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

4/26/88

006679

OFFICE OF
PESTICIDES AND TOXIC SUBSTANCES

MEMORANDUM

SUBJECT: Mancozeb - Rabbit Study Submitted under EPA Accession No. 404330 in Response to Data Call-In Notice
EPA ID No. 707-78

TB Project No.: 8-0371
Caswell No.: 913A

FROM: Irving Mauer, Ph.D. 4/14/88
Toxicology Branch
Hazard Evaluation Division (TS-769C)

TO: Lois A. Rossi, PM 21
Fungicide-Herbicide Branch
Registration Division (TS-767C)

THRU: Judith W. Hauswirth, Ph.D., Head Judith W Hauswirth 4/14/88
Section VI, Toxicology Branch
Hazard Evaluation Division (TS-769C)

and

4/26/88

Theodore M. Farber, Chief
Toxicology Branch
Hazard Evaluation Division (TS-769C)

Registrant: Rohm & Haas
Philadelphia, PA

Request

Review and evaluate the following rabbit teratology study, submitted in response to the Data Call-In Notice and data gap identified in the Mancozeb Registration Standard:

Oral (Gavage) Developmental Toxicity Study in Rabbits, performed at the Toxicology Department, Rohm & Haas, Spring House, PA,

Report No. 86R-021, dated March 31, 1987
(EPA Accession No. 404330-01).

TB Conclusion

A detailed review of this study is attached. In summary, TB judges the study Core Minimum, with the following parameters:

Maternal NOEL = 10 mg/kg (the authors of the study proposed 30 mg/kg)

Maternal LEL = 30 mg/kg (death)*

Developmental NOEL > 80 mg/kg (HDT)

A/D Ratio = 0.125

(Doses Tested: 0, 10, 30, and 80 mg/kg)

Attachment

*If the registrant can document more completely the circumstances of the death of the single doe in the mid-dose group (in addition to the "mis-dosage" cause alluded to, and the pathologist's statement about "yellow material within the thoracic cavity"), the maternal NOEL can be raised to 30 mg/kg.

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TOXICOLOGY BRANCH: DATA REVIEW

Reviewed By: Irving Mauer, Ph.D. 04-14-88
Toxicology Branch
Hazard Evaluation Division

TB Project: 8-0371

Date:

Through: Judith W. Hauswirth, Ph.D., Head
Section VI, Toxicology Branch
Hazard Evaluation Division
Judith W. Hauswirth 4/14/88

Chemical: Mancozeb

Caswell: 913A
EPA Chem: 014504

Study Type: Developmental Toxicity - Rabbit

Citation: Oral (Gavage) Developmental Toxicity Study in Rabbits

Authors: H.M. Solomon and M.F. Lutz

Accession No.: 404330-01

MRID: N/A

Sponsor: Rohm & Haas
Philadelphia, PA

Testing Lab: Rohm & Haas
Toxicology Department
Spring House, PA

Study No.: 86R-021

Study Date: March 31, 1987

TB Conclusions:

Doses Tested: 0, 10, 30, and 80 mg/kg/day
Maternal NOEL = 10 mg/kg
Maternal LEL = 30 mg/kg (death)
Developmental NOEL > 80 mg/kg (HDT)
A/D Ratio = 0.125

TB Evaluation/Classification: Core Minimum

DETAILED REVIEW

Test Article:

Dithane M-45, Lot No. D56530, 83.0% mancozeb, a coordination product of zinc ion and manganese ethylenebisdithiocarbamate (Confidential ID Sheet, APPENDIX J, however, states purity as [redacted] containing [redacted] ethylenethiourea (ETU); yellow powder with a slight sulfur odor, suspended in 0.5% aqueous methylcellulose for oral testing by gavage. Samples of daily preparations from the second, ninth, and last dose days were analyzed.

Test Organism:

Female New Zealand White rabbits, 4 to 5 months old and weighing 3.2 to 3.8 kg, from Hazleton Research Animals, Denver, PA; acclimated for 41 days before treatment.

