RB-1143
TR-2284

UNITED STATES ENVIRONMENTAL PROTECTION AGENCY

002284

DATE: February 28, 1979

SUBJECT: S-Ethyl hexahydro-1H-azepine-1-carbothioate (ORDRAM) - supplemental toxicology data. EPA Reg. No. 476-2107, Accession No. 236576, Caswell#444

FROM: Krystyna K. Locke
Toxicology Branch, HED (TS-769) Krystyna K. Locke 4-25-79

TO: W. Garner
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## SUMMARY OF DATA

On 11-11-77, Mr. R.B. Jaeger (HED, Tox. Branch) reviewed four studies on Ordram and raised many questions with regard to these studies (see letter from Mr. Jacoby to Stauffer Chemical Company, dated 12-12-77). In the current submission, "Ordram - Supplemental Toxicology Data" (dated 12-1-78; Acc. No. 236576), Stauffer provided a point-by-point response to the 12-12-77 correspondence. As a part of this response, the following material was also submitted:

(1) Data on the reproductive effects of Ordram in the male rats (Attachment I).
(2) Reproductive performance and rat litter data (Attachment II).
(3) Revised data tables for the life-long mouse study (Attachment III).

Additional histopathology on certain organs was also conducted by the Experimental Pathology Laboratories and a final report, expected in Jan., 1979, will be submitted by Stauffer to EPA.

Most of Mr. Jaeger's questions were answered satisfactorily in the current submission. However, deficiencies still exist in the data found in Attachments I and III. These deficiencies are specified below.

## DEFICIENCIES

Attachment I. Preliminary studies of the reproductive effect.

(1) Strain, age and weight of the rats used in all of these studies are unknown.

(2) The number of male rats used in the study concerning the effect of the dose level on the number of litters born is not given. It is also not stated for how long after mating the Ordram feeding was continued.

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(3) In the study concerning the change of dose levels, from the higher to the lower levels, it is not clear whether this study was a continuation of one mentioned under (2) above, or whether it was a separate study.

(4) No mention is made when (month, year) these experiments were conducted, or what was the Project Number, if any.

Attachment III. Revised data tables.

Although a correct count of animals in each group was submitted (p. 5), these values do not always agree with those found in Attachment III. These discrepancies are described below.

(1) Control group, male mice (Table on p. A-140).

There were 20 animals in this group, numbered chronologically from 1561 through 1580. Since only 15 mice were necropsied, there should obviously be 5 missing numbers (mice that were not necropsied) from this table. However, there are only 14 (not 15) entries within the 1561-1580 sequence and 6 numbers (not 5) are missing from the table on p. A-140. Since 15 animals were necropsied (statement on p. 5 of this submission), another animal was apparently added to this group, one numbered 1596. Yet, according to Attachment III (p. A-146), mouse #1596 should have been a control female.

(2) Males, 3.6 mg dose level, $F_1$ generation. (Table on p. A-145).

According to the "correct" count (p. 5 of this submission), there were 36 animals in this group. However, 37 animals are listed in this table.

(3) Female mice, 14.4 mg dose level, $F_1$ generation. (Table on p. A-152).

According to the "correct" count, there were 24 animals in this group. However, 31 mice are listed in this table. The same number of animals (that is, 31) was listed in the previous submission (Mr. Jaeger's review, 11-11-77).

(4) Female mice, 7.2 mg dose level, $F_1$ generation. (Table on p. A-153).

According to the "correct" count, there were 24 animals in this group. However, considering the numbers (2252-2276) listed in the table on p. A-153, as well as the numbers missing from that sequence, at least 26 mice should have been in this group.

(5) Female mice, 3.6 mg dose level, $F_1$ generation. (Table on p. A-154).

According to the "correct" count, there were 24 animals in this group. However, considering the numbers (2351-2377) listed in the table on p. A-154, as well as the numbers missing from that sequence, at least 27 mice should have been in this group.

In general, the ambiguities and discrepancies detailed in this section concern the following:

A. The total number of animals in the groups (1) through (5).

B. The listing of a nonsequentially numbered animal in group (1).

INFORMATION REQUESTED

With regard to Attachment I.

