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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: Molinate: Review of Fourteen Fertility Studies (Primates, Rabbits, and Rats)

TO: Ernestine Dobbins
Product Manager (52)
Generic Chemical Support Branch/
SRRD (H7508C)

FROM: Linda L. Taylor, Ph.D Linda Lee Taylor 12/17/92
Toxicology Branch II, Section II,
Health Effects Division (H7509C)

THRU: K. Clark Swentzel K. Clark Swentzel 1/6/93
Section II Head, Toxicology Branch II
Health Effects Division (H7509C)

and

Marcia van Gemert, Ph.D. M. van Gemert 1/13/93
Chief, Toxicology Branch II/HFAS/HED (H7509C)

Registrant:	ICI Americas Inc.
Chemical:	Molinate; S-ethyl hexahydro-1H-azepine-1-carbothioate
Synonym:	Ordram
Submission No.:	S422018
Caswell No.:	444
Schaughnessy #:	041402
DP Barcode.:	D180732
MRID No.:	MRID #'s 423613-01 through 423613-14
Action Requested:	None specified.

Comment: The Registrant has submitted supplemental toxicology data from investigations into the potential effects of Molinate on fertility in primates, rabbits, and rats, as well as four dermal absorption studies. None of these studies is a data requirement for reregistration under FIFRA. These data have been reviewed and, where applicable, a DER has been prepared and is attached. For those studies that were reported as summaries only (no raw data), a discussion is provided below.

1. MRID # 423613-01: Molinate: Overview of Primate, Rat and Rabbit Fertility Studies for Submission to the U.S. EPA. GA Wickramaratne,

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dated 6/11/92. The overview briefly cites (a) the original studies (early 1980's) that demonstrated (1) an adverse effect of Molinate on rat male reproduction and (2) the lack of an effect in primates and rabbits and (b) more recent studies, which the Registrant contends support their hypothesis that the fertility effects observed in rats are rodent-specific. No comment required.

2. MRID # 423613-02: Final Report: Molinate: Evaluation of Sperm Morphology in the Cynomolgus Monkey. U Zuhlke, dated 1/7/91, Report # CTL/C/2550 (DER appended). Under the conditions of the study, administration of Molinate to male monkeys at dose levels of 0.2, 10.0, and 50.0 mg/kg/ day for a total of 12 weeks resulted in reduced erythrocyte cholinesterase levels at the mid dose (10.0 mg/kg/day) in week 11 of treatment, and reduced erythrocyte and plasma cholinesterase levels at the high dose (50.0 mg/kg/day) in weeks 4 and 11 of treatment. Additionally, there was a slight ($p < 0.05$ not attained) increase in the level of brain cholinesterase activity in the high dose monkeys at sacrifice. No effects were observed on ejaculate weight, sperm count, or sperm morphology. Based on the limited parameters measured, no definitive statement regarding the potential of Molinate to affect fertility in male monkeys can be made. The NOEL for the parameters measured can be set at 0.2 mg/kg/ day, the LEL at 10.0 mg/kg/day, based on decreased erythrocyte cholinesterase activity levels. This study is classified Acceptable; it does not satisfy any guideline requirement, nor was it intended to.

3. MRID # 423613-03: Molinate: A Study to Assess Differences in Body-Burden Following a Range of Acute Inhalation Exposures. PM Hext, RW Lewis, KO Rodgers, dated 1/17/92, Report # CTL/L/4361. **Comment:** This is a summary report of an inhalation study designed to compare body burden (dose) of Molinate with the method of exposure (whole body vs nose-only) and the physical state of the test atmosphere. The dose was estimated by quantification of the major urinary metabolite of molinate (4-hydroxy molinate) and assuming that this represented 16% of the total dose (based on previous metabolism studies). The authors concluded that non-inhalation routes (oral and dermal) contribute significantly to the total dose received during inhalation exposure. From this, it was concluded that the dose derived from the 4-week inhalation "reproductive" toxicity study should be 3.53 mg/kg/day instead of 0.07 mg/kg/day. No DER has been prepared of this study since no raw data with respect to body weight, clinical signs, etc. were provided. This study does not satisfy any guideline requirement, nor was it intended to.

4. MRID #423613-04: Molinate: Fertility Study in Male Rabbits. DJ Tinston, dated 1/18/91, Report # CTL/P/3225 (DER appended). Under the conditions of the study, oral administration of Molinate at a dose level of 200 mg/kg resulted in deaths and, consequently, the assessment of fertility at this dose level was precluded. The limited data available for the mid-dose (100 mg/kg) male rabbit

suggest a reduction in fertility, which is associated with an increased incidence of sperm abnormalities. Additionally, there was an increase pre-implantation loss and a decrease in the number of live fetuses at this dose level at week 4. Although there were no differences noted at the low-dose (10 mg/kg) level compared to the control group, the duration of the study was only 8 weeks, with fertility being assessed only during week 4 of dosing. This study is classified Unacceptable; it cannot be upgraded, and it does not satisfy any guideline requirement.

5. MRID # 423613-05: Molinate: Second Fertility Study in Male Rabbits. DJ Tinston, dated 6/6/91, Report # CTL/P/3328 (DER appended). **Comment:** TB II agrees with the author that no definitive statement can be made with regard to the effects of Molinate on male rabbit fertility due to deaths and poor pregnancy performance. Under the conditions of the study, administration of Molinate to male rabbits at dose levels of 10, 100, and 200 mg/kg/day for a total of 12 weeks resulted in death at the mid- and high-dose levels and decreased body-weight gains at the high-dose level. Females inseminated by semen from these males at weeks -1, 4, 8, and 12 displayed poor pregnancy rates during the first weeks of the study, including the pre-dose period. Week 12 data suggest an increase in pre-implantation loss and fewer fetuses at the high-dose level compared to the controls. Although the number of females available at this dose level is too few for adequate assessment, TB II notes that increased pre-implantation loss and decreased number of fetuses are effects noted in the rat fertility studies on Molinate. The author stated that the design criteria for this study were not attained. This study is classified unacceptable (cannot be upgraded), and it does not satisfy any guideline requirement.

6. MRID # 423613-06: Molinate: Second Preliminary Study in Male Rabbits. DJ Tinston, dated 5/31/91, Report # CTL/T/2747. No DER was prepared of this study since only summary data were provided in the report. This study was performed to clarify discrepancies between two studies (MRID #'s 423613-07 and MRID # 423613-04) with respect to mortality. No treatment-related deaths were observed following administration of Molinate at dose levels of 40, 100, and 250 mg/kg/day for 28 days to male rabbits in this second preliminary study, which was consistent with the results of the first preliminary study (MRID # 423613-07). Therefore, the dose levels suggested for the second fertility study (MRID # 423613-05) were 10, 100, and 200 mg/kg [the same doses used in the first (MRID # 423613-04) fertility study].

7. MRID # 423613-07: Molinate: Preliminary Study in Male Rabbits. DJ Tinston, dated 5/31/91, Report # CTL/T/2745. This study was performed to determine dose level for a fertility study (MRID # 423613-04) in male rabbits. Only summary data were provided in this report; therefore, no DER was prepared. Dose levels of 100, 200, and 300 mg/kg were administered for 28 consecutive days to male rabbits. Evidence of toxicity (deaths and weight loss) was observed

at the 300 mg/kg dose level. Inhibition of erythrocyte cholinesterase activity was observed at all dose levels (dose-related), but no other effects were observed at the 100 and 200 mg/kg dose levels. The dose levels chosen for the fertility study (MRID # 423613-04) were 10, 100, and 200 mg/kg, based on these findings.

8. MRID # 423613-08: Molinate: Mechanistic Study in the Pregnant Rat. JM Horner, dated 3/3/92, Report # CTL/T/2769 (DER appended). Under the conditions of the study, oral administration of Molinate at dose levels of 0, 75, 135, or 200 mg/kg/day for three days to pregnant rats resulted in decreased survival, neurological effects, and increased adrenal weights at the mid- and high-dose levels, and decreased body weight/gain, an increase in the neutral lipid content of cells in the adrenal cortex, and microscopic lesions in the adrenal cortex and ovary at all three dose levels. There was no evidence of an effect on the ability to maintain a pregnancy. No no-effect level was demonstrated, nor was there discussion as to a possible cause of the deaths observed in this study at dose levels that had not been shown previously to result in deaths in this strain/sex of rat.

This study is a mechanistic study, and it does not satisfy any guideline requirement. It is classified unacceptable, since individual data were not provided.

9. MRID # 423613-09: Molinate: Overview of Three Studies in Male Rabbits Conducted at ICI Central Toxicology Laboratory. PH Rose, dated 9/28/92, Report # PHR331/SCI/LC. **Comment:** This overview compares the results reported in MRID #'s 423613-04, -06, and -07

Comparison of Results of Three Studies

Study	423613-07			423613-04			423613-06		
Dose mg/kg	100	200	300	10	100	200	40	100	250
deaths	0	0	4/5	0	4/10	6/10	0	1/5	0
body weight	-	-	↓	↓	↓	↓	-	-	-
RBC chol. act.	↓	↓	↓	-	↓	↓	-	↓	↓
Duration	28 days			12 weeks			28 days		

The Registrant concluded that more reliance should be placed on the two preliminary studies; consequently, dose levels of 10, 100, and 200 mg/kg were chosen for the repeat (Second) fertility study (MRID # 423613-05).

10. MRID # 423613-10: Molinate: In Vitro Percutaneous Absorption Through Sprague Dawley Rat Epidermis. HM Clowes, RC Scott, dated 8/2/91, Report # CTL/P/3256. Only summary data were provided. The report was reviewed (memo from R.P. Zendzian to L. Taylor, dated 8/17/92, copy appended), and the study is classified unacceptable.

11. MRID # 423613-11: Percutaneous Absorption of Ordram-^{14}C in Male Rats Under Occluded and Unoccluded Conditions. PA Holmes, dated 1/15/90, Report # T-10364. Only summary data were provided. The report was reviewed (memo from R.P. Zendzian to L. Taylor, dated 8/17/92, cited under # 10 above), and the study is classified unacceptable.

12. MRID # 423613-12: Molinate: In Vitro Absorption From Technical Grade Material Through Human and Rat Epidermis. RJ Ward, dated 8/7/90, Report # CTL/P/3070. Only summary data were provided. The report was reviewed (memo from R.P. Zendzian to L. Taylor, dated 8/17/92, cited above), and the study is classified unacceptable.

13. MRID # 423613-13: Molinate: IN Vitro Absorption From 1 100g kg^{-1} Granule Formulation Through Human and Rat Epidermis. RJ Ward, Report # CTL/P/3028. Only summary data were provided. The report was reviewed (memo from R.P. Zendzian to L. Taylor, dated 8/17/92, cited above), and the study is classified unacceptable.

14. MRID # 423613-14: Final Interim Report: Molinate: Evaluation of Sperm Morphology in the Cynomolgus Monkey. U. Zuhlke, dated 9/26/90, Report # CTL/C/2511. **Comment:** This report (73 pages) is of the first 4 weeks of the 12-week study that is reported in MRID #423613-02; therefore, no TB II review of this interim report was performed.

CONCLUSION

The Registrant has submitted these data to inform the Agency of their efforts "to investigate the potential effects of molinate in primate, rat and rabbit fertility studies." It is the Registrant's contention that the effects on reproduction associated with Molinate exposure are specific to rodents. TB II concludes that these data are not definitive. The studies in the nonrodent have been limited; i.e., few parameters have been assessed, only males were treated in the rabbit and monkey studies, and the studies on the monkey only looked at effects on sperm. Additionally, the data on the rabbit suggest a possible effect (increased pre-implantation loss, fewer fetuses).

