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

JUL 15 1992

MEMORANDUM

SUBJECT: Developmental and Reproductive Toxicity Peer Review
Of Molinate

FROM: Gary J. Burin, Ph.D., D.A.B.T. 5/25/92
Executive Secretary
Developmental/Reproductive Toxicity Peer Review Committee
Science Analysis and Coordination Branch
Health Effects Division (H7509C)

Karen Whitby, Ph.D.
Review Section II
Toxicology Branch II

TO: Jay Ellenberger, Chief
Accelerated Reregistration Branch
Special Review and Registration Division (H7508C)

The Health Effects Division Peer Review Committee (PRC) for Developmental and Reproductive Toxicity met on December 12, 1991 to discuss and evaluate the weight-of-the-evidence on molinate with particular reference to its potential for reproductive and developmental toxicity. This was the first evaluation of molinate by the PRC.

The Committee concluded that molinate causes effects on male reproduction in dogs, mice and rats. The lowest NOEL is 0.2 mg/kg/day, found in the rat, based upon effects on sperm measures and fertility. Female reproductive toxicity was observed in the rat and a NOEL of 0.3 mg/kg/day was established based upon histological changes in the ovary observed at higher dose levels. Additional testing was recommended to further investigate reproductive toxicity.

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A. Individuals in Attendance:

1. Peer Review Committee: (Signatures indicate concurrence with the peer review unless otherwise stated.)

Penelope A. Fenner-Crisp

Karl Baetcke

Marcia Van Gemert

Gary J. Burin

Bob Sonawane (ORD)

Thomas F.X. Collins (FDA)

Laurence D. Chitlik

Roger Gardner

James Rowe

Hugh Pettigrew

Stephen Dapson

2. Reviewers: (Non-committee members responsible for data presentation; signatures indicate technical accuracy of panel report.)

Karen Whitby

Linda Taylor

Clark Swentzel

3. Peer Review Members in Absentia: (Committee Members who were unable to attend the discussion; signatures indicate concurrence with the overall conclusions of the Committee.)

William L. Burnam

Jennifer Seed (OTS)

Reto Engler

Jennifer Orme Zavaleta (OW) Mary Bunny for Jennifer Orme Zavaleta

David Anderson David M Anderson

4. Other Attendees: Mark Dow, Larry Dorsey.

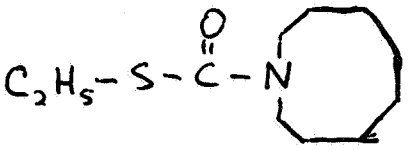
B. Material Reviewed:

The material available for review consisted of DER's, one-liners, and other data summaries prepared by Drs. Karen Whitby and Linda Taylor. The material reviewed is attached to the file copy of this report.

C. Background Information:

This is the first review of molinate by the PRC.

Molinate has the following chemical structure:



Molinate is a selective, pre-emergence, thiocarbamate herbicide for use on rice only. The chemical name is S-ethyl hexahydro-1H-azepine-1-carbothioate. It is also known by the synonym Ordram, and as R-4572. The molecular formula is $C_9H_{17}NOS$. The molecular weight is 187.3. Molinate is miscible with most organic solvents at 20°C such as acetone, ethanol, and xylene; it is soluble in corn oil. The water solubility is 800 ppm at 20°C. The product pH is 9.0.

D. Studies Pertaining to Developmental Toxicity

1. Developmental Toxicity in the Rat. Ciba- Giegy Study No. T-13266. Date of Study: April 14, 1989 (Study Initiation), May 19, 1989 (Study In-Life Termination)

Molinate technical (purity 97.6%) was administered on days 6 through 15 of gestation by gavage in a corn oil solution (5 ml/kg) to sperm-positive Crl: CD (SD) BRVAF/PLUS female rats, at 0, 2.2, 35, and 140 mg/kg. Dosing was based upon gestation day 6 body weight. There were twenty-six rats assigned to each group. Approximately one-half of each litter was eviscerated and subsequently processed and examined for skeletal anomalies. The second half

of the litter was decapitated and the heads were fixed in Bouin's fixative for subsequent examination (Wilson, 1965). The trunk of these fetuses was examined internally (Staples, 1974), eviscerated, and the trunk and limbs processed for possible skeletal examination.