Answers to comments (1) through (4), listed under DEFICIENCIES, are needed.

With regard to Attachment III.

Considering the ambiguities and the discrepancies detailed under DEFICIENCIES, (1) through (5), explanations and/or corrections are needed for the following:

(1) Listing of animal No. 1596 (apparently a female mouse) in the table on p. A-140 (male mice).

Absence of 6 (rather than 5) sequential numbers, representing non-necropsied mice, from the table on p. A-140.

(2) Total number of animals in this group (table on p. A-145).

How many animals were initially assigned to this group, 36 or 37? If 36 is correct (p. 5 of this submission, entry VIII M), then why 37 animals are still listed in this (presumably corrected) table?

(3) Total number of animals in this group (p. A-152).

How many animals were initially assigned to this group, 24 or 31? If 24 is correct (p. 5, entry VI F), where do the extra mice come from?

(4) Total number of animals in this group (p. A-153).

How many animals were initially assigned to this group, 24 or 26? According to p. 5, entry VII F, the correct number is 24. According to this table, there were 26 mice in this group.

(5) Total number of animals in this group (table on p. A-154).

How many animals were initially assigned to this group, 24 or 27? According to p.5, entry VIII F, the correct number is 24. According to this table, there were 27 mice in this group.

As far as Attachment III is concerned, the discrepancies and the ambiguities with regard to the histopathological findings still remain unexplained. These discrepancies and ambiguities were first noted in Mr. Jaeger's review, dated 11-11-77. It is hoped that a new report from the Experimental Pathology Laboratories, to be submitted by Stauffer to EPA, will provide the needed explanations.

## INTRODUCTION

On 11-11-77, Mr. R.B. Jaeger (HED, Tox. Branch) reviewed 4 studies on Ordram and the results were communicated to Stauffer Chemical Co. (Richmond, Ca.) on 12-12-77. These studies and their validation categories appear below.

(1) Suppression of fertility in male rats; Ordram technical; final report. Core-Supplementary Data

(2) Three-generation reproduction study in rats. Core-Supplementary Data.

(3) Ordram-safety evaluation by repeated oral administration to rats for 104 weeks. Core-Minimum Data.

(4) Ordram - repeated oral administration to mice for lifetime. Core-Invalid.

It should be noted that studies 1 and 2 were classified as "supplementary data", whereas study 4 was rejected.

On 1-18-78, two representatives from Stauffer met with Mr. Jaeger in order to discuss Mr. Jaeger's comments with regard to these 4 studies. According to Mr. W.R. Hillebrecht (letter to EPA, 12-1-78), "many of Mr. Jaeger's questions were answered" during that meeting. Stauffer also "agreed to provide a summary of the preliminary reproductive effect studies and certain other additional data to answer the questions raised in the 12-12-77 correspondence".

The current submission contains point-by-point answers to the 12-12-77 correspondence and the data listed below.

(1) Ordram - summary of Woodard Res. Corporation's preliminary studies of reproductive effect.

(2) Ordram three-generation rat reproduction study; reproductive performance and litter data.

(3) Ordram lifetime mouse feeding study; revised data tables.

(4) Reprints of 2 published studies, concerned with the metabolism of [ring - $^{14}C$] Ordram in the rat.

EVALUATION OF THE CURRENTLY SUBMITTED SUPPLEMENTARY DATA

I. Summary of Woodard Res. Corp. preliminary studies of the reproductive effect. (Attachment I).

These data were submitted in reference to the Litton Bionetics' study, "Suppression of Fertility in Male Rats", LBI Project No. 2621, dated 10-29-76. Acc. No. 231329.

Experimental

A. Groups of rats of (of unknown strain, age, weight) were fed diets providing 0, 8, 16 or 32 mg of Ordram/kg body weight. The animals were bred after 6 weeks of feeding. Fertility was evaluated by the number of litters born at each dose level. There were apparently 25 female rats per level, but it is unknown how many males were used in this study. It is also unknown for how long the Ordram feeding was continued after mating.

B. To determine whether Ordram affected male or female reproductive performance, treated male rats were bred with control females and vice versa. The doses used were 8, 16 and 32 mg of Ordram/kg body weight/day, and 8 pairs of rats per level were used.