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

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OFFICE OF
PESTICIDES AND TOXIC SUBSTANCES

MEMORANDUM

August 17, 1992

SUBJECT: Molinate, In Vivo and In Vitro Dermal Absorption Studies

TO: Linda Taylor, Ph.D
Pharmacologist
Rev Sec II, Tox Br II, HED

8/17/92

FROM: Robert P. Zendzian Ph.D.
Senior Pharmacologist
Health Effects Division (H7509C)

Compound; Molinate Registrant; ICI

MRID #423613-10, 11, 12 & 13

Action Requested

Reveiw the following four studies;

Molinate: In vitro percutaneous absorption through Sprague Dawley rat epidermis, H.M. Clowes & R.C. Scott, ICI Central Toxicology Laboratory, UK; Report No : CTL/P/3256, Study No: JV1370; Aug 2, 1991; MRID 423613-10

Percutaneous absorption of Ordam ^{14}C in male rats under occluded and unoccluded conditions; P.A. Holmes, ICI Americas Inc, Report Number T-10364, Jan 15, 1990; MRID 423613-11

Molinate: In vitro percutaneous absorption from technical grade material through human and rat epidermis, R.J. Ward & R.C. Scott, ICI Central Toxicology Laboratory, UK; Report No : CTL/P/3070, Study No: JV1353; Aug 7, 1990; MRID 423613-12

Molinate: In vitro percutaneous absorption from a 100 g kg^{-1} formulation through human and rat epidermis, R.J. Ward & R.C. Scott, ICI Central Toxicology Laboratory, UK; Report No : CTL/P/3028, Study No: JV1354; Sept 20, 1990; MRID 423613-12

Conclusions

The reports are incomplete, lacking individual animal/ sample data and are unacceptable. The studies have significant technical deficiencies that render them invalid.

Discussion

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The reports are of three in vitro studies from ICI Central Toxicology Laboratory,

UK; and one in vivo study from ICI America Inc. laboratories. All four reports are deficient in that they present only summary data as tables or graphs. Individual animal and/or sample data are not presented. Agency policy requires that all data generated in a study be presented. This allows the Agency scientist to present, evaluate or manipulate the data in various manners to develop secondary relationships within and between studies. For example in all four studies a measure of 'absorption' is plotted against time by computer graphics. Not only is the 'absorption' measure plotted on the wrong type of scale but the computer graphics, while pretty, are of no value in determining kinetic parameters and relationships from the derived line. In this type of kinetic study the 'absorption' data must be plotted as the log usually by utilizing a log scale (converting the data to log values is permissable but it complicates kinetic analysis and increases the risk of error by adding unnecessary mathmatics). The various processes followed contain or are first order processes such that log presentation will allow fitting of straight lines from which kinetic parameters may be obtained directly.

Also, the individual data are required so that laboratory audit may determine if the records of the study are accurately presented in the report of the study.

Technical problems with the studies are presented briefly, and only to the extent that they are considered sufficient to invalidate the studies. Other technical problems were noted and more may be expected to appear with presentation of the individual data.

Data available to the Agency have shown that in vitro penetration studies do not provide accurate absolute values for in vivo penetration. Also, usable comparative data are very scarce so that we lack sufficient data to predict the direction and size of the error. In vitro studies do appear to have value in comparing the penetration from different formulations through the same skin and comparing the penetration of the same formulation through different 'skins'. The in vitro studies reported have a fundimental error which make the comparative data highly suspect.

In the in vitro studies epidermal membranes were prepared from skin samples, fresh from rats and postmortum from human females, by standard procedures and stored frozen. Membrane integrity was determined by mounting the membrane in the diffusion cell, filling the receptor chamber with physiological saline (0.9% NaCl), placing tirtiated water in saline in the donor chamber and determining the permeability coefficient. Epidermal samples having coefficients of $\geq 2.5 \times 10^{-3}$ cm hr^{-1} for the human membrane were $\geq 1.5 \times 10^{-3}$ cm hr^{-1} for the rat membrane and considered damaged and rejected. For the experiment 50% v/v ethanol:distilled water was utilized in the receptor chamber. The integrity of the epidermal membrane was not tested against the receptor fluid used in the experiment. The comparative data generated indicate that "The results obtained in the study indicated that absorption through rat epidermis overestimated absorption through human epidermis by as much as 60 times," (JV1353). Numerous studies, in vitro and in vivo have shown that rat skin is more permiable than human skin but the conclusion of this experimental set is at least an order of magnitude larger than reported (about five times). It is suggested that the 50% ethanol solution altered the state of the epidermal membranes so that hair folicule openings in the membrane 'opened' to some extent and/or became filled with the alcholic receptor fluid in which the molinate is more soluble than water. The more numerous 'openings' in the rat

membrane would have significantly increased the rate of penetration.

The in vivo rat study contains several obvious errors of design that invalidate the data collected. A well designed preliminary study was performed to investigate, in vitro, evaporation from the application site. Two substrates were used, a glass plate and a section of shaved exised skin placed on a sheet of aluminum foil. The substrate was placed in a chamber, a measured amount of ^{14}C test material spread on and the chamber sealed. Air passed through the chamber and through 2-stage XAD columns which trapped the test material. The columns were sampled at 3, 6, 12, 24, 48 and 72 hours. There was one error in the design which can be expected to produce a large under estimate of evaporation. Since evaporation is highly temperature dependent, the substrates must be heated to approximate the temperature of the rat's back. The substrates were not heated in this study.

The in vivo portions of this study are poorly described but appear to be in three parts, a study comparing neat and mthanol diluted test material, an occluded study with three doses of test material diluted in methanol and a nonoccluded study with one dose of test material diluted in methanol. In general the studies did not in any manner model field expcsure conditions. Obvious errors in the studies were as follows;

Dosing as mg/kg rather than mg/cm^2.

Dilution of test material in methanol,rather than the field solvent.

Occlusive material in direct contact with the application site.

Application site washed with methanol which dose not occur in the field.

In the unoccluded study no provision was made to distinguish between evaporated material and expired label.

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Tox Chem No. 041402 Molinate 444 File Last Updated _____ Current Date 7/20/92

STUDY/LAB/STUDY #/DATE	MATERIAL	EPA MRID NO.	RESULTS: LD50, LC50, PIS, NOEL, LEL	TOX CATEGORY	CORE GRADE/DOC. #
Subchronic - monkey RX0513; # CTL/C/2550; HAZLETON Labs. Ger. GmbH; 1/7/91	Molinate (99%) w/w	423613-02	Under the conditions of the study, administration of Molinate to male monkeys at dose levels of 0.2, 10.0, and 50.0 mg/kg/ day for a total of 12 weeks resulted in reduced erythrocyte cholinesterase levels at the mid dose (10.0 mg/kg/day) in week 11 of treatment, and reduced erythrocyte and plasma cholinesterase levels at the high dose (50.0 mg/kg/day) in weeks 4 and 11 of treatment. Additionally, there was a slight ($p < 0.05$ not attained) increase in the level of brain cholinesterase activity in the high dose monkeys at sacrifice. No effects were observed on ejaculate weight, sperm count, or sperm morphology. Based on the limited parameters measured, no definitive statement regarding the potential of Molinate to affect fertility in male monkeys can be made. The NOEL for the parameters measured can be set at 0.2 mg/kg/ day, the LEL at 10.0 mg/kg/day, based on decreased erythrocyte cholinesterase activity levels. This study does not satisfy any guideline requirement, nor was it intended to.		ACCEPTABLE
Acute inhalation - rat HR2112; # CTL/L/4361; ICI Central Tox. Lab., UK; 1/17/92	Molinate (97.6% w/w)	423613-03	This is an inhalation study designed to compare body burden (dose) of Molinate with the method of exposure (whole body vs nose-only) and the physical state of the test atmosphere. The dose was estimated by quantification of the major urinary metabolite of molinate (4-hydroxy molinate) and assuming that this represented 16% of the total dose (based on previous metabolism studies). The authors concluded that non-inhalation routes (oral and dermal) contribute significantly to the total dose received during inhalation exposure. From this, it was concluded that the dose derived from the 4-week inhalation "reproductive" toxicity study should be 3.53 mg/kg/day instead of 0.07 mg/kg/day. No DER has been prepared of this study since no raw data with respect to body weight, clinical signs, etc. were provided. This study does not satisfy any guideline requirement, nor was it intended to.	009972	Unacceptable
Subchronic - rabbit RB0521; # CTL/P/3225; ICI Central Tox. Lab., Cheshire, UK; 1/18/91		423613-04	Under the conditions of the study, oral administration of Molinate at a dose level of 200 mg/kg resulted in deaths and, consequently, the assessment of fertility at this dose level was precluded. The limited data available for the mid-dose (100 mg/kg) male rabbit suggest a reduction in fertility, which is associated with an increased incidence of sperm abnormalities. Additionally, there was an increase pre-implantation loss and a decrease in the number of live fetuses at this dose level at week 4. Although there were no differences noted at the low-dose (10 mg/kg) level compared to the control group, the duration of the study was only 8 weeks, with fertility being assessed only during week 4 of dosing. This study does not satisfy any guideline requirement. It cannot be upgraded.		Unacceptable

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Subchronic - rabbit RB0533; # CTL/P/3328; ICI Central Tox. Lab., UK; 6/6/91	Molinate (99%) w/w	423613-05	No definitive statement can be made with regard to the effects of Molinate on male rabbit fertility due to deaths and poor pregnancy performance. Under the conditions of the study, administration of Molinate to male rabbits at dose levels of 10, 100, and 200 mg/kg/day for a total of 12 weeks resulted in death at the mid- and high-dose levels and decreased body-weight gains at the high-dose level. Females inseminated by semen from these males at weeks -1, 4, 8, and 12 displayed poor pregnancy rates during the first weeks of the study, including the pre-dose period. Week 12 data suggest an increase in pre-implantation loss and fewer fetuses at the high-dose level compared to the controls. Although the number of females available at this dose level is too few for adequate assessment, TB II notes that increased pre-implantation loss and decreased number of fetuses are effects noted in the rat fertility studies on Molinate. The author stated that the design criteria for this study were not attained. This study cannot be upgraded, and it does not satisfy any guideline requirement.		Unacceptable
Dermal absorption - rat JV1370; ICI Central Tox. Lab., UK; 8/2/91	Molinate (99%) radiolabeled	423613-10	Since only summary data were provided, no DER was prepared. memo dated 8/17/92 Rendzian To Taylor		unacceptable
Dermal absorption - rats Report # T-10364; ICI Americas, Inc.; 1/15/90	^{14}C-Ordram	423613-11	Since only summary data were provided no DER was prepared. review memo dated 8/17/92		unacceptable
Dermal absorption-rat/human epidermis; JV1353; ICI Central Tox. Lab., UK; 8/7/90	Molinate radiolabeled	423613-12	since only summary data were provided, no DER was prepared review memo dated 8/17/92		unacceptable
Dermal absorption-rat/human epidermis; JV 1354; ICI Central Tox. Lab., UK; 9/20/90	Molinate radiolabeled	423613-13	since only summary data were provided, no DER was prepared review memo dated 8/17/92		unacceptable
Mechanistic-pregnant rat Report # CTL/T/2769 ICI Central Tox. Lab., UK; 3/3/92	Molinate (98.1%)	423613-08	Under the conditions of the study, oral administration of Molinate at dose levels of 0, 75, 135, or 200 mg/kg/day for three days to pregnant rats resulted in decreased survival, neurological effects, and increased adrenal weights at the mid- and high-dose levels, and decreased body weight/gain, an increase in the neutral lipid content of cells in the adrenal cortex, and microscopic lesions in the adrenal cortex and ovary at all three dose levels. There was no evidence of an effect on the ability to maintain a pregnancy. No no-effect level was demonstrated, nor was there discussion as to a possible cause of the deaths observed in this study at dose levels that had not been shown previously to result in deaths in this strain/sex of rat. No individual data were provided. This study is a mechanistic study, and it does not satisfy any guideline requirement.		Unacceptable

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Reviewed by: Linda L. Taylor, Ph.D. Linda Lee Taylor 12/17/92
Section II, Tox. Branch II (H7509C)
Secondary Reviewer: K. Clark Swentzel K. Clark Swentzel 1/6/93
Section II Head, Tox. Branch II (H7509C)

DATA EVALUATION REPORT

STUDY TYPE: subchronic-sperm morphology-monkey TOX. CHEM. NO.: 444

MRID NO.: 423613-02 PC Code: 041402

TEST MATERIAL: Molinate

SYNONYMS: Ordram; S-ethyl hexahydro-1H-azepine-1-carbothioate

STUDY NUMBER: RX0513; HLD Report # 956-088-095; CTL/C/2550

SPONSOR: ICI Americas Inc.

