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

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OFFICE OF
PESTICIDES AND TOXIC
SUBSTANCES

MEMORANDUM

SUBJECT: Methylene bis(Thiocyanate) Developmental Toxicity Studies in Rats and Rabbits.

Project No.: 9-1831
Tox. Chem. No.: 565
Bar Code No.: 248426
EPA ID No.: CA 61756

TO: John Lee, PM #31
Registration Division (H7505C)

FROM: Nguyen Bich Thoa, Ph. D. 10/25/91
Review Section 1
Toxicology Branch 1
Health Effects Division (H7509C)

THRU: Roger Gardner, Section Head
Review Section 1
Toxicology Branch 1 10-25-91
Health Effects Division (H7509C)

KB 10/28/91

Action Requested

Review the following developmental toxicity studies (83-3):

1. Developmental Toxicity (Embryo/Fetal Toxicity and Teratogenic Potential) Study of Methylene bis (thiocyanate) Administered Orally via Gavage to Crl:CD®(SD)BR Presumed Pregnant Rats. MRID No. 411719-01.

2. Developmental Toxicity (Embryo/Fetal Toxicity and Teratogenic Potential) Study of Methylene bis (thiocyanate) Administered Orally (Stomach Tube) to New Zealand White Rabbits. MRID No. 411719-02.

Conclusions

1. Neither study indicated that developmental toxicity occurred at levels below maternally toxic doses. Therefore, the results of these studies do not exceed the flagging criterion for 6 (a)(2) (adverse effects) data in developmental toxicity studies (40 CFR, Part 158.34, Criterion No. 5).

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2. The NOEL for maternal toxicity in rats is 3 mg/kg/day; the LOEL is at 6 mg/kg/day (highest dose tested) and is based on slightly decreased maternal body weight gain and a small increase in the incidence of rales (a sign observed at higher dose levels in range-finding studies). No developmental toxicity was noted at 1 or 3 mg/kg/day dose levels, but discrepancies in reporting (see discussion below and attached Data Evaluation Record) preclude a final conclusion about the NOEL for developmental toxicity in this study. The classification of this study as Supplementary can be upgraded with an acceptable explanation of the discrepancy in the number of animals reported as partially dosed (5 as shown in tables of individual maternal data and 11 as indicated in a table of fetal data).

3. The maternal NOEL for MBT in pregnant rabbits was 3.5 mg/kg/day, and the developmental NOEL was > 5 mg/kg/day (highest dose tested). The LOEL for maternal toxicity was based on mortality at the initial dose level of 7 mg/kg/day (highest dose tested) and reduced maternal body weight gain after the initial dose level was reduced to 5 mg/kg/day (see discussion below and attached DER).

Summary of Studies

1. The Developmental Toxicity Study in Rats

The developmental toxicity potential of methylene bis (thiocyanate) was studied by dosing pregnant Crl:CD®BR rats by oral gavage with 0, 1, 3, and 6 mg/kg/day. Due to an error in dosing only 14 control, 12 low dose, 11 mid dose, and 17 high dose group dams were "fully dosed" (dosed from day 6-15). Dams partially dosed (from day 6-14) included 10, 12, 11 and 5 animals from the control, low, mid and high dose groups, respectively.

No maternal mortality was observed. When the data was analyzed regardless of the dosing error, maternal body weight, body weight gain, or feed consumption were slightly decreased at the 6 mg/kg/day dose level. The test material did not induce any gross pathological alterations. Pregnancy rate, average numbers of corpora lutea, implantations, percent of pre- and post-implantation loss, resorptions, fetuses/litter, viable number of fetuses/dam, fetal body weight, and percentage of male fetuses/litter were comparable between groups. No developmental toxicity was observed at the 1 and 3 mg/kg/day dose levels.

Based on results from two range-finding studies and slight effects on clinical signs (rales), food consumption, body weight and body weight gain, the 6 mg/kg/day dose level approached a maternally toxic level and is probably adequate for evaluation of

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the potential developmental toxicity of methylene bis(thiocyanate) in pregnant rats. Therefore, the NOEL for maternal toxicity is likely to be at 3 mg/kg/day.

Because of discrepancies in the report regarding the number of partially dosed animals in the high dose group, a conclusion about the NOEL for developmental toxicity in this study can not be made.

2. The Developmental Toxicity Study in Rabbits

The developmental toxicity potential of Methylene bis (thiocyanate) was studied by dosing pregnant Hra:(NZW)SPF rabbits, by stomach tube, with 0, 1, 3.5, and 7 mg/kg/day. The highest dose tested caused 5 deaths (25% of total) and was reduced after the fifth death to 5 mg/kg/day. The calculated average daily dosing for the high dose group's survivors was 5.31-5.77 mg/kg/day.

Five treatment-related deaths were observed in the high dose group following, respectively, 1, 2, 3, 4 and 5 dosings at 7 mg/kg/day. All 5 dead rabbits were pregnant. The conceptuses of the 3 that died after 3-5 dosings appeared normal for their developmental age. The viability state of the in utero implantations of the 2 that died after 1-2 dosings could not be determined because of the early developmental stage. All 4 high-dose does that died after 2-5 dosings showed severe gastric ulceration and/or gastric mucosal sloughing and hemmorhage at necropsy. No further mortality was observed after the high dose level was reduced.

Clinical signs preceeding the 5 deaths included respiratory difficulty, ataxia, decrease in motor activity, loss of righting reflex, tremors, convulsions, and/or dry feces. After the high dose was reduced, two survivors in the group showed single incidences of tremor (day 15 and day 17) and 8 showed incidences of dry feces lasting 2-4 days, six of those during the period from day 8 to 12.

Body weight gain and feed consumption in the high dose group was significantly ($p < 0.01$) decreased, but only during the period when the high dose was at 7 mg/kg/day (body weight gain decreased on day 6-7, 7-8, and 8-9; feed consumption additionally on day 9-10; respective number of high dose group does given 7 mg/kg/day on day 6-7, 7-8, 8-9, and 9-10 were 5,10,15, and 20).

MBT at 1, 3.5, and 5.31-5.77 mg/kg/day did not affect pregnancy rate, average numbers of corpora lutea, implantations, early and late resorptions, fetuses/litter, viable number of fetuses/litter, fetal body weight, and percentage of male fetuses. These doses did not cause external, soft tissue, or skeletal malformations or variations in the fetuses, and incidences of fetal changes were not statistically significantly increased in any of the treated groups.

The maternal no-observable effect level (NOEL) for MBT in pregnant rabbits was 3.5 mg/kg/day, and the developmental NOEL was >5 mg/kg/day.

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GUIDELINE: 83-3

Primary Review by: Nguyen B. Thoa, Ph.D. 10/25/91
Review Section 1, Toxicology Branch I/HED
Secondary Review by: Roger Gardner
Section Head, Review Section 1, Toxicology Branch I/HED 10-25-91

DATA EVALUATION RECORD

Study Type: Teratology - Developmental Toxicity
Species: Rats
Guideline: 83-3

EPA Identification No.s: EPA MRID No. 411719-01
EPA ID No. CA 61756
EPA Record No. 248426
EPA Shaughnessy No.
Caswell No. 565
HED Project No.9-1831
Document No.

Test Material: Technical Grade Methylene bis (thiocyanate); 99% ai
Synonyms: MBT

Sponsor: Methylene bis (thiocyanate) Task Force, ICI America Inc., Farmington, Connecticut

Study Number(s): 1419-001

Testing Facility: Argus Res. Labs., Horsham, PA 19004

Title of Report: Developmental Toxicity (Embryo/Fetal Toxicity and Teratogenic Potential) Study of Methylene bis (thiocyanate) Administered Orally via Gavage to Crl:CD®(SD)BR Presumed Pregnant Rats.

Author(s): Alan M. Hoberman, Ph.D., DABT.

Report Issued: 06-30-89

Bibliographic Citation: See attached copy.

