, 40 and 200 mg/kg by gavage in 5 mice/sex/dose.

B. Study Design

The methods followed in this study were previously reviewed (see "Background"). Deficiencies noted were a lack of justification for the doses that were tested, and no description of the route of administration of the positive control, cyclophosphamide. This reviewer is unable to discern from the submitted "revised" study report what the route of administration of cyclophosphamide was; it will therefore be assumed that the positive control was injected intraperitoneally, which is standard procedure for this type of study.

C. Results of the Range-Finding Study

Three separate toxicity trials were conducted. In the first, 3 male and 3 female mice were given single doses of 300 mg/kg by gavage. Clinical signs of clonic and tonic spasm, hyperreflexia, and straub tail were noted in these mice, of which 1 male and 1 female died.

In the second trial, doses of 250 mg/kg were administered to 3 mice/sex. Signs similar to those noted in the first trial were observed, and 2/3 males and 1/3 females died.

In the third trial, doses of 200 mg/kg were administered to 3 males and 3 females. No clinical signs or mortality were noted.

On the basis of these data, a high dose of 200 mg/kg was selected for the main micronucleus study.

Discussion

These data demonstrate that the highest dose tested (200 mg/kg) in the main micronucleus study is sufficiently close to a lethal dose to qualify as a Maximally-Tolerated Dose (MTD) in the mouse under the conditions of this experiment. As noted in the original review, the test material did not induce any clastogenic effects under the conditions of the study. The study is classified as Acceptable.