TESTING FACILITY: HAZLETON Laboratories Deutschland GmbH

TITLE OF REPORT: Evaluation of Sperm Morphology in the Cynomolgus Monkey

AUTHORS: U. Zühlke and W. Bee

REPORT ISSUED: January 7, 1991

QUALITY ASSURANCE: A quality assurance statement was provided.

CONCLUSIONS: Under the conditions of the study, administration of Molinate to male monkeys at dose levels of 0.2, 10.0, and 50.0 mg/kg/ day for a total of 12 weeks resulted in reduced erythrocyte cholinesterase levels at the mid dose (10.0 mg/kg/day) in week 11 of treatment, and reduced erythrocyte and plasma cholinesterase levels at the high dose (50.0 mg/kg/day) in weeks 4 and 11 of treatment. Additionally, there was a slight ($p < 0.05$ not attained) increase in the level of brain cholinesterase activity in the high dose monkeys at sacrifice. No effects were observed on ejaculate weight, sperm count, or sperm morphology. Based on the limited parameters measured, no definitive statement regarding the potential of Molinate to affect fertility in male monkeys can be made. The NOEL for the parameters measured can be set at 0.2 mg/kg/ day, the LEL at 10.0 mg/kg/day, based on decreased erythrocyte cholinesterase activity levels.

Classification: Acceptable. This study does not satisfy any guideline requirement.

A. MATERIALS

1. Test Compound: Molinate; Description: brown liquid; Batch #. BJB 2605; CTL reference # Y06367/009; Purity: 99% (w/w) Source: ICI Agrochemicals, S.C.B. Seneffe, Belgium.

Control/Vehicle: Corn oil.

2. Test Animals: Species: monkey; Strain: Cynomolgus (Macaca fascicularis); Age: not provided, wild, caught; Weight: ♂♂ 4.3-7.3; Source: Roberto C. Hartelust B.V., P.O. Box 21 70, 5001 CD Tilburg, The Netherlands; one monkey was from Shamrock Farms (Great Britain) Ltd., Victoria House, Small Dole, Henfield, Sussex BN5, England.

3. Statistics: Statistical evaluations used are described under 3.10 of the final report (copy appended).

B. STUDY DESIGN

1. Methodology: Groups of 10 male monkeys were administered (via gavage; 4 mL/kg/day from start of dosing until day 43 when 1 mL/kg/day was administered) Molinate at dose levels of 0, 0.2, 10.0, or 50.0 mg/kg/day for 12 weeks. Control males were administered an appropriate volume of corn oil. The monkeys were housed individually in stainless steel cages. Each monkey was offered ≈ 50-70 grams of Ssniff P 10 pellets once a day, and fresh fruit (≈ twice a week) and one slice of bread (once a week) were provided also. For conditioning purposes, each monkey received a tasty pellet (2 grams) of marmoset diet (Ssniff Marmoset Alleindiät) as a reward immediately after dosing. Water (tap) was available ad libitum. Upon arrival, the monkeys were quarantined for 6 weeks, during which time they were examined for ill health and suitability for testing. Additionally, the examination included laparoscopy, and antihelminthic therapy and tuberculin testing (on arrival and ≈ 2 and 6 weeks after arrival) were performed. Prior to the start of dosing, the monkeys were acclimated to the laboratory for a minimum of 50 days. The monkeys were allocated to the four groups by means of a stratified body weight procedure using a set of random permutations of the letters a,b,c,d, representing the four test groups. Prior to dosing (twice), and after 4, 8, and 12 weeks of treatment, a sample of semen from each monkey was collected for assessments of sperm morphology and number.

Dose preparation: It was stated that the amount of test material calculated and weighed was put into a measuring cylinder and made up to the final volume with corn oil. No other details were provided. Dosing solutions were prepared at ≈ 14-day intervals and stored at room temperature (assumed since stability studies were on formulations stored this way).

Samples of each test formulation and vehicle were analyzed once prior to the start of dosing for determination of homogeneity, 7- and 14-day stability assessments, and during weeks 3, 5, 8, and 10 to verify the concentration.

RESULTS

Molinate in corn oil was found to be stable for 14 days at room temperature. The dosing formulations were found to be within 7% of nominal concentrations. No data were found in the report regarding homogeneity of the formulations.

3. Clinical Observations: Each monkey was observed daily (twice) throughout the study for morbidity and mortality and at least once daily for appearance, behavior, feces, and general condition. Body weight was recorded once predose, weekly throughout the dosing period, and at sacrifice. Individual food consumption was estimated daily throughout the dosing period and was evaluated on a weekly basis.

RESULTS

Survival and Clinical Observations

There were 3 deaths during the study (1 each in the control, low-, and high-dose groups), which were not attributed to the test material. Soft feces and diarrhea were observed in most of the monkeys throughout the first 6 weeks of dosing. When the dosing volume was lowered to 1 mL/kg (day 43), the incidences were reduced. None of the findings are considered to be related to Molinate administration.

Body Weight and Food Consumption

The high-dose monkeys displayed a slight decrease in body weight compared to the controls, but statistical significance was not attained and the magnitude was small (≥ 94% of the control value). The overall weight gain was 0.1 kg in the control and low-dose groups, -1 kg at the mid dose, and no gain at the high dose. No assessment of body-weight gains was provided. Food consumption was reduced in all groups during the first weeks of treatment, compared to week 1 values, which the authors attributed to the vehicle and not to Molinate. An increase in food consumption was observed in all test groups following the lowering of the dosing volume on day 43 of the study. It is concluded that neither body weight nor food consumption was affected by Molinate administration.

4. Blood Analysis and Urinalysis: Erythrocyte and plasma cholinesterase activities were determined in blood samples from all monkeys (≈6 hours after dosing) twice predose before ejaculation and in weeks 4 and 11 of treatment (method of

obtaining blood not described). Brain cholinesterase activity was determined at scheduled sacrifice (one piece of the frontal part of the brain was deep frozen and used for the analysis). Urine (16-hour) was collected once predose and in weeks 4 and 11 of treatment. Total volume was measured.

Cholinesterase Activities: At week 4, there was a statistically significant decrease in plasma and erythrocyte cholinesterase activities at the high-dose level. At week 11, both the mid- and high-dose values were lower than control, but the mid-dose value was lower than the high-dose value. Brain cholinesterase activity was increased (193 % of control) at the high-dose level, but statistical significance was not attained.

Erythrocyte Cholinesterase Activity [U/L]

Dose/Week	0 mg/kg	0.2 mg/kg	10.0 mg/kg	50.0 mg/kg
Plasma				
-2	4906	5053	3967	4896
-1	6544	5843	5966	5536
4	5942	6926	5677	1867* (31%)
11	6262	6887	4613 (74%)♦	2176* (35%)
Erythrocyte				
-2	2688	2290	2602	2825
-1	3335	3230	3441	3663
4	3034	3009	3128	2586* 85%)
11	3450	3478	2083* (60%)	2323* (67%)
Brain	1.5	1.4	1.5	2.9 (193%)

♦ (% of control); * $p<0.05$

Urine Volume: No analysis of the volume data was provided, nor was any discussion. Appendix 7 gives the individual values (Table on page 144 lists the low-dose as 0.3 mg/kg/day). There was wide variation among the monkeys within the various groups, but in general, comparable ranges in volume were observed among the groups for each time point.

5. Semen Collection: A sample of semen from each monkey was collected twice prior to the start of dosing and in weeks 4, 8, and 12 of treatment for assessment of sperm morphology and count. The weight of each ejaculate was recorded and the coagulum was digested with a trypsin solution. Sperm number was determined in both the exudate and coagulum using a hematocytometer. It was stated that calculation of daily sperm production from representative testicular tissue collected at necropsy was not performed because the consultant for sperm analysis considered this to be redundant and to provide no further information in view of the clear results obtained by the other investigation methods. Sperm morphology was evaluated by light and scanning electron microscopy: Light - morphology assessed from Papanicolaou-stained exudate smears

(WHO guidelines, WHO, 1980); **scanning electron** - samples fixed in glutaraldehyde, separated by filtration, dehydrated, and prepared for critical point drying by "Frigen" treatment. After gold spattering, samples were examined on electron microscopes. Morphological assessments were made against the following criteria: total structure, head shape, middle piece shape, tail shape.

RESULTS

There were no differences noted in ejaculate weight, sperm counts, or sperm morphology (light and scanning electron microscopy).

Sperm Parameters (mean values)

Dose/Parameter/Week	0 mg/kg	0.2 mg/kg	10.0 mg/kg	50.0 mg/kg
Ejaculate Weight (g)				
Week -2	0.34	0.29	0.16	0.11
Week -1	0.32	0.53	0.49	0.28
Week 4	0.33	0.60	0.41	0.32
Week 8	0.37	0.66	0.26	0.37
Week 12	0.49	0.67	0.28	0.29
Sperm Counts ($x10^6$/mL)				
Week -2	80.6/60.7♦	126.1/52.8	113.4/50.0	278.7/67.3
Week -1	54.1/48.9	132.7/80.7	586.0/307.6♦	141.7/83.1
Week 4	86.4/72.3	268.5/103.9	155.9/138.1	144.2/72.1
Week 8	169.4/38.7	141.2/74.7	99.5/54.3	136.0/84.6
Week 12	70.2/83.1	130.0/115.2	116.4/63.7	117.3/81.3
Normally Formed Sperm (%)				
Week -2	78.4	66.4	61.9	65.7
Week -1	71.2	74.6	59.2	56.8
Week 4	68.1	70.6	58.1	56.5
Week 8	72.0	77.7	64.6	60.2
Week 12	76.0	67.4	57.4	59.7
Rolled Tails (%)				
Week -2	10.4	13.6	15.7	15.0
Week -1	16.5	10.1	17.9	22.8
Week 4	17.2	17.5	23.3	27.6
Week 8	13.4	6.9	21.4	15.0
Week 12	15.4	17.4	27.5	23.0
Bent Tails (%)				
Week -2	10.3	19.6	22.3	18.8
Week -1	8.5	13.0	21.8	19.1
Week 4	12.4	8.5	17.4	14.0
Week 8	11.9	14.1	12.8	23.0
Week 12	6.8	14.9	12.7	15.1
Broken Tails				
Week -2	0.4	0.3	0.1	0.2
Week -1	1.9	0.9	0.8	0.9
Week 4	0.9	1.6	0.2	1.1
Week 8	2.2	1.2	0.9	1.6
Week 12	0.9	0.4	2.2	2.2
Thickened Tails				
Week -2	0.5	0.1	0.0	0.3
Week -1	1.9	1.4	0.3	0.4
Week 4	1.4	1.8	1.0	0.8
Week 8	0.3	0.1	0.3	0.2
Week 12	0.1	0.0	0.2	0.0

♦ mean/median

6. Sacrifice and Pathology

At the scheduled sacrifice, each monkey was given a full external and internal examination, and all lesions were recorded. The testes, prostate, seminal vesicles, and epididymides were weighed (paired organs separately) before fixation. The seminal vesicles and prostate were preserved for histopathological examination in neutral buffered formalin, and the testes and epididymides were preserved in Bouin's fluid. These tissues were examined by light microscopy.