Conclusions: The developmental toxicity potential of methylene bis (thiocyanate) was studied by dosing pregnant Crl:CD®BR rats by oral gavage with 0, 1, 3, and 6 mg/kg/day. Due to an error in dosing only 14 control, 12 low dose, 11 mid dose, and 17 high dose group dams were "fully dosed" (dosed from day 6-15). Dams partially dosed (from day 6-14) included 10, 12, 11 and 5 animals from the control, low, mid and high dose groups, respectively.

No maternal mortality was observed. When the data was analyzed regardless of the dosing error, maternal body weight, body

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weight gain, or feed consumption were slightly decreased at the 6 mg/kg/day dose level. The test material did not induce any gross pathological alterations. Pregnancy rate, average numbers of corpora lutea, implantations, percent of pre- and post-implantation loss, resorptions, fetuses/litter, viable number of fetuses/dam, fetal body weight, and percentage of male fetuses/litter were comparable between groups. No developmental toxicity was observed at the 1 and 3 mg/kg/day dose levels.

Based on results from two range-finding studies and slight effects on clinical signs (rales), food consumption, body weight and body weight gain, the 6 mg/kg/day dose level approached a maternally toxic level and is probably adequate for evaluation of the potential developmental toxicity of methylene bis(thiocyanate) in pregnant rats. Therefore, the NOEL for maternal toxicity is likely to be at 3 mg/kg/day. Because of discrepancies in the report regarding the number of partially dosed animals in the high dose group, a conclusion about the NOEL for developmental toxicity in this study can not be made.

Core Classification: Supplementary. The classification of this study can be upgraded with an acceptable explanation of the discrepancy in the number of animals reported as partially dosed (5 as shown in tables of individual maternal data and 11 as indicated in a table of fetal data).

A. MATERIALS:

Test Compound: Purity: 99% ai
Description: yellow tan powder.
Lot No.: 7-0846M
Contaminant: Not listed
Storage: At room temperature.

Test Animal: Species/Strain: Rats/Crl:CD®BR
Source: Charles River Breeding Labs., Raleigh, NC.
Age (days) at receipt: 60 (F); 73 (M).
Weight at receipt (g): 152-208 (F); 214-295 (M)
Acclimation Period: 7 days

B. STUDY DESIGN:

Mating:

Young adult virgin female rats (150) were mated with male breeders on a 1:1 basis for 4 consecutive days. Each couple was housed in a wire-bottomed stainless steel cage suspended above absorbent paper. Presumed gestation day 0 was when the presence of spermatozoa in vaginal lavage or a copulatory plug in situ was first detected.

Group Arrangement:

On day 0, 100 healthy looking mated females were randomly (computer-generated weight-ordered process) assigned to the following experimental groups:

Group	Dose level mg/kg/day *	Number of animals
Control	0 (vehicle) **	25
Low dose (LDT)	1	25
Mid dose (MDT)	3	25
High dose (HDT)	6	25

* All doses were administered in a volume of 10 ml vehicle/kg of body weight/day on gestation days 6 through 15. Dosing suspensions were prepared daily and adjusted for individual body weight changes.

** Methyl cellulose (lot No. 114F-0354; 0.5% aqueous solution, w/w).

Selection of the doses tested was based on the results of two pilot studies (See Appendix A) (dates of studies not stated). In the pilot study with pregnant rats, death occurred at doses ≥ 10 mg/kg/day (death rate = 1/8 at 10 mg/kg/day; 6/8 at 30 mg/kg/day). Gastrointestinal tract lesions were observed in animals at doses ≥ 10 mg/kg/day. In the preliminary study with non-pregnant female rats, one death occurred in four animals given 9 mg/kg/day, and body weight gains were reduced at 5 mg/kg/day (day after first dosing) and at 7 and 9 mg/kg/day (through the 5-day dosing period).

The mated females were housed individually under conditions of 71-78° F; 31-50% relative humidity; HEPA filtered fresh air changes > 10/hour; and 12-hr fluorescent light/12-hr dark cycle. Feed (Purina Certified Rodent chow #5002) and deionized water (amended with ≤ 1.0 ppm chlorine as a bacteriostat) were given ad libitum. The investigators stated that "no agent was present in the feed and water, which was known to interfere with the results of the study".

Analyses of Test material Stability and Concentration in the vehicle:

Aliquots (10 ml) of all dosing suspensions were collected on every dosing day. They were immediately frozen on dry ice, and shipped to Lancaster Labs. Inc. for analysis of concentration and stability (HPLC UV method). According to the results of the analyses, the achieved concentrations of the test material in the dosing suspensions were respectively 81-105%, 87-108%, and 95-113%

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of target concentrations for the low, mid, and high dose suspensions.

Observations:

All experimental animals were checked twice daily for mortality, abnormal behavior, and clinical signs of toxicity from day 6 to day 20. Signs of abortion/premature delivery were checked once daily from day 16 to day 20. Body weight and feed consumption were recorded on day 0 and daily from day 6 to day 20. All were sacrificed by CO_2 asphyxiation on day 20, and were subjected to Caesarean section and subsequent gross necropsy. Dams found dead were immediately necropsied.

The following maternal observations were made: recording of individual intact gravid uterus weight, gross examination of thoracic and abdominal cavities, preservation of tissues with gross lesions in neutral buffered 10% formalin for possible future histology, recording of the number of corpora lutea, the number/placement of implantations and early/late resorptions, and the number of live/dead fetuses.

All fetuses were removed from gravid uteri and subjected to the following observations: recording of individual weight, sex, and gross external abnormalities.

All live fetuses were sacrificed. Fetuses from about one-half of each litter were sectioned by Wilson's technique, and examined for soft tissue alterations. The other half was eviscerated, cleared, stained with alizarin red S, and examined for skeletal abnormalities. Late resorptions were examined if possible.

Historical control data for Crl:CD®,BR rats, obtained from 1985-1986 (49 studies; 1210 control litters), were provided in APPENDIX G of the report, for comparison with concurrent controls.

Statistical Analysis of the Results:

A copy of the report section entitled "Statistical Methods" is attached as Appendix B to this DER.

Compliance:

The following signed Statements were provided: Statement of Confidentiality Claim, dated 07/13/89, Statement of Compliance with EPA GLP's (40 CFR 160), dated 07/13/89, and Quality Assurance Statement, dated 03/22/90.

C. RESULTS:

I. MATERNAL TOXICITY:

Mortality:

No mortality was observed in the study.

Clinical Observations:

A significant ($p \leq 0.01$) increase in the incidence of rales was observed in the "complete" HDT group (17 incidences/1 misdosed and 2 fully dosed HDT dams; 0 incidence in control group). One misdosed and one fully dosed dams were each affected with 4 incidences [day 10-13 (# 880); day 15-16 and 18-19 (# 886)]. The other fully dosed dam (# 889) was affected with 9 incidences (day 12-20). No other significant clinical signs were observed.

Body Weight:

Average body weight was comparable between groups, at all the time points measured (day 0, 6, 7, 8, 9, 12, 14, 15, and 20), irrespective of whether the data was analyzed to include all dams or only the fully dosed or the incompletely dosed animals. On day 16, 5 of the 7 HDT misdosed rats (3 pregnant + 2 non-pregnant) were not weighed by mistake (Table 15, pp 7 of report).

Body Weight Gain:

Mean body weight gain was comparable between groups, either during the pre-dosing (day 0-6), dosing (day 6-9; 9-12; and 12-15), or post-dosing (day 16-20) period, irrespective of whether the data was analyzed to include all dams or only the fully dosed or the incompletely dosed ones. During the post-dosing period, mean body weight gain of the HDT incompletely dosed animals was based on 2 dams only because body weights for 3 of the 5 pregnant animals were inadvertantly not taken according to the report.

A significant overall decrease in body weight gain (14% below control value; $p \leq 0.05$) was observed in the highest dosed group during the period from day 6 to day 15, but only when the misdosed dams were excluded from the group. Mean body weight gains of the excluded misdosed dams subgroup were equal or higher than those of the fully dosed dams HDT subgroup, both during the predosing as well as the common dosing period [day 0-6 (33% higher); day 6-9 (32% higher); day 9-12 (equal); day 12-14 (21% higher]). No other changes in body weight gain were observed.