C. Rats were fed Ordram at dose levels of 0, 8, 16 or 32 mg/kg body weight/day for 18 weeks, at which time the levels were decreased to 0, 0.63, 2.0 and 6.3 mg/kg/day, respectively. Three weeks later the animals were bred. Twenty five pairs of rats/dose level were used. The age and strain of animals is unknown. It is also not clear whether or not this study was a continuation of one described above (A).

Results

A. The number of litters decreased with an incerase in the level of Ordram fed. At dose levels of 0, 8, 16 and 32 mg/kg of body weight, 22, 4, 3 and 0 litters were born, respectively.

B. Ordram was affecting the reproductive performance of the male rats only. When treated male rats were bred with untreated females, only 2 litters were born at the 8 mg/kg level and no litters were born at the 16 mg/kg level and no litters were born at the 16 mg and 32 mg/kg levels. When treated female rats were bred with untreated males, the litter rate was 100% at all dose level used.

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C. Poor reproductive performance could be improved by lowering the level of Ordram fed to the rats. When the levels of Ordram were decreased from 0, 8, 16 and 32 mg/kg of body weight to 0, 0.63, 2.0 and 6.3 mg/kg, the number of litters born was 19, 12, 9 and 3, respectively.

COMMENTS

This summary is classified as SUPPLEMENTARY DATA for the following reasons:

(1) Omission of several experimental details, as indicated under A, B and C (Experimental).

(2) No mention is made when (year, month) these experiments were conducted or what was the Project No., if any.

Mr. Jaeger classified the study, "Suppression of Fertility in Male Rats", as Supplementary Data "because the requirements for a Dominant Lethal Study were not met" (11-11-77). Stauffer replied that the above-mentioned study was not intended as a Dominant Lethal Study, but "rather as a probe study to further evaluate the mechanism of reduced reproductive performance observed in preliminary studies" (12-1-78). The currently - submitted summary of these preliminary studies clearly illustrates the effect of Ordram on the reproductive performance of the male rats. Therefore, when the missing experimental details are supplied for this summary, the validation category will be changed from "Supplementary Data" to "Core-Minimum Data".

II. Reproductive performance and litter data. (Attachment II).

These data were submitted in reference to the Woodard Research Corporation's study, "Three-generation reproduction study in rats", dated 6-3-77. (No Project No.). Acc, No. 231331.

Mr. Jaeger raised several questions with regard to the study mentioned above, including teratological evaluation, and validated that study as "Supplementary Data" (11-11-77). These questions were satisfactorily answered, point-by-point, in the communication from Stauffer to Mr. Jacoby (cc to Mr. Jaeger), p. 2-4 of this submission, and in Attachment II.

Attachement II (1½ -page table) contains the following data:

1. Number of litters with live pups.
2. Gestation index.
3. Mean live litter size.
4. Mean pup weight.
5. Pregnancy rate.

For day 1 and generations $F_{1a}$, $F_{1b}$, $F_{2a}$, $F_{2b}$, $F_{3a}$ and $F_{3b}$.

1. Number of litters with live pups.
2. Mean litter size.
3. Mean pup weight.

For day 5 and generations $F_{1b}$, $F_{2b}$ and $F_{3b}$.

1. Number of litters with live pups.
2. Mean litter size.
3. Mean pup weight.

For day 21 and generations $F_{1a}$, $F_{2b}$ and $F_{3b}$.

| Percent survival for: | Days | Generations |
|---|---|---|
| | 1-5 | F1b, F2b, F3b |
| | 5-21 | F1a, F2b, F3b |
| | 1-21 | |

Data validation category: should be changed from "Supplementary Data" to CORE-MINIMUM DATA ("Three-generation reproduction study in rats").

III. Revised data tables (Attachment III).

These data were submitted in reference to the Woodard Research Corporation's study, "Ordram - Repeated oral administration to mice for lifetime", dated 6-3-77. (No Project No.). Acc. No. 231328. Mr. Jaeger considered that study "Invalid" because of numerous discrepancies in the reporting of the data (11-11-77). There were apparently discrepancies in the number of animals listed in various tables, organ weights, histopathological findings and organ-to-body weight ratios. In the current submission, Stauffer attempts to explain and/or correct these discrepancies, as indicated below.