RESULTS

Gross Pathology: None of the gross lesions observed could be attributed to treatment.

Organ Weights: Both the absolute and relative weights of the testes, epididymis, and prostate were comparable among the groups. The weights (absolute and relative) of the seminal vesicles were lower in the mid- and high-dose monkeys compared to the control values, but the decreases were not dose-related and statistical significance was not attained.

Histopathology: There were no differences noted among the groups with respect to the microscopic lesions observed.

C. DISCUSSION

There were no treatment-related effects noted on any of the sperm parameters evaluated (ejaculate weight, sperm count, light and scanning electron microscopical sperm morphology), and no lesions or sex organ weight changes were observed. The only effect of Molinate administration was a decrease in erythrocyte (mid- and high-dose) and plasma (high-dose) cholinesterase activity levels. No effects were observed at the low dose (0.2 mg/kg/day).

D. CONCLUSION

Under the conditions of the study, administration of Molinate to male monkeys at dose levels of 0.2, 10.0, and 50.0 mg/kg/day for a total of 12 weeks resulted in reduced erythrocyte cholinesterase levels at the mid dose (10.0 mg/kg/day) in week 11 of treatment, and reduced erythrocyte and plasma cholinesterase levels at the high dose (50.0 mg/kg/day) in weeks 4 and 11 of treatment. Additionally, there was a slight ($p < 0.05$ not attained) increase in the level of brain cholinesterase activity in the high dose monkeys at sacrifice. No effects were observed on ejaculate weight, sperm count, or sperm morphology. Based on the limited parameters measured, no definitive statement regarding the potential of Molinate to affect fertility in male monkeys can be made.

This study is classified acceptable; it does not satisfy any guideline requirement.

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Page _____ is not included in this copy.

Pages 17 through 18 are not included in this copy.

The material not included contains the following type of information:
Identity of product inert ingredients.

_____ Identity of product inert impurities.

_____ Description of the product manufacturing process.

_____ Description of quality control procedures.

_____ Identity of the source of product ingredients.

_____ Sales or other commercial/financial information.

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The information not included is generally considered confidential by product registrants. If you have any questions, please contact the individual who prepared the response to your request.

Reviewed by: Linda L. Taylor, Ph.D. Linda Lee Taylor 12/14/92
Section II, Tox. Branch II (H7509C)
Secondary Reviewer: K. Clark Swentzel K. Clark Swentzel 12/15/92
Section II Head, Tox. Branch II (H7509C)

009972

DATA EVALUATION REPORT

STUDY TYPE: fertility study- male rabbit TOX. CHEM. NO.: 444

MRID NO.: 423613-05 PC Code: 041402

TEST MATERIAL: Molinate

SYNONYMS: Ordram; S-ethyl hexahydro-1H-azepine-1-carbothioate

STUDY NUMBER: RB0533; Report # CTL/P/3328

SPONSOR: ICI Americas Inc.

TESTING FACILITY: ICI Central Toxicology Laboratory, Cheshire, UK

TITLE OF REPORT: Molinate: Second Fertility Study in Male Rabbits

AUTHORS: DJ Tinston

REPORT ISSUED: June 6, 1991

QUALITY ASSURANCE: A quality assurance statement was provided.

CONCLUSIONS: TB II agrees with the author that no definitive statement can be made with regard to the effects of Molinate on male rabbit fertility based on the results of this study. Deaths and poor pregnancy performance make interpretation difficult. The dose levels chosen for this study had demonstrated conflicting results previously, with some studies indicating no effects (deaths) at 200 mg/kg and others resulting in deaths at 100 and 200 mg/kg. The author stated that the design criteria for this study were not attained. Under the conditions of the study, administration of Molinate to male rabbits at dose levels of 10, 100, and 200 mg/kg/day for at total of 12 weeks resulted in death at the mid- and high-dose levels and decreased body-weight gains at the high-dose level. Females inseminated by semen from these males at weeks -1, 4, 8, and 12 displayed poor pregnancy rates during the first weeks of the study, including the pre-dose period. Week 12 data suggest an increase in pre-implantation loss and fewer fetuses at the high-dose level compared to the controls. Although the number of females available at this dose level is too few for adequate assessment, TB II notes that increased pre-implantation loss and decreased number of fetuses are effects noted in the rat fertility studies on Molinate.

Classification: Unacceptable (cannot be upgraded). This study does not satisfy any guideline requirement.

A. MATERIALS

1. Test Compound: Molinate; Description: amber liquid; Batch #: CTL reference # Y06367/009, Certificate of Analysis # was illegible; Purity: 99% (w/w); Source: ICI Agrochemicals, Seneffe, Belgium.
Control/Vehicle: Corn oil; Batch #: CTL reference # Y00790/004
Source: Kraft Foods Limited, UK.

2. Test Animals: Species: rabbit; Strain: New Zealand White; Age: ♂♂ - ≈ 7 months at start of dosing, ♀♀ - 4-7 months on arrival; Weight: ♂♂ 3-5 kg, ♀♀ 3.2-4.2 kg; Source: ♂♂ from Mellors, Chadderton, Oldham, Lancs., UK, ♀♀ from Interfauna UK Ltd, Huntingdon, Cambridgeshire, UK.

3. Statistics: Analysis of variance: Day 1 body weigh , brain cholinesterase, testes and epididymides weights, sperm data; thereafter: analysis of covariance on initial body weight; Analysis of covariance: body weight after Day 1 (to initial body weight), erythrocyte cholinesterase activities (pre-experimental values), testes and epididymides weights. Female and fetal data were analyzed as described on page 24 of the study report, copy appended.

B. STUDY DESIGN

1. Methodology: Groups of 10 male rabbits were administered (via gavage; 1 mL/kg) Molinate at dose levels of 0, 10, 100, or 200 mg/kg/ day for 84 days (12 weeks). Control males were administered an appropriate volume of corn oil. Prior to dosing (week -1), and after 4, 8, and 12 weeks of treatment, a sample of semen from each male was collected for insemination of an untreated female and for assessments of sperm morphology, number, and motility. The latter assessments of sperm morphology, number, and motility were measured for samples collected from each male during week -2 also. There were four groups of 40 females (10/dose level); one group was inseminated at each of the following weeks of treatment of the males: week -1, 4, 8, and 12. Each female (untreated) was inseminated with an undiluted semen sample (nominal volume 0.5 mL), immediately after collection from a designated male, and within 1 hour each was injected (i.v.) with 25 IU of chorionic gonadotrophin (PROFASI, Serono Labs. UK Ltd) to promote ovulation (previously injected by the suppliers 4 weeks prior to delivery of females to the testing facility to promote ovulation prior to insemination). The day of insemination was designated Day 1 of gestation. On Day 18 of gestation, the females were sacrificed and their ovaries and uteri were examined. NOTE: Males were acclimated for 7 weeks prior to the start of dosing due to their suspected immaturity; females were acclimated for one week prior to insemination. Feed (CRB pellets; Labsure Animal Diets, Lavender Mill, Manea,

Cambridgeshire UK) and water (tap) were available ad libitum.

Dose preparation: Weighed amounts of Molinate were mixed in corn oil to provide preparations containing 10, 100, or 200 mg/mL (every 2-3 weeks). Each preparation was hand-shaken until a solution was formed prior to being subdivided into aliquots. The concentration was adjusted to provide a constant dose volume of 1 mL/kg. Dosing solutions were stored at room temperature. A sample of each preparation was analyzed prior to the start of dosing to verify the concentration.

RESULTS

The chemical stability of Molinate in corn oil had been determined in a previous study [at 10 mg/mL for 12 weeks, at 100 and 300 mg/mL for ≈ 4 weeks]. The dosing formulations were found to be within 5% of nominal concentrations (95-100.8%).

3. Clinical Observations: Each male was observed daily (twice) throughout the study for changes in behavior and signs of toxicity. Each female was observed daily for health status. Male body weight was recorded daily, immediately prior to dosing, and at study termination. Food consumption was not monitored. Erythrocyte cholinesterase activity was determined in blood samples from all males taken by venepuncture in weeks -6 and -1 prior to dosing, and from the male survivors in weeks 4, 8, and 12 of treatment. Brain cholinesterase activity (males) was determined at scheduled sacrifice at 13 weeks. Plasma cholinesterase activity levels were not measured since Molinate had not affected plasma values in previous studies.

RESULTS

Survival and Clinical Observations

There were 7 deaths (males) during the study, three (1 at 100 mg/kg, 2 at 200 mg/kg) of these being attributed to treatment (unrelated deaths: 2 bone fractures and 2 dosing accidents). At study termination, there were 8 males in the 100 mg/kg group and 5 in the 200 mg/kg group. All control and low-dose males survived. No treatment-related clinical signs (other than moribund, killed in extremis, found dead) were discussed in the study report; two high-dose males displayed diarrhea from week 2 to 12, and one male at both the 100 and 200 mg/kg dose levels appeared thin (more frequently at the high dose).

Body Weight

The high-dose males displayed a statistically significant decrease in body weight only at day 78 compared to the control, although the magnitude was small (95% of control value). No assessment of body-weight gains was provided. TB II

calculated the weekly and overall body-weight gains for the groups. In general, the high-dose males displayed lower body-weight gains throughout the study compared to control values, with the exceptions of week intervals 57-71 and 78-85 (statistics not performed). The overall gain for the high-dose males was ≈ 14% of the control value.

MALE BODY WEIGHT GAIN

Interval /Group (mg/kg)	Body Weight Gain [gms]			
	0	10	100	200
1-8	-91	-16	42	-54
8-15	107	40	1	-10
15-22	32	38	38	21
22-29	43	37	24	16
29-36	63	52	133	46
36-43	68	57	75	-52
43-50	60	13	-61	-45
50-57	62	57	41	57
57-64	9	15	84	16
64-71	-40	10	26	34
71-78	57	9	-25	-73
78-85	66	89	97	105
1-85	435	401	474	61

* P<0.05; **P<0.01

Cholinesterase Activities: At week 4, there was a statistically significant decrease in erythrocyte cholinesterase activity at the mid- and high-dose levels (dose-related). At week 12, the high-dose value was lower than control but statistical significance was not attained.

Erythrocyte Cholinesterase Activity [U/L]

Dose/Week	0 mg/kg	10 mg/kg	100 mg/kg	200 mg/kg
-6	2758	2720 (99)♦	2462 (89)	2929 (106)
-1	2406	2461 (102)	2027 (84)	2585 (107)
4	2681	2378 (89)	2254** (84)	2123** (79)
8	1995	1981 (99)	1666* (84)	1915 (96)
12	1874	1796 (96)	1738 (93)	1611 (86)

♦ (% of control); * p<0.05; ** p<0.01

4. Blood Analyses, Clinical Chemistry and Urinalysis: No analyses were performed.

5. Fertility Assessments

On Day 18 of gestation, the females were sacrificed and their ovaries were examined for number of corpora lutea in each ovary; the uteri were examined for the number and position of implantations subdivided into live fetuses and early (showed decidual or placental tissue only) and late (showed embryonic or fetal tissue in addition to placental tissue) intra-uterine deaths.