Group mean corrected body weight gains (Day 20 maternal body weight gain minus gravid uterine weight) were not calculated.

Food Consumption:

A slight but statistically significant short-term decrease in feed consumption was observed in the HDT subgroup of fully dosed dams during the period from day 12-15 (10% decrease below control; $p \leq 0.01$). Marginal but statistically significant decreases were also observed in this group from day 6 to 15 (5% decrease below control; $p \leq 0.01$), and in the complete HDT group (6% decrease below control; $p \leq 0.01$) during the period from day 12-15/16 (see attached copy of Table 6, pps 1-3 of report, Appendix C of this DER). No other changes were observed.

Gross Pathological Observations:

No treatment-related gross pathological lesions were observed.

Hydrometra was observed in a non-pregnant misdosed HDT rat. A fully dosed HDT dam was observed to have a bladder with a thickended wall and containing four calculi. Although the report stated that such gross lesions were common for this strain of rats, the total incidence for "bladder thick wall, w/calculi" reported in APPENDIX G (1985-1986 historical control data obtained with 1210 control litters) was 0. No historical control data was cited for hydrometra total incidence.

Caesarean-Sectioning Observations:

Results as reported are summarized in Table 1. No treatment-related adverse effects were observed on pregnancy rate, average numbers of corpora lutea, implantations, early and late resorptions, and viable number of fetuses/dam. Fetal body weight and percentage of male fetuses/litter were also unaffected by the test material. All of these parameters were similar for all groups even when the dosing error was considered (see Table 1).

Table 1: Summary of Selected Maternal Observations

Observation	Doses (mg/kg/day) 0	1	3	6
Number assigned	25	25	25	25
Number pregnant	24	24	22	22
Corpora lutea per dam	16.2	15.7	16.5	17.2
Implantations per dam	14.3	14.4	15.3	15.?
Fetuses per litter	13.3	13.7	14.3	14.0
Live fetuses per litter	13.3	13.7	14.3	14.0
Resorptions per dam	1.0	0.0	1.0	1.2
Early (n)	1.0 (24)	0.8 (19)	1.0 (21)	1.1 (25)
Late	0.0	0.0	0.0 (1)	0.1 (2)
Completely dosed animals				
Number tested	14	12	13	18
Number pregnant	14	12	11	17
Corpora lutea per dam	16.2	16.8	15.8	17.4
Implantations per dam	14.8	16.2	14.8	15.3
Fetuses per litter	13.8	15.2	13.8	13.9
Live fetuses per litter	13.8	15.2	13.8	13.9
Resorptions per dam	1.0	1.0	1.0	1.4
Early	1.0 (14)	1.0 (12)	0.9 (10)	1.3 (22)
Late	0.0	0.0	0.1 (1)	0.1 (2)
Incompletely-dosed animals				
Number tested	11	13	12	7
Number pregnant	10	12	11	5
Corpora lutea per dam	16.3	14.6	17.3	16.6
Implantations per dam	13.7	12.8	15.7	15.0
Fetuses per litter	12.7	12.2	14.7	14.4
Live fetuses per litter	12.7	12.2	14.7	14.4
Resorptions per dam	1.0	0.6	1.0	0.6
Early	1.0 (10)	0.6 (7)	1.0 (11)	0.6 (3)
Late	0.0	0.0	0.0	0.0

II. DEVELOPMENTAL TOXICITY:

Fetal observations were based on complete groups only and included the following:

Observation	Doses (mg/kg/day) 0	1	3	6
Number assigned	25	25	25	25
Number of litters examined	24	24	22	22
Total fetuses examined				
Externally	320	328	314	308
For soft tissue alterations	154	158	158	146
For skeletal alterations	166	170	163	162

Fetal Gross External Alterations:

A single LDT fetus (# 850-5; litter of a fully dosed dam) was affected with bilateral bulging/depressed eyes, jaw micrognathia, and tongue agenesis.

Fetal Soft Tissue Alterations:

One cleft palate, 1 lung cardiac lobe's agenesis, and 1 moderate unilateral dilation of the kidney pelvis were observed in 3 individual fetuses from 3 control litters.

Hydrocephalus and bilateral microphthalmia were observed in LDT fetus # 850-5, the same fetus shown to be affected with external gross alterations.

One MDT fetus was observed with slight unilateral dilation of the pelvis of kidney.

The author stated that "because these observations were non-dosage-dependent single events, they were not attributed to the test substance.

Skeletal Alterations:

A few skeletal alterations were observed but there was no dose-effect relationship. They included: 1) 7th cervical rib present (1 control and 1 HDT fetuses), 2) thoracic vertebral centra bifid (1 control and 1 HDT fetuses), 3) wavy ribs (1 control fetus), 4) incomplete/non ossification of manubrium (1 control fetus), 5) incomplete/non ossification of ≥1 sternebra (fetuses/litters affected were 9/5 controls, 4/2 LDT, 3/3 MDT, and 4/4 HDT fetuses), 6) asymmetric sternebrae (1 MDT fetus), and 7) incomplete /non-ossification of pelvic pubes (2 control fetuses.

The numb... of fetal ossification sites were comparable between all litter groups. These included ossification of the hyoid, vertebrae (cervical, thoracic, lumbar, sacral, and caudal), rib, sternum (manubrium, sternal centers, and xiphoid), forepaws (carpals, metacarpals, and phalanges) and hindpaws (tarsals, metatarsals, and phalanges) (see attached copy of Table 13 of report).

D. DISCUSSION:

Author's Discussion/Conclusions:

The following conclusions are directly quoted from the Summary and Conclusions section of the report:

> The dosages tested in this definitive developmental toxicity study were selected on the basis of a dosage-range study. In this dosage-range study, MBT was lethal to one of 4 non-pregnant female rats given 9 mg/kg/day dosages, and to one of 8 pregnant female rats given 10 mg/kg/day dosages and 6 of 8 pregnant female rats given 30 mg/kg/day dosages.
>
> In this definitive developmental toxicity study, MBT was given via gavage to female rats on days 6 through 15 of presumed gestation. In the control, low, middle, and high dosage groups, 14, 12, 11, and 17 pregnant rats, respectively, were given the test substance on days 6 through 15 of gestation. Due to an error, 10, 12, 11, and 5 pregnant rats in these same respective dosage groups were given the test substance on only days 6 through 14 of gestation. Dosages of 0 (vehicle), 1, 3, and 6 mg/kg/day were given to the presumed pregnant rats (25/group).
>
> No death occurred during the study. There were 24, 24, 22, and 22 litters of live fetuses examined from the control, low, middle, and high dosage group dams, respectively. The 6 mg/kg/day dosage of MBT was toxic to the dams (adverse clinical signs and inhibited average maternal body weight gain and feed consumption). Administration of 1, 3, or 6 mg/kg/day dosage of the test substance to the dams was not toxic to embryo-fetal development and did not cause external, soft tissue, or skeletal malformations or variations in the fetuses. The maternal no-observable effect level (NOEL) for MBT in pregnant rats was 3 mg/kg/day, and the developmental NOEL was >6 mg/kg/day.

Reviewer's Discussion/Conclusions:

An independent analysis of individual body weight data from the report indicated the following effects on body weight gain during the study (No statistical analyses were conducted on these data.):

Group	Body weight gain (g) during gestation days				
	0 - 6	6 - 9	6 - 15	15 - 20	0 - 20
Control					
Mean	44.79	17.88	61.21	73.50	179.5
S. D.	7.247	5.278	9.264	15.70	24.60
High dose					
Mean	43.27	15.91	56.09	74.36	173.7
S. D.	10.03	4.608	11.43	15.05	27.62

These results showed no statistically significant differences (t test), but they suggested that during the initial period of dosing (gestation days 6 through 9) high dose group dams gained approximately 11% less weight than the control group dams. During the entire dosing period (days 6 through 15 regardless of the number of doses received) body weight gain for high dose group dams was 8% below controls. Although there were some animals in the high dose group that were not weighed on day 16 (the day after dosing was ended), an approximation of the overall post-dosing weight gain (gestation days 15 through 20) for high dose group dams was slightly higher than that of the control group (1% greater than controls).