1. A new (or "correct") count of animals, initially and at necrospy, is submitted (p.5).

2. No corrections are made with regard to the organ weight discrepancies. Stauffer insists that the reported data agree with the raw data. However, certain very high organ weights are explained in terms of the newly obtained pathological findings, such as lung carcinoma or distended uteri.

3. Additional histopathology on certain organs was conducted by the Experimental Pathology Laboratories. A final report, expected in January, 1979, will be submitted to EPA.

(4) Although nothing is said with regard to the ratios of organs-to body weight, examination of these data indicates that only the animals surviving the entire experimental period were used in obtaining these ratios.

Although a "correct" count of animals in each group was submitted (p.5), these values do not always agree with those found in Attachment III. These discrepancies are described below.

1. Control group, male mice (Table on p. A-140).

   There were 20 animals in this group, numbered chronologically from 1561 through 1580. Since only 15 mice were necropsied, there should obviously be 5 missing numbers (mice that were not necropsied) from this table. However, there are only 14 (not 15) entries within the 1561-1580 sequence and 6 numbers (not 5) are missing from the table on p. A-140. Since 15 animals were necropsied (statement on p.5 of this submission), another animal was apparently added to this group, one numbered 1596. Yet, according to Attachment III (p. A-146), mouse #1596 should have been a control female.

An explanation is, therefore, needed for the following:

1. Presence of animal #1596 in this group (control males).

2. Absence of 6 (rather than 5) sequential numbers (representing non-necropsied mice) from the table on p. A-140.

2. Males, 3.6 mg dose level, $F_1$ generation. (Table on p. A-145).

   According to the "correct" count (p.5 of this submission), there were 36 animals in this group. However, 37 animals are listed in this table. Explanation and/or correction is, therefore, required.

3. Female mice, 14.4 mg dose level, $F_1$ generation. (Table on p. A-152).

   According to the "correct" count, there were 24 animals in this group. However, 31 mice are listed in this table. The same number of animals (that is, 31) was listed in the previous submission (Mr. Jaeger's review, 11-11-77). Explanation and/or correction is, therefore, required.

4. Female mice, 7.2 mg dose level, $F_1$ generation. (Table on p. A-153).

   According to the "correct" count, there were 24 animals in this group. However, considering the numbers (2252-2276) listed in the table on p. A-153, as well as the numbers missing from that sequence, at least 26 mice should have been in this group. Explanation and/or correction is, therefore, required.

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(5) Female mice, 3.6 mg dose level, $F_1$ generation. (Table on p. A-154).

According to the "correct" count, there were 24 animals in this group. However, considering the numbers (2351-2377) listed in the table on p. A-154, as well as the numbers missing from that sequence, at least 27 mice should have been in this group. Explanation and/or correction is, therefore, required.

This study, "Ordram - Repeated oral administration to mice for lifetime", is still considered INVALID for the following reasons:

1. Discrepancies occur in the number of mice listed in various "revised data tables" (Attachment III).

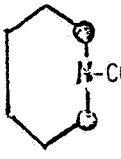
2. Discrepancies with regard to the histopathological findings, first noted in Mr. Jaeger's review (11-11-77), still remain unexplained. However, a new report from the Experimental Pathology Laboratories, to be submitted to EPA in 1979, will apparently clarify these discrepancies.

IV. Metabolism of [ring - $^{14}C$] Ordram (Molinate) in the Rat. 1. Balance and Tissue Residue Study. Jack R. DeBaun, Diane L. Bova, Kay A. Finley, and Julius J. Menn.*, J. Agric. Food Chem. 26:1096-1098, 1978.

*Biochemistry Dept., Stauffer Chemical Co., Mountain View Research Center, California. This study constitutes Attachment II of this submission.

Experimental procedures

1. Compound used:



N-CO-S-$C_2H_5$ Ordram

Position of $^{14}C$ label: ⊛

F.I.S.

2. Radiopurity: 98.7%, according to TLC in 2,2,4-trimethylpentane: p-dioxane, 2:1; 97.9%, according to TLC in benzene: ether, 7:3.