RESULTS

The number of pregnancies in the pre-treatment, week 4, and week 8 assessments was low, and evaluation of the data was precluded. At week 12, satisfactory pregnancy rates were achieved. The mean number of fetuses per litter was lower than the control value at the high-dose level at week 12, and this was associated with higher pre- and post-implantation losses (%).

Maternal Performance Data

Dose/Week	0 mg/kg	10 mg/kg	100 mg/kg	200 mg/kg
Week -1				
# ♀♀ inseminated	10	10	10	10
# ♀♀ pregnant	1	2	4	4
# total resorptions	1	0	0	2
Week 4				
# ♀♀ inseminated	10	10	10	8
# ♀♀ pregnant	2	4	6	5
# total resorptions	0	1	2	0
Week 8				
# ♀♀ inseminated	10	10	9	6
# ♀♀ pregnant	9	5	3*	2
# total resorptions	0	0	1	0
Week 12				
# ♀♀ inseminated	10	10	8	5
# ♀♀ pregnant	7	9	7	4
# total resorptions	0	0	1	0

* $p<0.05$

Pregnancy Data

Dose/Parameter/Week	0 mg/kg	10 mg/kg	100 mg/kg	200 mg/kg
pre-implant. loss mean %				
-1	67	37	18	45
4	36	6	16	27
8	27	26	62	51
12	9	19	19	54**
% ♀♀ affected				
-1	100	100	75	75
4	100	25	67	40
8	56	60	100	100
12	43	78	57	100
post-implant. loss mean %				
-1	100	10	16	50
4	0	25	33	0
8	2	7	37*	0
12	5	6	16	20
prop. ♀♀ affected				
-1	1/1	1/2	3/4	2/4
4	0/2	1/4	2/6	0/5
8	2/9	2/5	2/3	0/2
12	2/7	3/9	2/7	2/4
mean/total # live fetuses				
-1	0/0	5.5*/11	4.8*/19	1.3/5
4	4.5/9	5/20	5.3/32	6.6/33
8	7.1/64	7.4/37	3.3/10	4/8
12	8.9/62	7.8/70	7.7/54	4**/16

* $p<0.05$; ** $p<0.01$

6. Sacrifice and Pathology

At the scheduled sacrifice of the males, sperm samples from the left epididymides (from the proximal and distal ends of the cauda) were assessed for sperm morphology, number, and motility. The testes and epididymides were weighed (left and right separately) from terminal sacrifice males only. The right epididymis, seminal vesicles, prostate, heart, liver, and macroscopically abnormal tissues were fixed in appropriate fixative and processed routinely. The control and high-dose tissues were examined by light microscopy.

Sperm Numbers, Morphology, and Motility

Each ejaculated semen sample was diluted with M199H medium, applied to a hemocytometer, and the numbers of sperm and percentage motility were recorded. Additionally, smears were prepared on slides, fixed with acetone and stained with a multicolored single step stain (0.8% trypan blue, 0.4% naphthol yellow, 0.2% eosin Y in 1% acetic acid). Each slide was scanned (x 40 objective magnification) for 100 sperm, which were classified for abnormalities as follows: **Head** - no head, small head, reduced acrosome, double, pyriform; **Mid-piece** - abnormality: usually kinked; **Tail** - no tail, coiled/kinked, double; **Multiple** - multiple abnormalities of

the head, mid-piece, or tail.

RESULTS

Gross Pathology: None of the gross lesions observed could be attributed to treatment.

Organ Weights: No differences were observed in either the testes or epididymis weight.

Ejaculated samples: It was stated that although there was some evidence of a "relationship between pre-experimental data and subsequent values for each of the parameters assessed, the overall correlation was poor due to missing values and consequently analyses allowing for this are not presented." Motility was slightly higher in the high-dose group compared to the control value in week 4 only. Total abnormalities (%) were greater than control values at the high-dose level throughout the study (pre-dosing and during dosing), at the mid-dose at week -2, 4, and 12, and at the low dose at week -2. The increases were not dose related at any time point. Midpiece abnormalities were slightly increased over control values at weeks 8 and 12, but statistical significance was not attained.

Ejaculated Samples - Sperm Parameters (mean values)♦

Dose/Parameter/Week	0 mg/kg	10 mg/kg	100 mg/kg	200 mg/kg
% Sperm Motility				
Week -2	89.7 [7]	88.9 [9]	84.4 [8]	92.6 [10]
Week -1	88.9 [7]	89.7 [6]	89.5 [6]	90.6 [6]
Week 4	89.3 [9]	90.4 [9]	89.9 [8]	94.4* [7]
Week 8	92.6 [10]	90.3 [10]	87.9 [8]	91.0 [5]
Week 12	92.1 [9]	88.6 [10]	89.6 [7]	94.8 [5]
Sperm Counts ($x10^6$/mL)				
Week -2	86.9 [4]	40.2 [5]	114.4 [5]	109.1 [6]
Week -1	162.6 [8]	116.1 [9]	64.5 [9]	114.6 [6]
Week 4	194.9 [8]	186.2 [9]	101.2 [7]	210.6 [7]
Week 8	257.6 [10]	182.0 [10]	166.1 [6]	152.9 [4]
Week 12	249.1 [8]	388.5 [8]	185.3 [7]	228.4 [5]
Total Abnormalities (%)				
Week -2	36.6 [7]	35.6 [10]	48.1* [8]	46.6 [10]
Week -1	48.4 [7]	40.4* [7]	50.6 [8]	54.7 [6]
Week 4	39.0 [10]	39.9 [9]	46.1* [8]	42.3 [7]
Week 8	49.9 [10]	50.0 [10]	60.4 [8]	53.4 [5]
Week 12	57.3 [9]	62.6 [10]	66.8* [8]	62.6 [5]
Midpiece Abnormalities (%)				
Week -2	25.7 [7]	23.1 [10]	26.6 [8]	26.3 [10]
Week -1	30.4 [7]	19.9* [7]	22.4 [8]	27.5 [6]
Week 4	22.9 [10]	22.6 [9]	27.1 [8]	26.1 [7]
Week 8	31.3 [10]	25.3 [10]	33.5 [8]	35.2 [5]
Week 12	27.1 [9]	21.1 [10]	36.4 [8]	37.8 [5]

♦ [n]; * $p<0.05$

Epididymal samples : There was no evidence of an effect of Molinate on motility. The incidence of total abnormalities was

slightly higher than control values at the mid- and high-dose levels in proximal cauda epididymal samples. At the high dose, the incidence of midpiece abnormalities was slightly greater than control, although this was said to be due solely to one male. No effect was observed at the mid- or low-dose level.

Epididymal Samples - Sperm Parameters (mean values)

Dose/Parameter/Week	0 mg/kg	10 mg/kg	100 mg/kg	200 mg/kg
% Sperm Motility				
Proximal	79.9 [7]	84.0 [9]	84.1 [5]	83.1 [4]
Distal	84.4 [10]	87.3 [8]	87.1 [6]	84.0 [5]
Total Abnormalities (%)				
Proximal	60.1 [10]	53.6* [10]	68.1* [7]	73.4 ** [5]
Distal	54.0 [10]	49.1 [10]	61.3 [7]	63.2 [5]
Mid-Piece Abnormalities (%)				
Proximal	20.8 [10]	16.1 [10]	20.0 [7]	27.6 [5]♦
Distal	21.7 [10]	15.7 [10]	23.7 [7]	32.0 [5]♦

* $p<0.05$; ** $p<0.01$; ♦ values excluding male #35: proximal=23.3, distal=23.0

7. Histopathology: Sections of tissues noted above from the control and high dose (200 mg/kg) males were to be examined by light microscopy. **Scanning Electron Microscopy:** Samples of ejaculated semen or sperm from the cauda (distal end) of the left epididymis were mixed with phosphate buffer, placed on coverslips and fixed with osmium tetroxide. The samples were dehydrated, dried, and after mounting on stubs, the tissue was coated with gold prior to examination. Initially, semen samples were examined at low magnification to detect areas on the stub where sperm were present. These areas were examined (≈ 400-700x magnification) using a sliding cascade method. Individual sperm were examined at higher magnification (>800x) and classified as being either normal or abnormal. Abnormal sperm were further subdivided into those having head, mid-piece, mid-piece and tail, and tail abnormalities. This process was repeated until 20 sperm had been examined.

RESULTS

Scanning Electron Microscopy: No treatment-related effects were reported on the percentage total or midpiece abnormalities in ejaculated sperm. The percentage total and midpiece abnormalities at the high-dose level for the epididymal samples were lower than control values.

Microscopic observations: Only liver, heart, and abnormal lungs from the high-dose and control males were examined. None of the findings appear to be related to treatment.

C. DISCUSSION

Treatment-related deaths occurred at the mid- (one) and high-dose (two) levels, and there was an overall decrease in body-weight gain at the high-dose level. No consistent effect was observed on erythrocyte or brain cholinesterase activities, and neither testes or epididymal weight was affected. Poor pregnancy success rates and low sperm counts were observed in all groups in the first weeks of the study, which the author attributed to the immaturity of the males, as well as to the possible incompatibility of the males and females (different suppliers). The number of fetuses was decreased at week 12 at the high-dose level compared to the control group and was associated with high pre- and post-implantation losses. However, the author considered the number of females evaluated to be too low, in addition to the mortality observed in the males, for any meaningful assessment of the data. The incidence of mid-piece abnormalities in ejaculated sperm and total abnormalities in the epididymal sperm were slightly higher at the mid- and high-dose levels compared to the control value at week 12 when examined by light microscopy but not by scanning electron microscopy.

D. CONCLUSION

TB II agrees with the author that no definitive statement can be made with regard to the effects of Molinate on male rabbit fertility based on the results of this study. Deaths and poor pregnancy performance make interpretation difficult. The dose levels chosen for this study had demonstrated conflicting results previously, with some studies indicating no effects (deaths) at 200 mg/kg and others resulting in deaths at 100 and 200 mg/kg. The author stated that the design criteria for this study were not attained. Under the conditions of the study, administration of Molinate to male rabbits at dose levels of 10, 100, and 200 mg/kg/day for at total of 12 weeks resulted in death at the mid- and high-dose levels and decreased body-weight gains at the high-dose level. Females inseminated by semen from these males at weeks -1, 4, 8, and 12 displayed poor pregnancy rates during the first weeks of the study, including the pre-dose period. Week 12 data suggest an increase in pre-implantation loss and fewer fetuses at the high-dose level compared to the controls. Although the number of females available at this dose level is too few for adequate assessment, TB II notes that increased pre-implantation loss and decreased number of fetuses are effects noted in the rat fertility studies on Molinate.

This study is classified Unacceptable; it cannot be upgraded, and it does not satisfy any guideline requirement.

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Page 28 is not included in this copy.

Pages _____ through _____ are not included in this copy.

The material not included contains the following type of information:
Identity of product inert ingredients.

_____ Identity of product inert impurities.

_____ Description of the product manufacturing process.

_____ Description of quality control procedures.

_____ Identity of the source of product ingredients.

_____ Sales or other commercial/financial information.

_____ A draft product label.

_____ The product confidential statement of formula.

_____ Information about a pending registration action.

✓ FIFRA registration data.

_____ The document is a duplicate of page(s)

_____ The document is not responsive to the request.

The information not included is generally considered confidential by product registrants. If you have any questions, please contact the individual who prepared the response to your request.