Procedures:

Groups of 20 females each were injected intravenously with 0.10 mL/kg (20 USP units/kg) chorionic gonadotrophin to induce ovulation. Three hours later, the does were artificially inseminated with semen from stock bucks of proven fertility; the day of artificial insemination was designated Day 0 of gestation. Daily preparations of test material were administered by gavage on gestation Days 7 to 19 at dose levels of 0 (vehicle control), 10, 30, and 80 mg/kg/day* (in a constant volume of 5 mL/kg), based upon the most recently recorded body weights. The design of the study can be summarized as follows:

| Group | Test Material | Dose[a] mg/kg/day | Inseminated Females | Fetal Examinations[b] |
|---|---|---|---|---|
| 1 | Vehicle Control[c] | 0 | 20 | All |
| 2 | Mancozeb | 10 | 20 | All |
| 3 | Mancozeb | 30 | 20 | All |
| 4 | Mancozeb | 80 | 20 | All |

[a]All doses administered as mg ai/kg bwt/day on Days 7 to 19 of gestation.
[b]All fetuses were examined for external, visceral, and skeletal alterations.
[c]0.5% aqueous solution of methylcellulose.
*This dosage schedule was based upon the preliminary toxicity study performed by Rohm & Haas, "Mancozeb: Range-Finding (Gavage) Developmental Toxicity Study in Rabbits," Report No. 85R-244, dated March 9, 1987, in which the NOEL for does was 50 mg/kg (the LDT), and for fetuses 100 mg/kg.

Does were weighed on gestation Days 0, 7, 9, 11, 14, 17, 20, and 29. Animals were observed daily throughout pregnancy, but twice daily during the dosing period. Any that died during gestation, or appeared moribund and were killed, were subjected to a limited necropsy, restricted to evidence of pregnancy and any gross lesions present in the abdominal and thoracic cavities. The authors discarded the carcasses.

Surviving does were sacrificed on Day 29 by injection of "euthanasia solution"* into the heart. Thoracic and abdominal cavities were examined grossly, and the uterus (gravid and/or empty) was weighed. The uterus was resected and the distribution of live and dead fetuses, as well as resorptions was recorded. If the uterus appeared "nonpregnant," it was stained with ammonium sulfide for evidence of early resorptions. Corpora lutea were counted in does that had at least one viable fetus. Live fetuses were weighed, examined for gross (external) abnormalities, then killed by intraperitoneal injection of pentobarbital sodium**, examined for visceral alterations using Staples' technique***, and sexed. The brain was examined in a transverse section made between parietal and frontal bones; the eyes were also examined. Then each fetus was fixed in 95% ethanol, macerated in 2% potassium hydroxide and stained with alizarin red S for skeletal examination.†

Statistical Evaluation:

Following Haseman††, the authors considered the litter (proportion of affected fetuses per litter or the litter mean) as the experimental unit for statistical treatment of the data. As presented in the Report, pair-wise tests between control and each treated group were applied to each of the following parameters (the level of significance selected was in all cases $p \leq 0.05$).

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*T-61®, American Hoechst, Somerville, NJ.
**Nembutal, Abbott Laboratories, North Chicago, IL.
***R.E. Staples: "Detection of Visceral Alterations in Mammalian Fetuses" Teratology 9:A37 (1974).
†A.A. Dawson: "A Note on the Staining of the Skeleton of Cleared Specimens with Alizarin Red S." Stain Tech. 1:123-124 (1926).
††J.K. Haseman and M.D. Hogan "Selection of the Experimental Unit in Teratology Studies." Teratology 12:165-172(75).

| Parameters | Pair-Wise Test Between Groups |
|---|---|
| Pregnancy rate | Fisher's Exact |
| Clinical signs | Fisher's Exact |
| Maternal death | Fisher's Exact |
| Gross necropsy findings | Fisher's Exact |
| Litters with total resorptions | Fisher's Exact |
| Maternal body weight change | Dunnett's[a] |
| Maternal feed consumption | Dunnett's |
| Implantations | Mann-Whitney[b] |
| Live fetuses | Mann-Whitney |
| Dead fetuses | Mann-Whitney |
| Resorptions | Mann-Whitney |
| Corpora lutea | Mann-Whitney |
| Fetal weight | Mann-Whitney |
| Incidence of fetal alterations | Mann-Whitney |

To control bias, does were coded immediately before cesarean section on Day 29, and not decoded until all raw maternal and fetal data were collected. Thus, all maternal and fetal examinations were conducted "blind."