The above analysis and statistical analyses of maternal body weight, body weight gain, feed consumption, and Caesarean sectioning data from the report were conducted with "complete groups" (those animals getting 9 doses and those getting all 10 scheduled doses together). The following were observed when the data were analyzed in this manner:

1) The only treatment-related clinical sign observed was a minimal increase in the incidence of rales in the high dose group (Only three dams were affected, one given nine doses and two getting all ten doses. Two of these were only affected on four occasions. However, rales and other clinical signs associated with the breathing of treated animals were also noted in the range-finding studies at doses of 5 mg/kg/day and higher [see Appendix A below].);

2) Slight decreases in body weight gain were observed in the high dose group during the dosing period of the study (see the above discussion), and these changes were accompanied by slightly decreased food consumption (see Appendix C below);

3) no mortality was observed in this study;

4) no treatment-related gross pathological alterations were observed and none of the other maternal parameters were affected; and

5) no developmental toxicity was observed.

These points suggest that an adequate dose level was approached in the main study.

One statistically significant toxic effect was reported when the data was separately analyzed with regard to just the fully dosed dams, namely a decrease in body weight gain in the high dose group (14% overall decrease below control level; $p \leq 0.05$; day 6-15). If the decrease in maternal body weight gain observed in just the dams given all 10 doses at the highest dose level is to be used as an index of maternal toxicity, then the fetuses from these dams should also be evaluated separately. This would result in a reduction of the number of litters/fetuses examined for fetal alterations to numbers somewhat lower than those recommended for a developmental toxicity study in rats (at least 20 pregnant rats recommended per group).

Furthermore, there is a discrepancy in the number of dams reported to be fully dosed. Most of the tables in the report indicated 17 animals were fully treated, but Table 20 (Fetal Anomaly Data - Litter incidence) clearly showed the number fully treated to be 11 (dams # 889, 891-900). This discrepancy should be explained before a final conclusion regarding the developmental effects of methylene bis(thiocyanate) in rats can be reached.

Study Deficiencies:

1) The highest dose tested, 6 mg/kg/day caused marginal maternal toxicity (see discussion above).

2) Due to a dosing error only 14 control, 12 low dose, 11 mid dose, and 17/11(?) high dose group dams were treated with all ten scheduled daily doses (day 6-15).

3) There is a discrepancy regarding the number of fully dosed dams in the high dose group.

4) Body weight of 5 of the 7 partially dosed dams in the high dose group were not recorded on day 16 (Table 15; pp 7 of the report). The period day 15-16 is a critical period for body weight gain analysis because it is the first day of the post-dosing period, and body weight rebounds may occur when administration of the test material is terminated.

5) Body weight gain of the animals given only 9 doses in the high dose group was based on too few animals (2 dams) to provide meaningful results for analysis of the 10-dose and 9-dose subgroups separately.

APPENDIX A

Summary of Range-Finding Studies with Methylene bis(thiocyanate)

Range-finding studies:

Materials and Methods:

The methods used for the range-finding study with pregnant rats were similar to those used for the definitive study. The design of the experiment is summarized as follows:

Group Number	Dose (mg/kg/day)*	Number of animals
1	0 (vehicle)	8
2	0.3	8
3	1	8
4	3	8
5	10	8
6	30	8

* Doses were administered on days 6 through 15 of gestation in 0.5% (w/w) aqueous methyl cellulose.

The report noted that in this first range-finding study, no effects were observed at the 3 mg/kg/day dose level, and severe toxicity was seen at the next higher dose level (10 mg/kg/day). According to the report, a second range-finding study with non-pregnant female rats was conducted to determine an appropriate high dose level for the definitive developmental toxicity study. In the second study, groups of 4 animals were given 0 (vehicle), 5, 7 or 9 mg/kg/day by gavage for five consecutive days. (The vehicle control group contained 3 animals.)

Reported Results:

One animal in the 10 mg/kg/day dose group died after six doses were administered. Of the six 30 mg/kg/day dosed animals that died, two were found dead after two doses, two were dead after five doses, one died after the eighth dose, and one was dead after receiving ten consecutive daily doses.

The report described clinical signs attributed to MBT as follows:

> ...The 10 and 30 mg/kg/day dosages resulted in excess salivation, rales, labored breathing, red exudate on the nose, mouth and/or paws, chromorhinorrhea, ataxia, decreased grooming activity and alopecia. The 30 mg/kg/day dosage group also had decreased motor activity, impaired right reflex, urine-stained abdominal fur, bradypnea, ptosis, tremors, emaciation, chromodacryorrhea, and/or a clear nasal discharge. The onset of clinical observations was within 30 minutes of the initial dosage; the severity and incidence of effects increased with continued dosage; and some effects persisted for 24 hours after dosage.

Findings reported at necropsy included dose-related gastrointestinal lesions including discoloration, distention, mucosal sloughing, ulceration, hemorrhage and/or adhesions. Enlarged adrenal glands were also reported to occur at the 30 mg/kg/day dose level.

In the supplemental study with non-pregnant female rats, there was one death in the 9 mg/kg/day dosed group (after two consecutive daily doses). The report noted that this rat had rales and labored breathing after the second dose was given. Other clinical signs in this study included excessive salivation in all three treated groups, labored breathing in the groups given the 7 and 9 mg/kg/day dose levels, decreased motor activity, bradypnea, exophthalmos, and urine-stained abdominal fur in the 7 mg/kg/day dose group. Gross observations at necropsy which were noted in the report were confined to the 9 mg/kg/day dosed group, and they included dried residue associated with excess salivation, chromorhinorrhea, urine-stained abdominal fur, and enlarged adrenal glands.

Maternal and developmental observations in the range-finding study with pregnant animals are summarized in the table on the following page.

Summary of results from a range-finding study with MBT in pregnant rats

Observation	Dose level (mg/kg/day)					
	0	0.3	1	3	10	30
Number assigned	8	8	8	8	8	8
Number died	0	0	0	0	1	6
Number not pregnant	0	0	1	0	0	1
Number of litters examined (Day 20)	8	8	7	8	7	2
Corpora lutea per dam	16.6	16.9	16.3	18.0	16.4	16.0
Implantation sites per dam	14.4	14.9	14.3	16.2	14.0	8.5
Resorptions per dam	1.5	0.9	0.8	1.2	1.4	7.0
Early	1.5	0.9	0.7	1.2	1.4	7.0
Late	0.0	0.0	0.1	0.0	0.0	0.0
Fetuses per dam						
Live	12.9	14.0	13.4	15.0	12.6	1.5
Dead	0.0	0.0	0.0	0.0	0.0	0.0
Maternal feed consumption (g/day)						
Days 6 - 9	23.4	25.2	23.9	23.3	18.3	10.9
Days 6 - 16	24.1	26.4	25.2	24.7	21.1	16.0
Days 0 - 20	24.1	26.2	25.4	24.4	22.8	19.5
Maternal weight change (g)						
Days 6 - 9	15.8	13.8	11.4	14.8	-5.9	-37.0
Days 6 - 16	64.2	67.5	59.7	67.8	39.8	-26.7
Days 0 - 20	162.2	177.6	162.8	166.9	137.4	53.0

APPENDIX B

Description of Statistical Methods

5. Statistical Methods

For all evaluations, the minimum level of statistical significance reported was $P \leq 0.05$.