009972

Reviewed by: Linda L. Taylor, Ph.D. Linda Lee Taylor 12/14/92
Section II, Tox. Branch II (H7509C)
Secondary Reviewer: K. Clark Swentzel K. Clark Swentzel 1/6/93
Section II Head, Tox. Branch II (H7509C)

DATA EVALUATION REPORT

STUDY TYPE: mechanistic - pregnant rat TOX. CHEM. NO.: 444

MRID NO.: 423613-08 PC Code: 041402

TEST MATERIAL: Molinate

SYNONYMS: Ordram

STUDY NUMBER: none; Report # CTL/T/2769

SPONSOR: ICI Americas Inc.

TESTING FACILITY: ICI Central Toxicology Laboratory, Cheshire, UK

TITLE OF REPORT: Molinate: Mechanistic Study in the Pregnant Rat

AUTHORS: JM Horner. NOTE: There is no signature anywhere on the report of the study director/author (JM Horner); Page 4 lists contributors (Chemical Analyst, Pathologist, Bioanalyst, and Study Reviewer) and their signatures.

REPORT ISSUED: March 3, 1992

QUALITY ASSURANCE: No quality assurance statement was provided.

CONCLUSIONS: Under the conditions of the study, oral administration of Molinate at dose levels of 0, 75, 135, or 200 mg/kg/day for three days to pregnant rats resulted in decreased survival, neurological effects, and increased adrenal weights at the mid- and high-dose levels, and decreased body weight/gain, an increase in the neutral lipid content of cells in the adrenal cortex, and microscopic lesions in the adrenal cortex and ovary at all three dose levels. There was no evidence of an effect on the ability to maintain a pregnancy. No no-effect level was demonstrated, nor was there discussion as to a possible cause of the deaths observed in this study at dose levels that had not been shown previously to result in deaths in this strain/sex of rat.

Classification: Unacceptable; no individual data were provided. This study is a mechanistic study, and it does not satisfy any guideline requirement.

A. MATERIALS

1. Test Compound: Molinate; Description: amber liquid; Source: ICI Agrochemicals, Seneffe, Belgium; Batch #: not provided, was assigned CTL reference #: Y06367/024; Purity: 98.1% w/w.

Vehicle control: corn oil; Description: Kraft Wesson; Source: Kraft Foods Limited, UK; Batch #: assigned CTL reference # Y00790/004; Purity: 100%.

2. Test Animals: Species: rat; Strain: Crl:CD(SD)BR (Sprague-Dawley), (SPF); Age: ≈9 weeks old at study start; Weight: virgin female: 180-220 g; Source: Charles River UK Limited, Margate, Kent.

3. Statistics: Treatment group means for body weights and organ weights were compared with the control group mean using a two-sided Student's t-test.

B. STUDY DESIGN

1. Methodology: The female rats were mated with unrelated males of the same strain, overnight. Timed-mating (1♂ with 1♀) was performed. On the following morning, the females were examined for vaginal plugs for confirmation of successful mating. The day on which plugs were detected was day 1 of gestation; on day 6 of gestation, 40 females with confirmed vaginal plugs were sent to the testing facility in individual boxes. On arrival, each female was uniquely identified and randomly (not further defined) allocated to one of four experimental groups. The rats were housed individually and provided with food in glass jars (CT1 diet, supplied by Special Diets Services Limited, Stepfield, Witham, Essex, UK) and water ad libitum. The study was divided into ten replicates (randomized blocks), each replicate consisting one rat from each group. Computer-generated random number permutations were used to allocate the cages within each replicate to an experimental group; individual animal numbers were assigned sequentially within each group. The test material was administered via oral gavage daily (≈ the same time each day) at dose levels of 0, 75, 135, or 200 mg/kg (1.0 mL/100 g body weight, according to their daily individual body weights) during gestation days 7-9. NOTE: Dosing was scheduled for gestation days 7-10; due to effects on survival at the highest dose level, along with signs of toxicity at the mid-dose level, dosing was discontinued on gestation day 9.

2. Dose preparation: For each dose, an appropriate amount of corn oil was added to a weighed amount (adjusted for purity) of test sample and each was thoroughly mixed and subdivided into aliquots. Fresh aliquots were used each day, and the dosing solutions were shaken before dosing. The control vehicle was

also dispensed into aliquots. A sample of each preparation was analyzed prior to dosing to verify the achieved concentration, and samples of 0.5 mg/mL and 20 mg/mL were taken to determine the stability of Molinate in corn oil over a period of 29 days.

RESULTS

The mean achieved concentrations were found to be within 7% of nominal levels, and the preparations were found to be stable over the duration of use.

3. Clinical Observations: The rats were observed immediately prior to dosing for clinical signs, and cageside observations were made between 1 and 3 hours post dose daily. Detailed clinical observations were recorded on each animal when body weights were recorded. Body weight for each rat was recorded daily immediately prior to dosing (where applicable) on days 2, 4, 7-14 (inclusive).

RESULTS

Survival and Clinical Observations

There were fourteen treatment-related deaths during the study. Five of the mid-dose females were sacrificed on day 10, and nine high-dose females were sacrificed (1 each on days 8 and 9; 3 on day 10). Four high-dose females were found dead on day 10. Clinical signs observed prior to death for these animals included piloerection, signs of urinary incontinence, sides pinched in, subdued behavior, hunched posture, salivation, abnormal gait (high-stepping and splayed), peri-nasal and peri-oral staining.

Clinical signs displayed by the survivors at the 135 mg/kg dose level included head held twisted to one side (3 females) and rolling gait (1 female). One high-dose (200 mg/kg) surviving female also displayed head held twisted to one side. Discharge from the eyes was observed only in the treated animals (dose-related incidence), but this is not considered treatment-related or an adverse effect. No other differences in clinical signs were observed.

Body Weight

Prior to dosing (days 2-7), comparable body-weight gains were displayed among the groups. Following dosing, a marked treatment-related decrease in body weight gain at the mid- and high-dose levels and no gain at the low-dose level were observed. After day 10, the low-dose displayed a body-weight gain greater than that of the controls, which resulted in the

overall (day 2-14) gain being only marginally lower than the control.

BODY WEIGHT

Day/Group (mg/kg)	Body Weight (% of Control)		
	75	135	200
2	98	96	102
4	98	96	101
7	98	96	101
8	97	94**	96
9	95	92**	89*
10	95*	87**	85**
11	97	-	-
12	95	-	-
13	95	-	-
14	97	-	-

* P<0.05; **P<0.01

BODY WEIGHT GAINS

Day/ Group (mg/kg)	Body Weight Gain (grams)			
	0	75	135	200
2-4	27.7	28.3	25.8	26.4
4-7	18.7	18.4	18.9	19.6
7-8	3.8	1.3	-3.9	-9.4
8-9	3.7	-1.9	-1.6	-6.2
9-10	3.0	0.7	-10.1	-16.0
10-11	-1.0	4.8	-	-
11-12	8.8	4.6	-	-
12-13	8.4	7.3	-	-
13-14	2.0	7.3	-	-
2-14	75.1	70.8	-	-
2-10	56.9	46.8	29.1	14.4

• calculated by reviewer from summary Table 5 of report; no statistics performed

CUMULATIVE BODY WEIGHT GAINS

Day/ Group (mg/kg)	Body Weight Gain (grams)			
	0	75	135	200
2-4	27.7	28.3	25.8	26.4
2-7	46.4	46.7	44.7	46.0
2-8	50.2	48.0	40.8*	36.4**
2-9	53.9	46.1*	39.2**	31.2**
2-10	56.9	46.8*	29.1**	15.0**
2-11	56.4	51.6	-	-
2-12	65.2	56.2	-	-
2-13	73.6	63.5	-	-
2-14	75.6	70.8	-	-
% gain 2-10	27%	23%	14%	7%
% gain 2-14	36%	35%	-	-

4. Clinical Chemistry: At sacrifice, plasma progesterone levels were measured for each rat from which blood (via cardiac puncture) was obtained (using a commercial radioimmunoassay kit).

RESULTS

It was reported that no consistent trends were observed in plasma progesterone levels that could be related to treatment. TB II notes that values from only 2 high-dose females were obtained and these values are less than those of the control. Additionally, the mid-dose females displayed lower values than the controls. No definitive conclusion can be made with respect to an effect of Molinate on progesterone levels.

Progesterone Levels

DOSE (mg/kg)	0	75	135	200
Progesterone levels (ng/mL)	41.2 (10)♦ [5]♥ 53.8 (14)♦ [4]	60.9 [9]	30.6 [8]	27.8 [2]

♦ controls sacrificed on day ; ♦ controls sacrificed on day 10; ♥ [n];

5. Sacrifice and Pathology

All animals were subjected to a macroscopic post mortem examination. The adrenal glands and ovaries were weighed (left and right of each separately). The intact gravid uterus (minus ovaries and trimmed free of connective tissue) were removed. The ovaries and uterus were examined and the following data were recorded: (1) an external assessment of the number of corpora lutea in each ovary; (2) the number and position of

implantations subdivided into (a) live fetuses, (b) early intra-uterine deaths (showed decidual or placental tissue), and (c) late intra-uterine deaths (showed embryonic or fetal tissue in addition to placental tissue).

RESULTS

Gross Pathology: No differences were noted at necropsy that could be attributed to treatment. No consistent treatment-related effect was reported on the number of implantation sites.

UTERINE DATA♥

Dose/Day/Parameter	0 mg/kg	75 mg/kg	135 mg/kg	200 mg/kg
Day 10				
# corpora lutea	16.4	-	16.4	16.0
# implantations	13.2	-	12.4	13.8
% pre-implant. loss	17.6	-	24.2	13.8
Day 14				
# corpora lutea	14.8	13.8	-	-
# implantations	12.8	12.0	-	-
% pre-implant. loss	11.8	20.1	-	-

♥ # pregnant females in each group was 9; control + into 5/4

Organ Weights: There was a dose-related increase in absolute adrenal weights (statistically significant at all dose levels) compared to the control values, and the relative weights also displayed a dose-related increase over the control values, although no statistics were provided for the relative weights. A slight increase in ovarian weight was observed at the high-dose level, but statistical significance was not attained.

Organ Weights (g)

Dose/Organ	Control-low♣	Low	Control-mid/high♣	Mid	High
Adrenal					
absolute	0.080	0.097*	0.070	0.100**	0.134**
relative♦	0.028	0.035	0.026	0.043	0.059
Ovary					
absolute	0.104	0.096	0.090	0.094	0.119
relative♦	0.037	0.035	0.033	0.041	0.052

♦ relative values calculated by reviewer (x100); no statistics performed; ♣ 5 controls were sacrificed on day 10 with the mid- and high-dose animals; the remaining 5 controls were sacrificed at study termination

Histopathology: The left ovary and adrenal gland were submitted for routine histopathological examination (H & E staining). The right ovary and adrenal gland were submitted snap frozen in liquid nitrogen and stained with (a) Oil Red O for neutral lipid or (b) Burnt Sudan Black for neutral lipid and cholesterol, including cholesterol esters. All sections were examined by light microscopy. **Lipid Analysis:** Identification of the lipids (ovaries and adrenals that had

been frozen in liquid nitrogen) was performed using thin-layer chromatography to identify the lipid profile, and gas chromatography-mass spectrometry to identify the fatty acid profiles. It was stated that the method used for the extraction of the lipids was not exhaustive but designed to extract the majority of neutral and polar lipids.