The highest dose tested (HDT), 140 mg/kg, produced a significant inhibition of body weight gain during the dosing period, the post dosing period, and from day 0 to study termination. Corrected body weight gains for days 6-21 and 0-21 were also significantly reduced at the HDT. Maternal food consumption was significantly reduced at 140 mg/kg from day 6 until the conclusion of the study.

There was a significant increase ($p < 0.01$) in salivation at the HDT. Nine of the 23 dams in this group displayed this finding. Dehydration was observed in 1/23, 1/23, and 5/24 dams in the 0, 35, and 140 mg/kg groups, respectively. There was a dose-related reduction in RBC cholinesterase activity which was significant in the 140 mg/kg group.

Data obtained during caesarean sections indicated a significant reduction in the number of live fetuses per dam, fetal weight, and reduced weight of the reproductive tract at the HDT. Significant increases in postimplantation loss and the number of litters with resorptions (100%) were also observed in the HDT. The mean litter percent of normal pups observed during the soft-tissue examination was significantly reduced in the HDT and soft tissue variations observed in the trunk via the Staples technique were significantly decreased. Increases were observed in the incidence of dilated cerebral ventricles at the HDT. There was a dose-related increase in the mean litter percentage of the number of runts (runts were defined as fetuses that weighed less than 3/4's of the litter mean) with values of 0, 0.71, 1.13 and 4.38 reported for the 0, 2.2, 35 and 140 mg/kg/day dose levels, respectively. The PRC concluded that the control value was unusually low in this study and that the increase in runting was not biologically significant at the low dose level. Other effects observed at the HDT were a significant reduction in the weight of fetuses (both sexes combined), a statistically significant decrease in the mean litter percentage of normal pups and an increase in variants during the external examination. Pointed snout was reported in one low and one high dose fetus. The fetus with the pointed snout in the high dose group also had arrested development and a domed cranium.

Observations at the lowest dose tested (including an increase in runting, and the fetus with pointed snout noted above) were not considered by the PRC to be related to treatment due to their very low incidence and the lack of a dose response. The PRC concluded that the NOEL for developmental toxicity was 2.2 mg/kg in this study based upon an increase in runting at 35 and 140 mg/kg dose levels. The NOEL for maternal toxicity was 35 mg/kg/day based upon increased salivation, dehydration and decreased RBC acetylcholinesterase activity at the highest dose level.

2. Developmental toxicity study in the rabbit. Stauffer Chemical Company, Study No. T-11866, Farmington, Connecticut, December 20, 1984.

Molinate technical (purity 98.8%) was administered on days 7 through 19 of gestation by

gavage in a corn oil solution (0.5 ml/kg) to sperm positive New Zealand White [D1a:(NZW) SPF] female rabbits at 0, 2, 20, and 200 mg/kg. Dosing was based upon gestation day 7 body weight. There were 16 rabbits assigned to each group (except for the HDT which had 17).

All fetuses were weighed and examined for external abnormalities. The head of each fetus was removed and fixed in Bouin's fixative for examination by a modified Wilson technique (1965). The trunk of each fetus was examined, and the sex determined, by a modified Staples technique (1974). The fetuses were then eviscerated and processed for skeletal examination by a modification of the Kimmel and Trammel (1981) technique.

Maternal toxicity was observed at the 200 mg/kg level in the form of an increased incidence of abortions, a significant reduction in maternal body weight gain during days 14-21 and significantly increased absolute and relative liver weights. An increase in the observation of dark brown livers was also observed in does. The NOEL for maternal toxicity was 20 mg/kg.