Results:

A. Analysis for Test Samples

The authors stated that the analytical report for the dose suspension samples sent to Rohm & Haas' Analytical Chemistry Department was not ready when the March 31

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[a]When one-way ANOVA was significant.
[b]When more than 75% of litters were unaffected for a fetal parameter, Fisher's Exact Test was used instead of Mann-Whitney.

report was issued. However, the chemical analyses* were submitted to the Agency as Amendment No. 1, dated October 23, 1987 (given Accession No. 404330-02), which revealed dose levels that averaged 100 percent of theory (as actually reported: low-dose = 100%; mid-dose = 99%; high-dose = 100%).

B. Maternal Clinical Data

Two high-dose (80 mg/kg) females (one pregnant, one nonpregnant) became moribund during the treatment period and had to be killed (on Days 22 and 19, respectively--Report Table 1; Appendix B). The authors considered these deaths to be treatment related, and maintained that no compound-related deaths occurred in the lower treatment groups (10 and 30 mg/kg).

> [NB: Whereas no mortalities occurred in the low dose or control groups (Table 1; Appendix B), a pregnant animal on 30 mg/kg died on Day 22. This death was recorded as "spontaneous" due to having been "mis-dosed." The circumstances, however, were not further described in text (e.g., as to the amount mis-gavaged, etc.). The only information about this animal was the notation in the pathology report: "yellow material within thoracic cavity" (test compound, due to perforation of the esophagus?)]

During the treatment period, significant increases were recorded in the high-dose group over controls in the incidence of alopecia (4 vs. 0), anorexia (8 vs. 1), and scant feces (10 vs. 1), but the authors considered there were no significant increases in such adverse clinical signs among 10 or 30 mg/kg does (Final Report Table 2, attached to this review). During this period, high-dose animals also manifested increased but not statistically significant increased incidences of other clinical signs considered by the authors as treatment-related--such as anuria, lack of feces, ataxia, reddish discharge on cageliners and soiled perineum--each of which was either not noted, or found only as isolated instances among does treated at the mid or low dose, and comparable to controls. During the posttreatment period (Days 20 to 29), significant increases in anorexia, scant or no feces, and red discharge onto cageliners (as well as abortions, as noted in Report Table 2) persisted

*Raw analyses were conducted at Enviro-Bio-Tech (EBT), Ltd., using the Rohm & Haas (R&H) Protocol for Dithane analysis; EBT returned the raw data, which were further processed by R&H.

in high-dose animals; again, even though increases in alopecia and ataxia in this group were not significant, the authors considered that these effects were also treatment-related. None of the other groups showed any treatment-related increases over control in adverse clinical signs.

C. Maternal Body Weight

Body weight changes in pregnant does that survived to terminal cesarean section at Day 29 (15 controls, 12 at 10 mg/kg, 10 at 30 mg/kg, and 8 at 80 mg/kg) were similar in all groups during the treatment period (115, 137, 118, and 162 g/rabbit, respectively) as well as after treatment (113, 140, 108, and 141 g/rabbit, respectively, Report Table 3; Appendix C). Among the five high-dose does that aborted during the treatment period, however, body weight losses ranged from 210 to 720 g, and were considered compound-related.

D. Maternal Feed Consumption

Maternal feed consumption followed a similar course. When measured in pregnant animals with viable fetuses, test groups consumed similar amounts to controls, during both treatment (147.4, 140.9, and 147.7 g/rabbit in 10, 30, and 80 mg/kg, respectively, vs. 146.1 g/control), as well as in the posttreatment period (144.8, 139.2, and 149.9 g/test rabbit, vs. 143.4 g/control) (Report Table 4; Appendix E). On the other hand, food consumption was reduced in the five 80 mg/kg does that aborted, but only during the posttreatment period, by an average of 48.12 g/rabbit (range, +0.5 to 94.4 g/rabbit) (Appendix E).

E. Maternal Postmortem Findings

Gross pathological findings were comparable in all groups, consisting of single instances of minor organ involvement equally distributed (total affected does per 20 does examined per group = 5 controls, 6 low dose, 6 mid-dose and 4 high-dose--Report Table 5; Appendix F).

[NB: Whereas the stomach, spleen, kidneys, liver, ovaries, intestines, and abdominal cavity were examined and/or weighed, the thyroid was not.]