RESULTS

The incidence and severity of cellular swelling and vacuolation in the zonae fasciculata and reticularis of the adrenal cortex in the mid- and high-dose animals were increased (dose-related). One mid-dose and 5 high-dose animals also displayed minimal or slight multifocal degeneration with necrosis and loss of cells in the zona fasciculata. Additionally, a dose-related increase in the neutral lipid content of cells in these two regions of the adrenal cortex was apparent in all treatment groups (Oil Red O). In the ovary, a dose-related increase in the incidence and/or severity of fatty vacuolation of corpora luteal cells was detected in all treated groups (Oil Red O). One high-dose animal displayed slight necrosis in the liver.

Microscopic Pathology Observations♥

Dose/Organ (mg/kg)	0	75	135	200
ADRENAL GLAND				
fatty cytoplasmic vacuol., ZF♦	10	10	10	10
minimal	3	1	1	2
slight	5	6	2	0
moderate	2	1	5	3
marked	0	2	2	5
fatty cytoplasmic vacuol., ZR♦	7	10	10	9
minimal	3	5	3	1
slight	4	3	4	2
moderate	0	2	3	6
cellular swelling & ↑ vacuol., ZF♦	0	0	8	10
minimal	0	0	2	0
slight	0	0	5	7
moderate	0	0	1	3
cellular swelling & vacuol., ZR♦	0	0	7	9
minimal	0	0	1	1
slight	0	0	6	5
moderate	0	0	0	3
degeneration, ZF♦	0	0	1	6
minimal	0	0	0	5
slight	0	0	1	1
OVARY				
fatty cytoplasmic vacuol., CL♦	6	9	10	10
minimal	5	3	0	0
slight	0	5	1	1
moderate	1	1	7	5
marked	0	0	2	4

♥ #'s total # of animals (out of 10/group) displaying lesion; ♦ zona fasciculata; ♣ zona reticularis; ♠ corpora lutea

Lipid Analysis: As stated in the report (no individual data provided), there was visual evidence of a dose-related

increase in free fatty acids, diglycerides, monoglycerides, phosphatidyl ethanolamine, phosphatidyl choline, and 3 other unidentified phospholipids for adrenal extracts only. The most marked increase was in a polar lipid, which was tentatively identified as sphingomyelin. There were no apparent dose-related changes in either cholesterol or cholesterol esters. With regard to fatty acid profiles, similar profiles were reported for both ovaries and adrenals at all dose levels both for free and total fatty acids. The total amount of free fatty acids determined by GC-MS from extracts of adrenals showed a four-fold dose-related increase between the controls and high-dose groups. There were no dose-related changes noted for the ovary extracts. It was stated that there was an indication of a slightly higher proportion of unsaturated fatty acids at the high-dose level for both organs, but a similar trend was not apparent for free fatty acids. Also noted was an increase (2-3 fold) in a minor fatty acid, tentatively identified as $C_{22:5}$, at the mid- and high-dose levels (in the total fatty acid and free fatty acid fractions; adrenals only).

C. DISCUSSION

The objective of the study was to investigate the potential histopathological ovarian changes following a short-term (days 7-10 of gestation inclusive), high-dose regime, in pregnant rats. Due to treatment-related deaths after 3 doses (5 mid-dose and 9 high-dose females), dosing was stopped after day 9. On day 10, in addition to the surviving mid- and high-dose females that were sacrificed at this time, half (5) of the control females were sacrificed to provide control data for the intercurrent deaths. Necropsy did not reveal the cause of death, and the deaths were inconsistent with previous data from studies (same strain of rat) from which the dose levels were chosen. Neurological signs (head rolled to one side, rolling gait) were observed at the mid- and high-dose levels, and body weight was decreased (dose-related) at all dose levels. There were no apparent effects of Molinate on the numbers of corpora lutea and implantation sites, and there was no treatment-related effect on pre-implantation loss.

The adrenal cortex appears to be a target organ of Molinate, as evidenced by the increase in organ weight and microscopic findings (cellular swelling and increased vacuolation in the zonae fasciculata and reticularis; multifocal degeneration with necrosis and loss of cells in the zona fasciculata) and an increase in the neutral lipid content of cells in these two regions. The ovary is also a target organ for Molinate, as evidenced by the increased fatty vacuolation of corpora luteal cells.

The author concluded that the morphological changes observed indicate a functional disturbance in adrenocortical and

corpora luteal cells, which "would be consistent with a perturbation of steroid hormone synthesis by both tissues." However, due to the limitations of sample preparation and sensitivity of the lipid analysis methodology used, any changes in steroid synthesis or accumulation within the adrenal gland and ovary were not detectable.

D. CONCLUSION

Under the conditions of the study, oral administration of Molinate to pregnant rats resulted in decreased survival, neurological effects, and increased adrenal weights at the mid- and high-dose levels, and decreased body weight/gain, an increase in the neutral lipid content of cells in the adrenal cortex, and microscopic lesions in the adrenal cortex and ovary at all three dose levels. There was no evidence of an effect on the ability to maintain a pregnancy. No no-effect level was demonstrated, nor was there discussion as to a possible cause of the deaths observed in this study at dose levels that had not been shown previously to result in deaths in this strain/sex of rat.

This study is a mechanistic study, and it does not satisfy any guideline requirement. It is classified unacceptable, since individual data were not provided.

Reviewed by: Linda L. Taylor, Ph.D. Linda Lee Taylor 12/17/92
Section II, Tox. Branch II (H7509C)
Secondary Reviewer: K. Clark Swentzel K. Clark Swentzel 1/7/93
Section II Head, Tox. Branch II (H7509C)

009972

DATA EVALUATION REPORT

STUDY TYPE: fertility study- male rabbit TOX. CHEM. NO.: 444

MRID NO.: 423613-04 PC Code: 041402

TEST MATERIAL: Molinate

SYNONYMS: Ordram; S-ethyl hexahydro-1H-azepine-1-carbothioate

STUDY NUMBER: RB0521; Report # CTL/T/3225

SPONSOR: ICI Americas Inc.

TESTING FACILITY: ICI Central Toxicology Laboratory, Cheshire, UK

TITLE OF REPORT: Molinate: Fertility Study in Male Rabbits

AUTHORS: DJ Tinston

REPORT ISSUED: January 18, 1991

QUALITY ASSURANCE: A quality assurance statement was provided.

CONCLUSIONS: Under the conditions of the study, oral administration of Molinate at a dose level of 200 mg/kg resulted in deaths and, consequently, the assessment of fertility at this dose level was precluded. The limited data available for the mid-dose (100 mg/kg) male rabbit suggest a reduction in fertility, which is associated with an increased incidence of sperm abnormalities. Additionally, there was an increase in pre-implantation loss and a decrease in the number of live fetuses at this dose level at week 4. Although there were no differences noted at the low-dose (10 mg/kg) level compared to the control group, the duration of the study was only 8 weeks, with fertility being assessed only during week 4 of dosing.

Classification: Unacceptable; it cannot be upgraded. This study does not satisfy any guideline requirement.

A. MATERIALS

1. Test Compound: Molinate; Description: amber liquid; Batch #: CTL reference # Y06367/009; Purity: 99% (w/w); Source: ICI Agrochemicals, Seneffe, Belgium.

   Vehicle/Control: corn oil; Batch #: CTL reference # Y00790/004; Source: Kraft Foods Limited, UK.

2. Test Animals: Species: rabbit; Strain: New Zealand White; Age: ♂♂ - 6-8 months (start of dosing), ♀♀ - 4-6 months (at time of insemination); Weight: ♂♂ 3-5 kg, ♀♀ 3-4 kg; Source: Interfauna UK Ltd, Huntingdon, Cambridgeshire, UK.

3. Statistics: Analysis of variance: Day 1 body weight, sperm data, female data, fetal data; Analysis of covariance: body weight after Day 1 (on initial body weight), plasma and erythrocyte cholinesterase activities (on pre-experimental values); Fisher's Exact Test: proportion of females (a) pregnant, (b) with pre-implantation loss, (c) with post-implantation loss, (d) with early intra-uterine deaths, (e) with late intra-uterine deaths (comparing treated groups to control group). All statistical tests for males and females were two-sided. Additional details (pages 22-23 of report).

B. STUDY DESIGN

1. Methodology: Groups of 10 male rabbits were administered (via gavage; 1 mL/kg; volume adjusted daily according to body weight) Molinate at dose levels of 0, 10 and 100 for 49 days, or 200 mg/kg/day for 16 days (intended duration was 12 weeks). Control males were administered an appropriate volume of corn oil. There were two groups of 40 untreated females (10/dose level); one group was inseminated prior to the start of treatment and the other at week 4. The animals were acclimated for 14 days prior to the start of the study. Feed (CRB pellets; Labsure Animal Diets, Lavender Mill, Manea, Cambridgeshire UK) and water (tap) were available ad libitum. A randomization procedure was used to allocate the animals to the various groups.

2. Dose preparation: An appropriate amount of corn oil was added to a weighed amount of Molinate to provide preparations containing 10, 100, or 200 mg/mL (prepared at 2-3 week intervals). Each preparation was hand-shaken until a solution was formed and was then subdivided into aliquots, which were stored at room temperature. A new aliquot was used ≈ every 3 days. Prior to the start of dosing, a sample of each preparation was analyzed to verify achieved concentrations of Molinate.

RESULTS

The stability of Molinate in corn oil was determined previously and found to be stable for at least 4 weeks at 100 and 300 mg/mL (incorrectly listed as mg/kg on page 14 of the report). The dosing formulations were found to be within 5% of the nominal concentrations.

3. Clinical Observations: Males were checked twice a day during the study, and any changes in behavior or clinical condition were recorded. Male body weight was recorded daily (immeditaely prior to dosing) and at termination. Blood samples were obtained (venepuncture) from all males prior to the start of dosing and from the surviving males in week 4. Additionally, samples were obtained from the surviving high-dose males prior to termination in week 3 and from the surviving control, low-, and mid-dose males prior to termination in week 8. Plasma and erythrocyte cholinesterase activities were determined using a Vitatron PA800 analyzer.

RESULTS

Survival and Clinical Observations

There were 10 deaths during the study, which were attributed to treatment. Three high-dose (in week 2) and two mid-dose (in week 3) males were found dead, and 3 high-dose (in week 2) and 2 mid-dose (in weeks 3 and 4) males were sacrificed *in extremis*. The remaining high-dose males were sacrificed in week 3 and the control, low-, and surviving mid-dose males were sacrificed in week 8. There were no clinical abnormalities observed in those dying on test, and no signs of toxicity were observed in those sacrificed moribund until the day of sacrifice.

Body Weight

All groups lost weight during the first week of the study. The high-dose males lost weight throughout the study, and all treated groups had a negative weight gain at terminal sacrifice.

Body-Weight Gains (grams)

Week/Dose	0 mg/kg	10 mg/kg	100 mg/kg	200 mg/kg
2	-75.7	-120.3	-114.6	-181.3
3	3.9	-172.8	-151.9	-212.0
4	-72.6	-152.9	-271.0	
5	-67.0	-169.7	-254.0	
6	-35.0	-92.2	-136.2	
7	32.6	-51.3	-120.3	
8	16.3	-63.2	-170.3	
overall gain (wk 1-8)	16.3	-63.2	-323.8	-330.7♦

♦ weeks 1-3

Cholinesterase Activities: A dose-related decrease in plasma cholinesterase activity was displayed in all groups in week 1 and the high-dose also displayed a decrease in week 3, but statistical significance was not attained. The high-dose group displayed a decrease (≈ 20%) in the erythrocyte cholinesterase activity compared to the control value at week 3, and the mid-dose group showed a similar decrease in week 4. Due to the limited number of animals and the variability of the data, no definitive statement can be made regarding an effect of Molinate on erythrocyte cholinesterase activity in this study. NOTE: The pre-dosing values for plasma and erythrocyte cholinesterase activities were not provided.