A decrease in the % of does with live fetuses was observed at the HDT. The percentage of pregnant does with live fetuses at term was 94, 93, 85, and 71% for the 0, 2, 20, and 200 mg/kg groups, respectively, and the percentage of pregnant does that aborted was 6, 7, 8, and 24% for the 0, 2, 20, and 200 mg/kg groups, respectively. There was a statistically significant reduction in the mean litter percentage of incompletely ossified 5th sternebrae in all of the treated groups and a statistically significant reduction in the mean litter percentage of other sternebrae incompletely ossified at the HDT. These findings were not considered by the PRC to be biologically significant. The incidence of unossified 5th sternebrae in the 200 mg/kg group was not significantly increased, but was greater than that observed in the concurrent or historical control. Although there was a decrease in supernumery ribs at 200 mg/kg/day, it was not possible to conclude the decrease was associated with molinate exposure.

The NOEL for developmental toxicity was 20 mg/kg based upon a decrease in the percentage of pregnant does with live fetuses at term at the HDT. The LEL for this study was considered to be 200 mg/kg. The NOEL for maternal toxicity was also 20 mg/kg.

The PRC noted that heads of fetuses were examined for external malformations and sectioned using the Wilson technique but were not stained and examined for defects in ossification. The lack of any suggestion of cranial defects from gross external examination and from careful examination through soft tissue sectioning (Wilson technique) and the absence of effects on bone development at other sites in this study and in the rat developmental toxicity study led the majority of the PRC to conclude that the study is acceptable despite this deficiency. Some members of the PRC noted that the development of cranial bones may be altered without other signals from external or soft tissue sectioning and that the lack of examination of cranial bones after clearing and staining is a serious deficiency.

B. Reproduction Studies

1. 3-Generation Reproduction Study in Rats. Testing Facility: Woodard Research Corp.

Laboratory and Consulting Service Herndon, VA. Report Date: June 3, 1977

Molinate was added to the diet of Charles River CD rats at concentrations of 0, 0.063, 0.2, and 0.63 mg/kg. The F_o animals were mated at 100 days of age for 10 days. Half of the F_{1a} pups were fixed in Bouin's fixative and later examined for visceral abnormalities. The remaining half were stained with Alizarin Red for skeletal analysis. The F_{1b} were placed on test diet at weaning and then mated at approximately 100 days of age. The F_{2a} generation was carried through lactation before being examined and discarded. The F_{2b} generation was on treated diets for approximately 50 and 85 days respectively, before they were mated to produce the F_{3a} and the F_{3b} generation.

No effects were reported on food consumption. Mean body weight for the F_{1b} males was slightly reduced in the high dose group. The mean body weight for the F_{3b} male and female pups at weaning was 10-20% lower than controls. Mean absolute heart, liver, and kidney organ weight were reduced relative to control whereas the relative organ weights were unaffected.

The majority of the standard reproductive indices were not reported for this study. Adequate data are not available for calculation of separate gestation and fertility indices. A Pregnancy Rate Index for number of litters/number of females available for breeding and a modified gestation index (number of litters with live pups X 100/number females available for breeding) was provided. Survival at the highest dose level in the F_{1b} generation may have been adversely affected as indicated by the reduced number of females available for mating in subsequent generations.

No treatment-related findings were found in the skeletal and soft-tissue examinations performed with the F_{1a} pups and no treatment related findings were reported. Autopsies were conducted on the F_{3b} pups. Evaluation of the pups at birth and weaning from the various litters revealed no gross abnormalities. There were no obvious developmental findings observed in either surviving or non-surviving pups. Histological examination of pups did not reveal any difference between control and experimental groups. Microscopic appearance of the testes indicated that the male pups had not yet reached sexual maturity; no difference was detected between Molinate and control pups.