F. Reproductive Outcome

Except for a significant increase in the number of abortions among high-dose does (5 vs. 0 in control; also, none at 10 and 30 mg/kg), with a corresponding

significant decrease in the number of litters produced (8 vs. 15 in control), all other reproductive parameters in this group were comparable to control (Report Table 6, attached; Appendix G). There were no apparent treatment-related changes found by the authors in any reproductive parameter examined between controls and the low- or mid-dose groups; i.e., the following were reportedly similar in all these groups: Numbers of abortions (0) and litters (15, 12, and 10); mean numbers per litter of corpora lutea, implantations, resorptions, dead and live fetuses; sex ratio.

G. Fetal Data

No significant differences from controls were recorded in the following test group fetal parameters: mean fetal weights (Report Table 6, attached; Appendix G); types or incidence of malformations (Report Table 7, attached; Appendix H); types or incidence of developmental variations, whether arising in otherwise normal fetuses or as a consequence of retarded development (Report Tables 8, 9, and 10, the last attached to this review; Appendix I).

Gross malformations were found in controls as well as in the 10 and 30 mg/kg groups, but not at the HDT (Table 7). The types listed included:one or two instances of anasarca; omphalocele; cataract; cardiomegaly; diaphragmatic hernia; ringed aorta; hemivertebrae with supernumary rib; fused ribs, vertebrae or sternebrae.

Author's Conclusions:

From the results of the oral (gavage) administration of Dithane M-45 (83% mancozeb) to pregnant rabbits on Days 7 through 19 of gestation at daily dose levels of 10, 30, and 80 mg/kg, the authors concluded that the maternal NOEL was 30 mg/kg. Since neither fetal toxicity nor increase in the incidence of malformations was found at any dose level (including the HDT, 80 mg/kg), the authors did not cite a developmental NOEL.

TB Evaluation:

This study was well designed and appears to have been adequately conducted, such that one may have confidence in the data generated. The initial number of does per group inseminated (20) was sufficient to assure an adequate number of pregnancies for meaningful analysis as deemed desirable by Guidelines criteria (i.e., at least 12). As the in-life pretreatment portion proceeded, however, group size decreased from the 20 does per group originally inseminated in the four groups so that the numbers of live pregnancies available for treatment were 17 (control), 15 (low-dose), 11 (mid-dose), and 15 (high-dose) (see Report Table 6 attached). Maternal

death, abortion, and complete litter resorptions during treatment reduced the number of litters that could be examined to 15, 12, 10, and 8, respectively, the decrease in the high-dose compared to control being significant. The eight high-dose litters contained 42 live fetuses, a number sufficient to detect any developmental anomalies (but see below).

It is evident that 80 mg/kg mancozeb produced adverse maternal and reproductive effects, including death, alopecia, anorexia, fecal alteration, ataxia, anuria, and abortion. The authors contended that both lower doses, 10 and 30 mg/kg, showed no compound-related adverse effects; hence, 30 mg/kg was considered the maternal NOEL. Apparently there were no adverse fetal effects at all, even at the HDT, a conclusion based on examination of a total of 267 fetuses in 45 litters. Compared to the observation of a small number of grossly malformed fetuses in controls and the two lower test groups, none were reportedly found at the high dose (Table 7), unless they were "hidden" among the five abortions recorded in this group (APPENDIX B, citing individual clinical findings, however, lists those issues only as "aborted material").

On the other hand, we believe a reassessment should be made of the effects on pregnant does at 30 mg/kg, since this level may also represent a maternal effect level. Support for this reassessment would include a more satisfactory explanation for the death of a mid-dose pregnant doe on Day 11, recorded as "spontaneous" because of "mis-dosage" the same day (Table 1). The circumstances surrounding this death were not further described in the text; the only information recorded was the pathology report which noted that this animal had "yellow material within the thoracic cavity" (Appendix F) (perhaps test compound?). Therefore, unless further documentation is provided that resolves the concerns above, TB concludes the NOEL for maternal clinical effects is more properly 10 mg/kg.

TB Conclusion:

Doses Tested: 0, 10, 30, and 80 mg/kg daily on Days 7 through 19 of gestation.

Maternal NOEL = 10 mg/kg

Maternal LEL = 30 mg/kg (death)

Developmental NOEL > 80 mg/kg (HDT)

A/D Ratio = 0.125

The study is judged Core Minimum.

Attachments

SUMMARY TABLES

FROM

STUDY

Page_____ is not included in this copy.

Pages 12 through 15 are not included.

The material not included contains the following type of information:

____ Identity of product inert ingredients.

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