The following schematic represents the statistical analysis of the data:

Type of Test[a]

I. Parametric[b]

A. Bartlett's[d]

- Significant at $P \leq 0.05$ → Nonparametric
- Not Significant → Analysis of Variance
  - Significant at $P \leq 0.05$ → Dunnett's Test
  - Not Significant

B. Analysis of Covariance

- Significant at $P \leq 0.05$ → Covariance Analyses T-test
- Not Significant

II. Nonparametric[c]

A. Kruskal-Wallis (≤75% ties)

- Significant at $P \leq 0.05$ → Dunn's Test
- Not Significant

B. Fisher's Exact (>75% ties)

III. Proportion Data

Variance Test for Homogeneity of the Binomial Distribution

---

a. All tests evaluated at $P \leq 0.05$ and $P \leq 0.01$.
b. Used only to analyze data with homogeneity of variance.
c. Proportion data were not included in this category.
d. Test for homogeneity of variance.

Adult data were evaluated with the individual rat as the unit measured. Litter values were used in evaluation of Caesarean-sectioning and fetal ossification site data. The incidence of fetal alterations was examined in terms of the litter and fetal percentages.

Maternal clinical sign and necropsy observation data and the incidences of pregnancy and total resorption were analyzed using the Variance Test for Homogeneity of the Binomial Distribution[6].

Maternal body weight, body weight change and feed consumption data were based on surviving pregnant dams and analyzed using Bartlett's Test of Homogeneity of Variances[7] and the Analysis of Variance[8]. If the Analysis of Variance was significant and appropriate, i.e., it passed Bartlett's Test ($P>0.05$), then Dunnett's Test[9] was used to identify the statistical significance of individual groups. If Bartlett's Test was significant ($P\leq 0.05$) and there were less than or equal to 75% ties in a group, then the Kruskal-Wallis Test[10] was used; in cases where statistical significance occurred, Dunn's Method of Multiple Comparisons[11] was used to identify statistical significance of individual groups. If Bartlett's Test was significant ($P\leq 0.05$), and there were greater than 75% ties in a group, then the Fisher's Exact Test was used[12]. These same methods were used for the analyses of fetal body weights, anomaly average data and ossification site data.

The Analysis of Covariance[13] was used to evaluate the average maternal body weight change from day 0 to 6 and 20 of gestation, and from day 6 to days 9, 12, 16, 18 and 20 of gestation.

Data obtained at Caesarean-sectioning of the dams were evaluated using the Kruskal-Wallis Test[10]. In cases where statistical significance occurred ($P\leq 0.05$), Dunn's Method of Multiple Comparisons[11] was used to identify the statistical significance of individual groups.

Proportion data for Caesarean-sectioning and fetal observations were analyzed using the Variance Test for Homogeneity of the Binomial Distribution[6].

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APPENDIX C

Reported Food Consumption Data

PROTOCOL 1419-001: DEVELOPMENTAL TOXICITY (EMBRYO/FETAL TOXICITY AND TERATOGENIC POTENTIAL) STUDY OF METHYLENE BIS(THIOCYANATE) ADMINISTERED ORALLY VIA GAVAGE TO CRL:CD(SD)BR PRESUMED PREGNANT RATS

TABLE 5 (PAGE 1): MATERNAL FEED CONSUMPTION (g/day) - SUMMARY

	DOSAGE GROUP	0(VEHICLE) MG/KG/DAY	1 MG/KG/DAY	3 MG/KG/DAY	5 MG/KG/DAY
RATS - TESTED	N	25	25	25	25
RATS - PREGNANT	N(%)	24(96.0)	24(96.0)	22(88.0	22(88.0
MATERNAL FEED CONSUMPTION (g/day)					
DAYS 0 - 6	MEAN ± S.D.	21.5 ± 1.5	22.8 ± 1.9	21.7 ± 1.8	22.0 ± 2.1
DAYS 6 - 7	MEAN ± S.D.	23.4 ± 4.7	25.0 ± 2.8	23.9 ± 3.4	24.2 ± 4.7
DAYS 7 - 8	MEAN ± S.D.	23.7 ± 2.1	25.1 ± 3.0	24.5 ± 2.4	24.2 ± 2.4
DAYS 8 - 9	MEAN ± S.D.	24.2 ± 3.2	25.4 ± 3.1	24.8 ± 2.8	24.5 ± 2.4
DAYS 6 - 9	MEAN ± S.D.	23.8 ± 2.7	25.2 ± 2.4	24.4 ± 2.3	24.3 ± 2.5
DAYS 9 - 12	MEAN ± S.D.	24.9 ± 2.3	25.9 ± 2.2	25.5 ± 1.8	24.9 ± 2.2
DAYS 12 - 15	MEAN ± S.D.	27.2 ± 2.4	28.1 ± 2.8	27.1 ± 2.5	25.5 ± 2.4
DAYS 15 - 16	MEAN ± S.D.	27.9 ± 3.1	29.9 ± 3.2	28.3 ± 3.2	27.0 ± 3.6
DAYS 12 - 16	MEAN ± S.D.	27.4 ± 2.1	28.5 ± 2.8	27.4 ± 2.3	25.9 ± 2.8
DAYS 15 - 20	MEAN ± S.D.	28.5 ± 2.2	29.3 ± 2.9 [23]	28.5 ± 3.2	28.1 ± 2.1
DAYS 16 - 20	MEAN ± S.D.	28.6 ± 2.3	29.2 ± 3.1 [23]	28.6 ± 3.6	28.4 ± 2.4
DAYS 6 - 9	MEAN ± S.D.	23.8 ± 2.7	25.2 ± 2.4	24.4 ± 2.3	24.3 ± 2.5
DAYS 6 - 12	MEAN ± S.D.	24.3 ± 2.4	25.6 ± 2.2	24.9 ± 1.9	24.6 ± 1.8
DAYS 6 - 15	MEAN ± S.D.	25.3 ± 2.3	26.4 ± 2.3	25.7 ± 2.0	24.9 ± 2.0
DAYS 6 - 16	MEAN ± S.D.	25.6 ± 2.1	26.7 ± 2.3	25.9 ± 2.0	25.1 ± 1.9
DAYS 6 - 20	MEAN ± S.D.	26.4 ± 2.0	27.6 ± 2.4	26.7 ± 2.2	26.0 ± 1.9

This table restricted to pregnant animals.
[ ] = Number of values averaged.
Dosage occurred on days 6-14 and 6-15 of gestation.
DAYS refers to days of gestation.
* Significantly different from the vehicle control group value ($P \leq 0.05$).

GUIDELINE: 83-3

Primary Review by: Nguyen B. Thoa, Ph.D. 10/25/91
Review Section 1, Toxicology Branch I/HED
Secondary Review by: Roger Gardner 10-25-91
Section Head, Review Section 1, Toxicology Branch I/HED

DATA EVALUATION RECORD

Study Type: Teratology - Developmental Toxicity
Species: Rabbits
Guideline: 83-3

EPA Identification No.s: EPA MRID No. 411719-02
EPA ID No. CA 61756
EPA Record No. 248426
EPA Shaughnessy No.
Caswell No. 565
HED Project No.9-1831
Document No.

Test Material: Technical Grade Methylene bis (thiocyanate); 99% ai
Synonyms: MBT

Sponsor: Methylene bis (thiocyanate) Task Force, ICI America Inc., Farmington, Connecticut

Study Number(s): 1419-002

Testing Facility: Argus Res. Labs., Horsham, PA 19004

Title of Report: Developmental Toxicity (Embryo/Fetal Toxicity and Teratogenic Potential) Study of Methylene bis (thiocyanate) Administered Orally (Stomach Tube) to New Zealand White Rabbits.

Author(s): Alan M. Hoberman, Ph.D., DABT.

Report Issued: 06-30-89

Conclusions: The developmental toxicity potential of Methylene bis (thiocyanate) was studied by dosing pregnant Hra:(NZW)SPF rabbits, by stomach tube, with 0, 1, 3.5, and 7 mg/kg/day. The highest dose tested caused 5 deaths (25% of total) and was reduced after the fifth death to 5 mg/kg/day. The calculated average daily dosing for the high dose group's survivors was 5.31-5.77 mg/kg/day.