3. Dosing solutions: $^{14}C$-Ordram and technical Ordram (99.1% AI) were dissolved at a ratio of 1:26.9 in 1,2-propanediol (28.8 mg/ml).

4. Treatment of animals. Balance Study.

Two female (184g and 194g body weight) and two male (196g and 203g body weight) Simonsen rats (Wistar-derived) were fasted for 24 hrs prior to administration of 0.5 ml of the $^{14}C$-Ordram dosing solution by oral gavage.

This dose ( ~ 72 mg/kg; ~ 50 X $10^6$ dpm) is approximately one-tenth of the oral $LD_{50}$ value. After dosing, the rats were placed in metabolic cages for 72 hrs. Ground Purina Chow and water were available ad libitum for the duration of the study.

Urine samples were collected at 8, 24, 32, 48, 56 and 72 hrs after dosing, and were radio-assayed directly, in duplicate. Samples of feces were collected at 24, 48 and 72 hrs after dosing, and were radioassayed in triplicate, after combustion. Sodium hydroxide air traps were samples at 4, 8, 24, 32, 48 and 72 hrs. The cage washes were assayed at 24, 48 and 72 hrs after dosing.

Tissue residue study.

Six female and six male Simonsen rats (200g) were dosed by oral gavage with $^{14}C$-Ordram, as described above. After dosing, the rats were placed in metabolic cages and provided with food and water ad libitum. Two male and two female rats were sacrificed 1, 3 and 7 days after dosing. The following organs and tissues were assayed: esophagus, stomach, small intestine (proximal and distal), cecum, liver, kidney, lungs, heart, spleen, gonads, muscle (gastrocnemius), bone (femur), brain, fat (abdominal), hide, carcass, and blood.

In order to determine the relationship between dose and residual $^{14}C$ in blood, three male rats were given $^{14}C$-Ordram orally at doses of 5, 20, and 80 mg/kg of body weight. Each dose contained 32.4 X $10^6$ dpm. The blood, removed by heart puncture, was radioassayed 7 days after dosing.

Results: balance study.

The distribution of $^{14}C$ in excreta, after oral administration of Ordram, is shown in the table below.

| Sample | Interval Hrs. | % of administered dose Average | Cumulative Av. |
|---|---|---|---|
| Urine | 24 | 38.8 | 67.5 |
| | 48 | 8.2 | 79.7 |
| | 72 | 1.3 | 82.1 |
| Feces | 24 | 8.4 | 8.4 |
| | 72 | 0.2 | 10.6 |
| Expired $^{14}CO_2$ (air traps) | 24 | 0.3 | 0.7 |
| | 72 | 0.0 | 0.9 |
| Cage washes | 24 | 5.2 | 5.2 |
| | 72 | 0.1 | 5.8 |

Recovery of activity: 99.2%

Most of the radioactivity was rapidly excreted in urine. There was no significant sex difference with regard to rates and routes of excretion.

Results: tissue residue study.

The distribution of $^{14}C$ in various tissues is shown in the table below.

| Tissue | Ordram equivalents, ppm 1 Day* | 7 Days* |
|---|---|---|
| Esophagus | 9.4 | 2.7 |
| Stomach | 7.2 | 1.2 |
| Small intest. (prox.) | 26.5 | 2.1 |
| Small intest. (distal) | 9.3 | 1.4 |
| Cecum | 13.0 | 0.6 |
| Liver | 31.6 | 4.9 |
| Kidney | 21.5 | 3.8 |
| Lungs | 17.2 | 9.5 |
| Heart | 10.3 | 3.8 |
| Spleen | 15.5 | 6.0 |
| Gonads | 8.3 | 1.6 |
| Muscle | 4.4 | 0.9 |
| Bone | 4.1 | 0.8 |
| Brain | 5.1 | 1.7 |
| Fat | 5.9 | 0.8 |
| Hide | 5.3 | 3.6 |
| Carcass | 6.1 | 1.4 |
| Blood | 35.4 | 28.3 |

*Days after dosing. These are average values for male and female rats.