The information contained in this study was considered by the PRC to be inadequate to determine a NOEL for reproductive toxicity primarily due to lack of data necessary to calculate reproductive indices.

2. Two Generation Reproduction Study in the Female Rat. Ciba-Geigy Environmental Health Center. Farmington, CT. Study No. T-13218, September 2, 1988.

Molinate technical (purity 97.6%) was administered in the diet with 0.1% corn oil at 0, 6, 50, and 450 ppm to 25 female P_o and P_1 Crl:CD (SD) BRVAF/Plus rats per group. The females were mated after 60 days of treatment in a 1:1 ratio with untreated proven males. P_o females were treated until necropsy. Twenty five females were randomly selected from the

offspring and were treated for 63 days (approximately 108 days of age), then mated with freshly introduced untreated proven males in a 1:1 ratio. The P_1 females were necropsied after weaning at approximately 201 days.

Weekly body weights were recorded for unmated females during the mating period. Maternal body weight and food consumption were measured on gestation days 0, 6, 13, and 20. Maternal food consumption was recorded on postnatal days 4, 7, 14, and 21. On postnatal days 0, 4, 7, 14, and 21 maternal body weight, total litter size, number of live and dead pups, external pup anomalies, sex, and weight of each pup were recorded. Litters were culled to 4 males and 4 females where possible on postnatal day 4. The culled and weaned pups were subject to an internal exam by a modification of the Staples technique (1974). The heads of the culled pups were fixed in Bouin's fixative and examined by the Wilson technique (1965).

Based on decreased body weight, body weight gain, food consumption, and significant changes in absolute brain [P_1 generation ($p<0.01$ two tailed test)] and relative kidney weights [P_o generation ($p<0.01$ two tailed test)], the NOEL for systemic toxicity was 6 ppm (LDT) and the LEL was 50 ppm (MDT). The findings at 450 ppm included reduced body weight, body weight gain, food consumption, fecundity (uterine implants and litter size) and an increased incidence of vacuolation/hypertrophy of the ovary in both generations. In the high dose of the P_o generation, 16 animals were found to have vacuolation at grades 1 & 2 and 9 at grade 3. No animals had a grade higher than 3. For the P_1 generation, 18 animals in the HDT were found to be grades 1 & 2 and 9 for grade 3. At the mid dose of the P_o generation, 2 animals were diagnosed as grades 1 & 2 and 4 animals of the mid dose of the P_1 generation were found to be grades 1 & 2.

The NOEL for female reproductive toxicity was 6 ppm (equivalent to 0.3 mg/kg/day). The LEL for reproductive toxicity was 50 ppm based on the reduced fecundity and increased incidence of ovarian histopathological findings.

3. 13 - Week Inhalation Toxicity Study and Reproduction - Fertility Study of R-4572 in the Rat. Testing Facility: Bio/dynamics Inc. East Millstone, NJ, Study No. : 78-7153 & 78-2346. Date of Study: March 1979 (Necropsy)

Eighty Sprague-Dawley rats (10/sex/group) were equally divided into four groups. The nominal exposure was 0, 2, 10, and 50 mg/m^3. The animals were exposed to Ordram (purity not given) by inhalation for 6 hours/day five days a week. The mean actual exposure was 0, 2.2, 11.1, and 42 mg/m^3.

All animals survived the 13 week study. The male and female rats in the HDT had significant decreases in mean body weight on weeks 1-13. Brain cholinesterase was significantly decreased in the males of the MDT and both sexes of the HDT at week 13. Increased adrenal weight was observed in the males of the MDT and both sexes of the HDT. Decreased pituitary weight was observed in both sexes at the HDT. Females in the HDT also had increased thyroid weights. The testes of 3 rats in the HDT were smaller than normal. Microscopic examination

revealed testicular degeneration in 8 high dose, 3 mid dose, and 4 low dose, and 0 control rats. The degeneration was focal and mild in affected low and mid dose animals. The lesion was more severe and extensive in the high dose animals. Significant numbers of abnormal sperm were observed in the epididymides of all high dose, one mid dose, and two low dose rats. The sperm count was also significantly decreased in six high dose and one low dose male rat. An NOEL could not be established for this phase of the study.