Five treatment-related deaths were observed in the high dose group following, respectively, 1, 2, 3, 4 and 5 dosings at 7 mg/kg/day. All 5 dead rabbits were pregnant. The conceptuses of the 3 that died after 3-5 dosings appeared normal for their developmental age. The viability state of the in utero implantations of the 2 that died after 1-2 dosings could not be

determined because of the early developmental stage. All 4 high-dose does that died after 2-5 dosings showed severe gastric ulceration and/or gastric mucosal sloughing and hemmorhage at necropsy. No further mortality was observed after the high dose level was reduced.

Clinical signs preceeding the 5 deaths included respiratory difficulty, ataxia, decrease in motor activity, loss of righting reflex, tremors, convulsions, and/or dry feces. After the high dose was reduced, two survivors in the group showed single incidences of tremor (day 15 and day 17) and 8 showed incidences of dry feces lasting 2-4 days, six of those during the period from day 8 to 12.

Body weight gain and feed consumption in the high dose group was significantly ($p < 0.01$) decreased, but only during the period when the high dose was at 7 mg/kg/day (body weight gain decreased on day 6-7, 7-8, and 8-9; feed consumption additionally on day 9-10; respective number of high dose group does given 7 mg/kg/day on day 6-7, 7-8, 8-9, and 9-10 were 5,10,15, and 20).

MBT at 1, 3.5, and 5.31-5.77 mg/kg/day did not affect pregnancy rate, average numbers of corpora lutea, implantations, early and late resorptions, fetuses/litter, viable number of fetuses/litter, fetal body weight, and percentage of male fetuses. These doses did not cause external, soft tissue, or skeletal malformations or variations in the fetuses, and incidences of fetal changes were not statistically significantly increased in any of the treated groups.

The maternal no-observable effect level (NOEL) for MBT in pregnant rabbits was 3.5 mg/kg/day, and the developmental NOEL was >5 mg/kg/day.

Core Classification: Minimum

A. MATERIALS:

Test Compound: Purity: 99% ai
Description: yellow tan powder.
Lot No.: 7-0846M
Contaminant: Not listed
Storage: At room temperature.

Test Animal: Species/Strain: Rabbits/[Hra:(NZW)SPF]
Source: Hazleton Res. Animals, Denver, PA.
Age at receipt: About 5 months
Weight at group assignment: 3.03-4.94 kg
Acclimation Period: 7 weeks

Artificial Insemination:

During the acclimation period, 79 healthy young adult virgin females rabbits were randomly (computer-generated weight ordered process) assigned to 4 dosage groups. On day 0 the rabbits were primed with an iv injection of HCG (pregnyl; 20 USP units/kg; Organon Inc lot No. 1187315). Three hours later they were artificially inseminated with semen (diluted with 0.9% saline; 0.25 ml containing 6 X 10^6 spermatozoa) from untreated proven male rabbit breeders of the same strain and from the same supplier.

STUDY DESIGN:

Groups of artificially inseminated and presumed pregnant rabbits were randomly assigned to the study as follows:

Group	Dose level mg/kg/day [1]	Number of animals
Control	0 (vehicle) [2]	19
Low dose (LDT)	1	20
Mid dose (MDT)	3.5	20
High dose (HDT)	7	20

[1] All doses were administered in a volume of 10 ml vehicle/kg of body weight/day on gestation days 6 through 18. Dosing suspensions were prepared daily and adjusted for individual body weight changes.

[2] Methyl cellulose (lot No. 96F-0470; 0.5% aqueous solution, w/w).

Dosing:

Selection of the doses for the main study was based on the results of 2 range-finding studies (Study No. 1419-002P; dated 11/06/87): a pilot study at dose levels of 0, 0.3, 1, 3, 10, or 30 mg/kg/day administered on gestation days 6-18 with 4 does/group; and a supplementary study with dose levels of 0, 5, or 7 mg/kg/day administered for 7 consecutive days to groups of 3 non-pregnant rabbits.

According to the results of the pilot study with pregnant rabbits, doses of 0.3-3 mg/kg/day did not have any maternal or developmental effects. The 10 mg/kg/day dose caused 3 deaths within 4 days of dosing, and the 30 mg/kg/day dose caused 100% death within 30 minutes of the first dosing. Clinical signs preceeding death included decrease of motor activity, ataxia, loss of righting reflex, tremor, convulsion, and bradypnea. All dead does showed gastric lesions (ulceration, hemorrhage, and/or necrosis) at necropsy. All 4 rabbits treated with 10 mg/kg/day

were pregnant, but the vital status of the conceptuses could not be determined because of the early developmental stage. The surviving doe of this dose group aborted 3 conceptuses on day 20 of gestation (developmental status unknown because of cannibalism) and showed 2 early resorptions at necropsy. Only 2 does treated with 30 mg/kg/day were identified as pregnant (vital status of conceptuses undetermined due to early developmental stage). The two other does had corpora lutea without apparent implantations.

According to the results of the supplementary study, the only effect observed was a decrease in body weight gain (on day 1 at 5 mg/kg/day and on days 1, 2 and 3 with 7 mg/kg/day). The 7 mg/kg/day dose was chosen as the highest dose for the definitive study.

The highest dose in the main study, 7mg/kg/day, caused 5 deaths , so the dose was reduced to 5 mg/kg/day to prevent additional mortality. These reductions in dosage were made according to the following table:

Animal numbers *	Number of doses given		Average daily dose (mg/kg)
	7 mg/kg/day	5 mg/kg/day	
12560-12564[1]	5	8	5.77
12565-12569	4	9	5.62
12570-12574[2]	3	10	5.46
12575-12579[3]	2	11	5.31

[1] Animal numbers 12562 and 12563 died on day 10 of the study.

[2] Animal number 12573 died on study day 7 and 12570 died on study day 9.

[3] Animal number 12578 died on day 6 of the study and animal 1256

All doses were in a volume of 10 ml vehicle/kg of body weight/day. Dosing suspensions were prepared daily (day 6 -18). Dosing was readjusted daily for individual body weight changes.

Environmental Conditions:

The female rabbits were housed individually, (64-70° F; relative humidity = 38-64%; 12 HEPA filtered fresh air changes per hour; 12-hr fluorescent light/12-hr dark cycle). Feed was Purina Certified Rabbit chow #5322 (180g/day/animal). Water (deionized and amended with ≤ 1.20 ppm chlorine as a bacteriostat) was given ad libitum. The investigators stated that "no agent was present in the feed and water, which was known to interfere with the results of the study".

Analyses of Test material Stability, Concentration, and homogeneity in the vehicle:

Aliquots (10 ml) of all dosing suspensions were collected on every dosing day. They were immediately frozen on dry ice, and were shipped to Lancaster Labs. Inc. for analysis of homogeneity, concentration, and stability (HPLC UV method). According to the results of the analyses, the achieved concentrations of the test material in the dosing suspensions averaged respectively 76, 85, and 91% of target concentrations for the LDT, MDT, and HDT suspensions. The homogeneity tests showed that averages RSDs (relative standard deviations) of 1.95 and 2.79% were obtained with the LDT and the HDT suspensions.

Observations:

All experimental animals were checked daily for clinical signs during the prestudy period and from day 0-5. They were checked twice daily for mortality, abnormal behavior, and clinical signs of toxicity from day 6 to day 29. Signs of abortion/premature delivery were checked immediately prior to and 15-45 minutes following each dosing from day 16 to day 18 and daily from day 19 to day 29. Body weight and feed consumption were recorded on day 0 and daily from day 6 to day 29. All surviving does were sacrificed on day 29 by iv pentobarbital sodium and were subjected to Caesarean section and subsequent gross necropsy. Does found dead prematurely were immediately necropsied and their uterine contents were recorded.

The following maternal observations were made: recording of individual intact gravid uterus weight, gross examination of thoracic and abdominal cavities, preservation of tissues with gross lesions in neutral buffered 10% formalin for possible future histology, recording of the number of corpora lutea, the number/placement of implantations and early/late resorptions, and the number of live/dead fetuses.

All fetuses were removed from gravid uteri and subjected to the following observations: recording of individual weight, sex, and gross external abnormalities.