With the exception of blood, residues associated with most tisses substantially decreased over the 7-day period after dosing. The disappearance of Ordram from the blood-rich tisses, such as liver, kidney, lungs, heart and spleen, was also slow. No sex differences were observed in the tissue distribution of Ordram.

The amount of $^{14}C$ present in blood 7 days after treatment was dose-related.

Study validation category: CORE-MINIMUM DATA

V. Metabolism of [ring - $^{14}C$] Ordram (Molinate) in the Rat. 2. Urinary Metabolite Identification. Jack R. DeBaun, Diane L. Bova, Chien K. Tseng, and Julius J. Menn., J. Agric. Food Chem. 26:1098-1104, 1978.

This study constitutes Attachment V of this submission.

## Experimental procedures

In this study, rat urine collected in the "Balance and Residue Study" amendment IV) was used. The methanolic extracts of the 0-48 hr urine were used for determining the distribution of metabolites in male and female rats. For the isolation of metabolites, the combined ether extracts of the 0-24-hr and 0-48-hr urine were used. The metabolites were identified by combinations of the following procedures: column chromatography, TLC (15 solvent systems), enzymatic hydrolysis (β-glucuronidase), mass spectra and nuclear magnetic resonance spectra.

## Results

Ordram was readily degraded by the rat to more polar products which were excreted primarily in the urine. Unchanged Ordram accounted for only 0.1% of the urinary $^{14}C$ after an oral dose (72 mg/kg) of [ring - $^{14}C$] Ordram. A metabolic pathway, proposed by the authors, appears below.

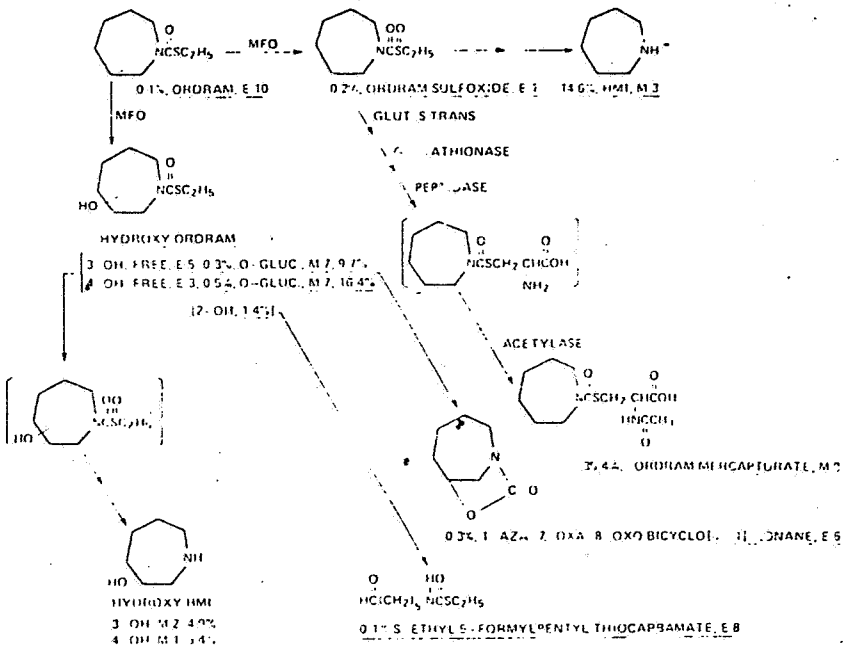



Figure 13. Proposed metabolism of [*ring*-$^{14}C$]Ordram in the rat. Percentages are average values for female and male 0-48 h urine after oral dosing with 72 mg/kg. Expressed as percent urinary $^{14}C$.

The major metabolites were as follows:

| Metabolite | % Urinary $^{14}C$ |
|---|---|
| 1. Mercapturic acid derivative | 35.4 |
| 2. 3-and 4-Hydroxy Ordram derivatives | |
| Free | 0.8 |
| O-glucuronides | 21.6 |
| 3. Hexamethyleneimine | 14.6 |
| 4. 3- and 4-Hydroxyhexamethyleneimine | 10.3 |

Although there were small quantitative differences, the metabolism of [ring-$^{14}C$] Ordram in female and male rats was qualitatively the same.

Study validation category: CORE-MINIMUM DATA