- Reproduction phase.

Ten males were assigned to the same treatment regimen as above in the inhalation study. This study had four mating intervals:

a) Following a one month exposure to test substance, each male was caged with two unexposed females nightly for 10 consecutive days.
b) Each male was caged with two unexposed females nightly for the last 10 consecutive days of the 3 month treatment period.
c) Each male was caged with two unexposed females nightly for 10 consecutive days one month after the last day of treatment.
d) Each male was caged with two untreated females nightly for 10 consecutive days 3 months after the last day of treatment.

The body weight of the mid and high dose males were slightly lower than controls. There was a significant reduction in testicular weight at 50 mg/m^3. At the LDT (2 mg/m^3) there were significant dose related reductions in the mean number of implantations and number of fetuses at the one and three month treatment periods. Significant treatment-related effects were not observed at the one or three month posttreatment recovery matings.

5. Suppression of Fertility in Male Rats. Litton Bionetics, Inc. Kensington, MD. LBI Project No. 2621, Report Date Oct. 29, 1976.

Molinate technical (purity not specified) was administered in the diet to Charles River (Crl:COBS CD (SD) BR) rats as follows:

Group	No. of Male Rats	Dose (mg/kg)
1	20	0
2	10	0.2
3	10	1.0
4	20	5.0
5	10	0
6	10	5.0

The animals in groups 2, 3, and 4 received these diets for nine days. The test material was

then suspended in corn oil and administered by gavage for five days during mating. The dose was miscalculated during gavage for group 4; the actual dose administered was 2.0 mg/kg. Animals in this group which were retained for recovery matings continued to receive the test material in the diet for this five day period. Group 6 received the test diet for 14 days. Group 1 males were gavaged with 10 mg/kg of corn oil. Males in groups 1 and 5 received basal diet throughout the study. Ten to 14 days after the initiation of treatment 10 male rats of groups 1 - 4 were paired for mating with 2 untreated females of the same strain and source. Groups 1 and 4 were also mated 2 and 4 weeks after treatment stopped. Groups 5 and 6 were mated immediately after treatment stopped.

Females were sacrificed 13 days after removal from the mating cage. The number of live and dead fetuses, resorption sites, and corpora lutea were counted. Males were sacrificed after mating was completed; their testes were removed and weighed. Sperm samples were obtained and evaluated, and the testes, seminal vesicles, coagulation gland, and prostate were fixed and evaluated for histopathological changes.

Mean body weight was reduced in the males of group 4 during the first week. Thereafter the weight of these animals was significantly higher than the control group weeks 2 through 6. The mean body weight of group 6 was significantly increased relative to the control at week 1 (the increase was also present at week 2 but was not significant). The number of implants was frequently found to exceed the number of corpora lutea. Analyses of semen indicated that agglutination of the semen was more intense in groups 4 and 6. Testicular weight was similar in all groups. Histopathological examination showed increased clumping of sperm in the epididymis in 4/5 rats in group 6. No effects were observed in groups 2 and 3.

6. Fertility Study in Male Rats Mechanism/Site of Action Report. Testing Facility: Stauffer Chemical Co. Environmental Health Center, Farmington, CT Study No.: T-10421. Date of Study: October 24, 1980.

This investigation consisted of four parts, the objectives of which were:

Part I: to determine which phase(s) of spermatogenesis are affected;
Part II: to evaluate effect on fertility after 10 weeks of treatment;
Part III: to evaluate effect on male fertility after 5 weeks of treatment;
Part IV: to evaluate effect of low dose on male fertility after 5 weeks of treatment and to determine a NOEL.