All live fetuses were sacrificed by ip pentobarbital sodium and examined for visceral alterations (cranial, abdominal, thoracic, and peritoneal cavities). The brain was examined in situ following a cross-section at the anterior fontanelle level, between the frontals and parietals. Abnormal fetal tissues were preserved in neutral buffered 10% formalin. All fetuses were cleared, stained with alizarin red S, and examined for skeletal abnormalities. All skeletal preparations were stored in 80% glycerin amended with thymol crystals for retardation of fungal growth. Late resorptions and aborted fetuses were examined to the extent possible (because of autolysis).

Historical Control Data:

Historical control data for [Hra:(NZW)SPF] rabbits, obtained from 1983-1986 [Cesarean data (567 control litters); gross external alterations (570 control litters; 4088 specimen), soft tissue and skeletal alterations (516 control litters; 3738 specimen)], were provided in APPENDIX G of the report for comparison with concurrent controls. Fetal data consisted of average litter and fetal incidences values (No. and %) with no ranges.

Statistical Analysis of the Results:

A copy of the report section entitled "Statistical Methods" is included as Appendix A of this DER.

Compliance:

The following signed Statements were provided: Statement of No Confidentiality Claim (dated 07/13/89), Statement of Compliance with EPA (40 CFR 160) GLP (dated 07/13/89), and Quality Assurance Statement (dated 06/30/89).

C. RESULTS:

The highest dose tested was reduced from 7 to 5 mg/kg/day after 5 deaths occured (see discussion above under "Dosing").

MATERNAL TOXICITY:

Mortality:

The five treatment-related deaths were observed in the high dose group; rabbits 12578, 12573, 12570, 12562, and 12563 succombed after 1, 2, 3, 4 and 5 dosings at 7 mg/kg/day, respectively. Accidental intubation (confirmed at necropsy) caused the death of 1 control rabbit. All dead rabbits were pregnant, and the

conceptuses of does 12562 (9), 12563 (6), and 12570 (9) appeared normal for their developmental ages. The viability of the in utero implantations of doe 12573 (9), 12578 (3), and 12515 (13) could not be determined because of the early developmental stage.

Abortion and Premature Delivery:

One low dose group doe (12531) aborted on day 19 and cannibalized its litter of one conceptus. This doe did not show any clinical signs prior to the abortion or any gross lesion at necropsy. The report stated that "this event was not attributed to the test substance as single incidences of abortion or premature delivery commonly occurs in this size population of rabbits". The historical control (27 studies; 1985-1987) incidence for abortion rate in this strain of rabbits was 2.4% with a range of 0-12.5%.

Clinical Observations:

Clinical signs preceeding the death of the 5 high dose group does include respiratory difficulty, ataxia, decrease in motor activity, loss of righting reflex, tremors, convulsions, and/or dry feces. Two surviving does from the high dose group were affected with tremor (12560 on day 15 and 12576 on day 17). Eight other survivors in the group were affected with dry feces for 2 to 4 days [12561 (day 11-14), 12564 (day 9-10), 12565 (day 21-24), 12566 (day 10-11), 12568 (day 10-12), 12571 and 12572 (day 9-10), and 12577 (day 8-12)]. Other clinical signs were comparable between groups.

Body Weight:

Average body weight was comparable between groups, at all the time points measured (day 0 and daily from day 6 to 19 and day 24 to 29.

Body Weight Gain:

Mean body weight gain was comparable between groups during the predosing period (day 0-6). Overall mean body weight gain for the entire dosing period (day 6-19) was also comparable between groups. A statistically significant body weight loss was observed in the high dose group, but only during the first 3 days of dosing when 15 of the 20 rabbits in the group were dosed from 1 to 3 days with 7 mg/kg/day. Group mean corrected body weight gains (Day 29 maternal body weight gain minus gravid uterine weight) were not calculated. The main changes of body weight in the control and the high dose group are shown in the table below.

	Body weight gain (g) during gestation days					
Group	0-6	6-7	7-8	8-9	10-11	6-19
Control						
Mean	200	-20	-20	20	10	100
S. D.	110	40	40	40	30	110
High dose						
Mean	240	-120**	-100**	-60**	50	-30
S. D.	90	80	50	60	60	200

** Statistically significantly different from controls, $p < 0.01$.

Food Consumption:

Mean feed consumption was comparable between groups during the predosing period (day 0-6). Overall mean feed consumption for the entire dosing period (day 6-19) was also comparable between groups. A statistically significant decrease in feed consumption was observed in the high dose group, but only during the first 4 days of dosing when all 20 rabbits in the group were dosed from 1 to 4 days with 7 mg/kg/day. The main differences in feed consumption between the control and the HDT group are shown in the table below.

	Feed consumption (g/day) during gestation days					
Group	0-6	6-7	7-8	8-9	9-10	6-19
Control						
Mean	163.2	152.7	160.9	154.7	158.0	149.4
S. D.	22.5	31.7	25.0	29.8	29.7	31.1
High dose						
Mean	168.2	77.7**	40.9**	45.5**	72.6**	112.0
S. D.	19.6	66.7	67.2	72.6**	61.1	42.9

** Statistically significantly different from controls, $p < 0.01$.

Gross Pathological Observations:

The 4 high dose group does that died after 2 or more 7 mg/kg/day dosings all showed severe gastric ulceration and/or gastric mucosal sloughing (1 doe) and hemmorhage (1 doe) at necropsy.

All other gross pathological observations were not treatment-related because the incidences were either comparable between groups (paraovarian cysts: 11 control, 8 from the low dose, 13 mid dose, and 6 high dose group animals), were within historical control ranges (minute gastric ulcerations: 1 low dose animal), or were confined to the control group [(enlarged left kidney and atrophied right kidney with a nodule: 1 control), (agenesis of

uterine horn: 1 control), and (accidental lung perforation: 1 control)].

Caesarean-Sectioning Observations:

Caesarean observations are summarized in Table 1. No treatment-related effects were observed on pregnancy rate, mean numbers of corpora lutea, implantations, and early and late resorptions/doe, fetuses/litter, viable number of fetuses/doe, fetal body weight, and percentage of male fetuses/litter.

Table 1: Summary of Selected Maternal Observations

	Doses (mg/kg/day)			
Observation	0	1	3.5	7/5
Number assigned	19	20	20	20
Number pregnant	15	17	19	19
Pregnancy rate (%)	79	85	95	95
Number died/pregnant	1	0	0	5
Number totally resorbed	0	1*	1*	0
Number aborted	0	1	0	0
Number of litters examined	14	15	18	14
Corpora lutea per dam				
Mean	9.8	11.6	11.3	11.4
S. D.	1.8	2.6	1.9	2.0
Implantations per dam				
Mean	6.8	6.9	7.9	8.2
S. D.	2.4	3.8	2.6	2.7
Fetuses per litter				
Mean	6.3	5.8	7.6	6.6
S. D.	2.0	3.6	2.8	3.0
Early resorptions per dam				
Mean	0	0.2	0.2	0.8
S. D.	0	0.4	0.4	1.6
Late resorptions per dam				
Mean	0.6	0.9	0.2	0.7
S. D.	1.0	2.7	0.4	2.4
Number with any resorptions (%)	4 (29)	6 (38)	7 (37)	6 (43)
Fetal weight (g)				
Mean	44.25	43.87	43.19	45.13
S. D.	8.9	7.4	4.1	6.9
Sex ratio (% male)				
Mean	41.7	58.9	44.0	59.4
S. D.	20.5	25.9	16.8	17.1

* Litter had only one conceptus.

II. DEVELOPMENTAL TOXICITY (Tables II-IV):

No developmental toxicity was observed in the study. The report stated, "The differences in the incidences of litters that had fetuses with alterations were neither dosage-dependent nor statistically significant. The percentage of fetuses with alterations and the percentage of altered fetuses per litter were lower in each of the MBT dosage groups than in the control group". The total number of altered fetuses observed were respectively 50 (56.8%), 40 (43.0%), 60 (41.7%), and 35 (37.6%) and the number of litters with altered fetuses were respectively 14 (100%), 14 (93.3%), 18 (100%), and 13 (92.8%) for the control, low, mid, and high dose groups. Individual fetal alterations included the following:

Fetal Gross External Alterations (Table II):

Only 4 fetuses from 3 litters showed gross external malformations; one in the mid dose group (12547-4; scoliosis) and 3 high dose fetuses from 2 litters [(12560-8; umbilical hernia) (12577-4 and 12577-6; short tail). These incidences were not statistically significant ($p > 0.05$).