Cholinesterase Activity [U/L]

Parameter/Week/Dose	0 mg/kg	10 mg/kg	100 mg/kg	200 mg/kg
Plasma cholinesterase				
1	688	583 (85)♦	575 (84)	574 (83)
3	688	-	-	560 (81)
4	621	505 (81)	588 (95)	-
7	598	515 (86)	674 (113)	-
8	678	551 (81)	564 (83)	-
RBC cholinesterase				
1	1793	1678 (94)	1710 (95)	1884 (105)
3	1845	-	-	1471 (80)
4	2216	2137 (96)	1781 (80)	-
7	3660	2436 (67)	2978 (81)	-
8	2424	2460 (101)	2035 (84)	-

♦ (% of control value)

4. Hematology: Blood samples were obtained (venepuncture) from surviving high-dose males prior to termination in week 3 and from surviving males in the control, low- and mid-dose groups prior to termination in week 8. There was no statement of whether food was withheld prior to sample collection. Blood films were prepared for Romonowsky-staining, and a differential white cell count was performed. The CHECKED (X) parameters were examined.

X	Hematocrit (HCT)		Leukocyte differential count
X	Hemoglobin (HGB)	X	Mean corpuscular HGB (MCH)
X	Leukocyte count (WBC)	X	Mean corpusc. HGB conc.(MCHC)
X	Erythrocyte count (RBC)	X	Mean corpusc. volume (MCV)
X	Platelet count		Reticulocyte count
	Blood clotting measurements		Red cell morphology
	(Thromboplastin time)		
	(Activated partial thromboplastin time)		
	(Prothrombin time)		

RESULTS

There were no apparent effects on any of the measured parameters, and the cause of death/moribundity was not elucidated from these data.

4X
41

5. Clinical Chemistry: Blood samples were obtained as stated above. The CHECKED (X) parameters were examined.

X	Electrolytes:	X	Other:
X	Calcium	X	Albumin
	Chloride	X	Blood creatinine
	Magnesium	X	Blood urea nitrogen
X	Phosphorous	X	Cholesterol
	Potassium		Globulins
	Sodium	X	Glucose
	Iron		Phospholipids
	Enzymes	X	Total bilirubin
X	Alkaline phosphatase (ALK)	X	Total serum Protein (TP)
	Cholinesterase (ChE)	X	Triglycerides
X	Creatine kinase (CK)		Lipids, total
	Lactate dehydrogenase (LAD)		Triiodothyronine, total T3
X	Serum alanine aminotransferase		
X	Serum aspartate aminotransferase		
	Gamma glutamyl transferase (GGT)		
	Glutamate dehydrogenase (GLDH)		
	Ornithine carbamyltransferase (OCT)		
	Serum protein electrophoresis		
	Thyroxine, total T4		

RESULTS

There were no apparent effects on any of the measured parameters, and the cause of death/moribundity was not elucidated from these data.

6. Fertility Assessments

Each female (untreated) was inseminated with an undiluted semen sample (nominal volume 0.5 mL), immediately after collection from a designated male, and within 1 hour each was injected (i.v.) with 25 IU of chorionic gonadotrophin (PROFASI, Serono Labs. UK Ltd) to promote ovulation (previously injected by the suppliers 2 weeks prior to delivery of females to the testing facility to promote ovulation prior to insemination). The day of insemination was designated Day 1 of gestation. On Day 18 of gestation, the females were sacrificed and their ovaries and uterus were removed and examined for number of corpora lutea in each ovary; the uteri were examined for the number and position of implantations subdivided into live fetuses and early (showed decidual or placental tissue only) and late (showed embryonic or fetal tissue in addition to placental tissue) intra-uterine deaths. Pre- and post-implantation losses were calculated.

RESULTS

Prior to dosing, the number of pregnancies in the mid- and high-dose groups was too low to adequately assess fertility at

this time point. At week 4, no differences were noted in the number of pregnant females/number inseminated among the surviving groups (control, low-, and mid-dose). The mid-dose group displayed a significantly lower number of implantations and live fetuses compared to the control group, which was due to increased pre-implantation loss. There was a dose-related increase in pre-implantation loss, but the increase at the low-dose level did not attain statistical significance. Post-implantation loss was unaffected by treatment.

Maternal Performance Data

Dose/Week	0 mg/kg	10 mg/kg	100 mg/kg	200 mg/kg
Week -1				
# ♀♀ inseminated	10	10	10	10
# ♀♀ pregnant	6	8	4	1
Week 4				
# ♀♀ inseminated	10	10	7	-
# ♀♀ pregnant	7	7	6	-

* p<0.05

Pregnancy Data

Dose/Parameter/Week	0 mg/kg	10 mg/kg	100 mg/kg	200 mg/kg
mean # corpora lutea	9.83	9.38	10.50	9.00
mean # implantations	6.67	6.75	8.00	8.00
pre-implant. loss mean %				
-1	35.2	31.2	24.2	11.1
4	19.2	26.4	45.6*	-
proportion ♀♀ affected				
-1	6/6	5/8	4/4	1/1
4	5/7	6/7	6/6	-
post-implant. loss mean %				
-1	6.8	2.5	2.5	0.0
4	6.1	14.1	10.8	-
proportion ♀♀ affected				
-1	3/6	2/8	1/4	0/1
4	2/7	4/7	2/6	-
mean/total # live fetuses				
-1	6.17/37	6.50/52	7.75/51	8.00/8
4	7.57/53	7.14/50	4.17*/25	-

* p<0.05; ** p<0.01

7. Semen Collection: A sample of semen from each male was collected prior to the start of dosing and in week 4 for insemination of an untreated female and for assessments of sperm morphology and motility. When the volume was not adequate for both objectives, an additional sample was collected. When 2 samples were taken, scanning electron microscopy and sperm numbers, morphology, and motility were measured for the same sample, which was also used for insemination, where possible.

Sperm Numbers, Morphology, and Motility

Each semen sample was diluted with M199H medium, applied to a hemocytometer, and the numbers of sperm and percentage motility were recorded. Additionally, smears were prepared on slides, fixed with acetone and stained with a multicolored single step stain (0.8% trypan blue, 0.4% naphthol yellow, 0.2% eosin Y in 1% acetic acid). Each slide was scanned (x 40 objective magnification) for 100 sperm, which were classified for abnormalities as follows: **Head** - no head, small head, reduced acrosome, double, pyriform; **Mid-piece** - abnormality: usually kinked; **Tail** - no tail, coiled/kinked, double; **Multiple** - multiple abnormalities of the head, mid-piece, or tail.

Scanning Electron Microscopy

Samples of semen were mixed with phosphate buffer and then fixed with osmium tetroxide in phosphate buffer. The samples were dried and then coated with gold prior to examination. One hundred sperm per sample were examined for any with abnormalities.

RESULTS

There were no differences noted in percentage motility, and sperm counts showed wide variability with no significant differences noted. There was a statistically significant increase in the incidence of mid-piece and total abnormalities at the mid-dose level compared to the control values in week 4 (no data for the high dose). Additionally, the values for these parameters were higher than the pre-experimental values. Scanning electron microscopy revealed no treatment-related effects among the groups with respect to the percentage of abnormal sperm.

Sperm Parameters (mean values/excludes outliers)♦

Dose/Parameter/Week	0 mg/kg	10 mg/kg	100 mg/kg	200 mg/kg
% Sperm Motility				
Week -1	82.1/91.1♦	92.3	91.6	87.5/93.5
Week 4	93.5	88.3/91.1	90.8	-
Sperm Counts ($x10^6$/mL)				
Week -1	223/149	147	165/137	204
Week 4	371/166	165	170/106	-
Total Abnormalities (%)				
Week -1	31.8	27.1	32.5	35.3
Week 4	23.2	27.5	39.1**	-
Midpiece Abnormalities (%)				
Week -1	13.5	13.4	16.8	21.2
Week 4	9.2	13.3	27.7**	-

** p<0.01

8. Sacrifice and Pathology

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All rabbits were subjected to a necropsy examination. The following organs/tissues were submitted in fixative from males of the 100 and 200 mg/kg groups dying during the study: epididymis, prostate, seminal vesicles, testis, and macroscopically abnormal tissues. For the remaining 4 high-dose males sacrificed after 14 days of treatment, the following were submitted: epididymis, prostate, seminal vesicles, testis, stomach, duodenum, jejunum, ileum, cecum, colon, rectum, liver, kidney, and macroscopically abnormal tissues. For the rabbits surviving to study termination (53 days), the lung was submitted in addition to the tissues listed above. With the exception of the testis and epididymis, which were fixed in Bouin's fluid, all tissues were fixed in 10% neutral buffered formol saline. For the high-dose group, samples were taken from those organs of the survivors with macroscopic lesions and cultured for evidence of bacterial infection. Additionally, samples were also taken from the cecum and cultured for fecal pathogens.

RESULTS

Gross Pathology: There were no differences noted in the findings among the groups surviving to study termination in week 4. However, there were numerous changes noted in those dying on test or sacrificed *in extremis* that were not observed in the survivors. These included enlarged heart (one high-dose, 2 mid-dose), pale areas on the myocardium and rigidity of the heart wall (1 mid-dose), and excess pericardial and peritoneal fluid (1 high-dose).

Microbiology: There was no evidence of bacterial infection in the high-dose rabbits sacrificed in week 3.

Organ Weights : No organs were weighed.

Histopathology: Several microscopic changes in the heart, liver, and lungs were observed in those rabbits dying on test. None were observed in the low-dose rabbits or those surviving to study termination. In the liver, diffuse and periportal fatty vacuolation was observed in the mid- and high-dose rabbits dying on test.

C. DISCUSSION

The objective of the study was to investigate the effects of Molinate on the fertility of male rabbits when administered orally. The study was to have continued for 12 weeks but, because of unanticipated deaths at the mid- and high-dose levels, was terminated in week 8 (week 3 for the high-dose animals). Attempts to identify the cause of the deaths were unsuccessful. In general, the findings at necropsy and the microscopic examination are common, non-specific findings in moribund animals. Additionally, the minor inhibition of erythrocyte cholinesterase activity was not of sufficient

magnitude to compromise the animals. In the liver, periportal fatty vacuolation was observed in the mid- and high-dose rabbits dying on test. Since fatty vacuolation commonly seen in moribund animals has a distribution that is characteristically centrilobular, the possibility exists that this lesion was due to Molinate. Although there was a reduction in the fertility of male rabbits, which was associated with an increased incidence of sperm abnormalities, no definitive statement can be made with respect to the potential of Molinate to affect male fertility due to the problems (deaths and premature termination of the study) encountered in this study. However, TB II notes that increased pre-implantation loss and decreased number of fetuses are effects noted in the rat fertility studies on Molinate.

D. CONCLUSION

Under the conditions of the study, oral administration of Molinate at a dose level of 200 mg/kg resulted in deaths and, consequently, the assessment of fertility at this dose level was precluded. The limited data available for the mid-dose (100 mg/kg) male rabbit suggest a reduction in fertility, which is associated with an increased incidence of sperm abnormalities. Additionally, there was an increase in pre-implantation loss and decrease in the number of live fetuses at this dose level at week 4. Although there were no differences noted at the low-dose (10 mg/kg) level compared to the control group, the duration of the study was only 8 weeks, with fertility being assessed only during week 4 of dosing.

This study is classified Unacceptable; it cannot be upgraded, and it does not satisfy any guideline requirement.