Molinate Technical (purity 98.2%) was administered by gavage to male Charles River Sprague-Dawley rats using corn oil as the vehicle. At terminal sacrifice, blood, sperm samples, and reproductive tissues were taken for evaluation.

The study design was as follows:

TABLE 1: Study Design

Part	# males	Time dosed (mg/kg)	Design	Parameters monitored
I	12/group	5 days 0, 12, 60	mated w/ new ♀ each week for 10 wk	♀ sac. 9-10 d after cohabitation # corpora lutea # implants # viable fetuses # resorptions
II	20/group	10 weeks 0,12	mated w/ 2 ♀/wk for 2 wk after treatment	same as in Part I, T_3, T_4, TSH, LH, FSH,testosterone conc., sperm viability, motility,morphology, concentration, adrenal & testes/epididymides wt. and histology
III	12/group	5 weeks 0, 12, 30	mated w/ 2 ♀ for 1 wk	♀ sacrificed 15 d after cohabitation, otherwise design same as in Part II
IV	12/group	5 weeks 0, 0.2, 4	mated w/ 2 ♀ for 1 wk	same as in Part III

Part I of this investigation found a significant reduction in the following at 60 mg/kg/day: number implants & viable fetuses/litter, the implantation index, number of pregnancies at the third mating; a significant increase in preimplantation loss at the third mating; and a significant reduction in number implants/litter during the 4th mating. Part I suggests that the mid to late stages of the spermatogenic cycle were affected by treatment; the major effect was on the late spermatid stage.

The raw data for this investigation contains numerous incidences where the number of implants exceeds the number of corpora lutea. Females with higher counts of implants than corpora lutea, and those with corpora lutea and no implants (as well as those with no data) were excluded from the calculations for preimplantation and postimplantation losses.

TABLE 2: Fertility Indices

PART I			
Dose (mg/kg)	**0**	**12**	**60**
Week of Mating:			
1	83	75	75
2	83	92	75
3	100	92	67*
4	100	92	92
5	100	100	83
6	100	92	100
7	100	100	100
8	100	100	100
9	92	92	100
10	100	100	100

PART II		
Dose (mg/kg)	**0**	**12**
Week of Mating:		
1 ♂ Fertility Index	95	89
1 ♀ Fertility Index	79	75
2 ♂ Fertility Index	95	83
2 ♀ Fertility Index	84	58*

PART III			
Dose (mg/kg)	**0**	**12**	**30**
Week of Mating:			
1 ♂ Fertility Index	100	100	50*
1 ♀ Fertility Index	92	86	38*

PART IV			
Dose (mg/kg)	**0**	**0.2**	**4.0**
Week of Mating:			
1 ♂ Fertility Index	100	100	73
1 ♀ Fertility Index	79	96	62

* Significantly different from control ($p < 0.05$ by Fisher's Exact Probability test).

All phases of the study showed a reduction in male fertility. Data from phases II-IV show a good correlation between impaired male fertility and statistically significant alterations in sperm viability, morphology and motility. Significant increases were observed in Phase III in

T_4 at 30 mg/kg, testosterone at 12 and 30 mg/kg and FSH at 30 mg/kg.
Sperm abnormalities at 5 and 10 weeks of treatment at dose levels of 4 mg/kg and greater included:

(1) detached sperm heads and tails;
(2) heads and tails bent at abnormal angles;
(3) rupture of sperm membranes at head-midpiece junction and midpiece-tail junction

The percentage of viable sperm, motile sperm and abnormal sperm were all decreased at 4 mg/kg. Sperm counts, numbers of implants, pre-implantation loss and resorptions were also decreased at this dose level.

The PRC considered the NOEL to be 0.2 mg/kg. The LEL was set at 4 mg/kg based upon the effects noted above. The apparent increase in the number of resorptions/litter in the 0.2 mg/kg group (1.1) was considered by the PRC to probably be due to the unusually low number of resorptions in the concurrent control (0.4) and was not considered to be of biological significance.