Three control (1/litter), 1 low dose, and 1 high dose group fetuses showed flexed paws, a gross external variation resulting from in utero compression.

Fetal Soft Tissue Alterations (Table III):

Soft tissue alterations included circumcorneal hemorrhage (1 mid dose group fetus), agenesis of the intermediate lobe of the lung [2 controls (1/litter), 2 low dose fetuses (1/litter), 4 mid dose group fetuses (3 litters), and 6 fetuses in the high dose group (5 litters), ectopic kidney (1 control and 1 high dose fetuses), kidney agenesis (1 mid dose group fetus), and hydronephrosis (1 high dose fetus). The report stated, "No soft tissue alteration in any group demonstrated a significant increase ($p > 0.05$), as compared with the litter and fetal incidences for the control group. Although the incidences of agenesis of the intermediate lobe in the lungs increases in the middle and high dosage groups, this variation was not attributed to the test substance because the increase was not significant ($p > 0.05$), as compared with the concurrent control values, and was within the incidence range observed historically [Fetal incidence (FI)(%) = 9(0.24); Litter (L) I(%) = 6(1.16)]".

Skeletal Alterations (Table IV):

Fetal and/or litter incidences of irregular ossification in the skull were significantly lower ($p < 0.01$) in the low and high dose groups than in the control group. Litter and fetal incidences of other skeletal alterations in the treated groups were not

significantly increased ($p > 0.05$) above those of the concurrent control group. Most non-skull skeletal alterations were observed in 2 high dose littermates, one (fetus 12577-4) of which was also affected with multiple external and soft tissue alterations.

The numbers of ossification sites for the hyoid, vertebrae (cervical, thoracic, lumbar, sacral, and caudal), rib, (manubrium, sternal centers, and xiphoid), forepaws (carpals, metacarpals, and phalanges) or hindpaws (tarsals, metatarsals, and phalanges) were comparable between groups.

D. DISCUSSION:

Author's Discussion/Conclusions:

The following conclusions are directly quoted from the Summary and Conclusions section of the report:

> The dosages tested in this definitive developmental toxicity study were selected on the basis of a dosage-range study (1419-002P) (APPENDIX E) in which the test substance was lethal and caused gastric ulc ration in 3 out of 4 female rabbits given 10 mg/kg/day dosages and lethal to each of 4 female rabbits given 30 mg/kg/day dosages. THe surviving 10 mg/kg/day dosage group doe aborted its litter. Dosage-dependent inhibitory effects on body weight gain and feed consumption occured in does given 5 mg/kg/day dosages and above of the test substance.
>
> The 7 mg/kg/day dosage of MBT (in the definitive study) resulted in maternal toxicity (death, clinical signs, necropsy observations, inhibited average maternal body weight gain and feed consumption. Toxic signs persist in surviving does after the dosage was decreased from 7 mg/kg/day to 5 mg/kg/day. Administration of 3, 3.5, and 7 mg/kg/day dosage of the test substance to the does was not toxic to embryofetal development and did not cause external, soft tissue, or skeletal malformations or variations in the fetuses.
>
> The maternal no-observable effect level (NOEL) for MBT in pregnant rabbits was 3.5 mg/kg/day, and the developmental NOEL was > 5 mg/kg/day.

Reviewer's Discussion/Conclusions:

Based on the incidence of clinical signs, reduced body weight gain and feed consumption, the maternal no-observable effect level (NOEL) for MBT in pregnant rabbits was 3.5 mg/kg/day. The developmental NOEL in this study was >5 mg/kg/day.

The primary deficiency of this study was that the 7 mg/kg/day dosage had to be reduced during the study. The initial dose level was excessively toxic causing 5 deaths (25% mortality) within 5 days. All other significant toxic effects (clinical signs, decreased body weight gains and feed consumption, and pathological observations at necropsy) were also noted during the first 5 days of dosing. Body weight gain and feed consumption of the HDT and

the control groups were comparable as soon as the high dose was reduced to 5 mg/kg/day (day 10-11). The reduced dose level did not appear to cause any toxic developmental or maternal effects. The 2 individual tremor incidences on day 15 and 17 may be residual effects from the dosings with 7 mg/kg level. Six out of the 8 incidences of dry feces occured during the early period of dosing (day 8-12) and did not persist through the period of reduced dosing with the 5 mg/kg level (day 11-18).

The maternal NOEL, 3.5 mg/kg/day was only half of the dose that caused 25% mortality after 1 to 5 dosings. This incidence of mortality is a deviation from acceptance criteria for developmental toxicity studies (guidelines 83-3), but the narrow dose range of MBT within which effects become severe may make the choice of an adequate high dose level difficult. Because of this complication and because other significant acceptance criteria were satisfied (number of pregnant rabbits/group, minimum maternal clinical signs observed, and establishment of maternal and developmental NOELs), the 25% mortality rate does not make the study unacceptable.

APPENDIX A

Statistical Methods Used in Analysis of Results from a Rabbit Developmental Toxicity Study with Methylene bis(Thiocyanate)

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5. Statistical Methods

For all evaluations, the minimum level of statistical significance reported was $P \leq 0.05$.

The following schematic represents the statistical analysis of the data:

Type of Test[a]

I. Parametric[b]

A. Bartlett's[d]

- Significant at $P \leq 0.05$ → Nonparametric
- Not Significant → Analysis of Variance
  - Significant at $P \leq 0.05$ → Dunnett's Test
  - Not Significant

B. Analysis of Covariance

- Significant at $P \leq 0.05$ → Covariance Analyses T-test
- Not Significant

II. Nonparametric[c]

A. Kruskal-Wallis (≤75% ties)

- Significant at $P \leq 0.05$ → Dunn's Test
- Not Significant

B. Fisher's Exact (>75% ties)

III. Proportion Data

Variance Test for Homogeneity of the Binomial Distribution

a. All tests evaluated at $P \leq 0.05$ and $P \leq 0.01$.
b. Used only to analyze data with homogeneity of variance.
c. Proportion data were not included in this category.
d. Test for homogeneity of variance.

Adult data were evaluated with th individual rabbit as the unit measured. Litter values were used in evaluation of Caesarean-sectioning and fetal ossification site data. The incidence of fetal alterations was examined in terms of the litter and fetal percentages.

Maternal clinical sign and necropsy observation data and the incidences of pregnancy, death and total resorption were analyzed using the Variance Test for Homogeneity of the Binomial Distribution[6].

Maternal body weight, body weight change, feed consumption, fetal body weight, anomaly average and ossification data were based on pregnant does and their litters and analyzed using Bartlett's Test of Homogeneity of Variances[7] and the Analysis of Variance[8]. If the Analysis of Variance was significant and appropriate, i.e., it passed Bartlett's Test ($P>0.05$), then Dunnett's Test[9] was used to identify the statistical significance of individual groups. If Bartlett's Test was significant ($P\leq 0.05$) and there were less than or equal to 75% ties in a group, then the Kruskal-Wallis Test[10] was used; in cases where statistical significance occurred, Dunn's Method of Multiple Comparisons[11] was used to identify statistical significance of individual groups. If Bartlett's Test was significant ($P\leq 0.05$), and there were greater than 75% ties in a group, then Fisher's Exact Test[12] was used.

Data obtained at Caesarean-sectioning of the does were evaluated using the Kruskal-Wallis Test[10]; in cases where statistical significance occurred ($P\leq 0.05$), Dunn's Method of Multiple Comparisons[11] was used to identify the statistical significance of individual groups.

Proportion data for Caesarean-sectioning and fetal observations were analyzed using the Variance Test for Homogeneity of the Binomial Distribution[6].

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