7. Ordram Antifertility Study in Mice. Testing Facility: Stauffer Chemical Co. Environmental Health Center, Farmington, CT. Study No. : T-10121. Date of Study: April 15, 1980

Molinate Technical (purity 98.2%) was administered by gavage to male CD-1 mice, using corn oil as the vehicle. One hundred males of proven fertility were assigned to five dose groups which received either 0, 2, 20, 100, and 200 mg/kg, respectively. The males were dosed daily by gavage for 7 weeks. Fertility was assessed by mating one male to two females after 2, 4, and 6 weeks of treatment and again after a four week recovery period. An interim sacrifice was performed on 5 males/group at the completion of dosing and a final sacrifice was performed on the remaining males after the end of the recovery period. Testes plus epididymides were weighed. Thyroids, pituitaries, testes, and epididymides were collected and subjected to microscopic examination.

The NOEL for this investigation was 20 mg/kg. There were no significant differences in weekly body weights, and no dose related mortalities. The mating index was reportedly not affected by treatment. Pregnancies from matings with the treated males were significantly fewer than the matings with the control males in the 100 mg/kg dose after 2 weeks of dosing, the 200 mg/kg group after 4 weeks of dosing, and the 100 and 200 mg/kg groups after 6 weeks of dosing. There was a significant downward trend with increasing dose for the fertility indices after 2, 4, and 6 weeks of dosing. No significant trend of this kind was observed after the 4-week recovery period. Significant reductions in the numbers of implants and viable fetuses in the 100 mg/kg treatment group after 2 weeks of dosing and in the 100 mg/kg (implants only at week 4) and 200 mg/kg groups after 4 and 6 weeks of dosing; the number of resorptions remained the same in these groups. No significant differences were observed after the 4 week recovery period. No histological alterations of the male reproductive tissues were attributable

to test compound.

8. Ordram Antifertility Study in Rabbits. Testing Facility: Stauffer Chemical Company Environmental Health Center, Farmington, CT. Study No.: T-10176. Date of Study: August 1980 (Study Termination)

Ordram technical (98.2% purity) was administered to male Dutch Belted rabbits in gelatin capsules. Corn oil was used in dose preparation. Ten males were assigned to the vehicle control group, and 9 males each were assigned to the 2, 20, and 200 mg/kg dose groups. The test material was administered for 6 weeks. During the sixth week of dosing, the males' fertility was tested by mating with the same females he was paired with during the predose fertility test. At the end of the dosing period, 5 males from the control group and 4 males from each treatment group were necropsied. The remaining males were allowed to recover and their fertility was tested again during the fifth week of their recovery period.

Mating behavior was not affected by treatment. The predose male fertility index was 77%. The male fertility index for all groups during the postdose or postrecovery periods was 100%. The predose female fertility index was 54%. The lowest female fertility index during the postdose or postrecovery period, was 60% in the post recovery controls. The lowest female fertility index for treated animals was 81% in the 2 mg/kg postdose group. The litter results from the postdose fertility test did not reveal any treatment related differences.

There were no apparent treatment-related differences in organ weight and histopathology from the interim and final sacrifice. A significant increase was observed in eye irritation with increasing dose, which was reversed after the completion of dosing. The NOEL for antifertility effects was > 200 mg/kg (HDT).

9. The Effect of Ordram on Nonhuman Primate Sperm Production. Testing Facility: Stauffer Chemical Company Environmental Health Center, Farmington, CT. Study No. : T-10714. Date of Report: December 1981.

Ordram technical (98.6% purity) was administered to adult male Macaca fascicularis (crab-eating or cynomolgus macaques) monkeys via a nasal gastric tube with a corn oil vehicle. Seven animals were assigned to each dose group (0, 0.2, 10.0, or 50.0 mg/kg). The animals were dosed five days a week for 12 weeks. Blood was drawn every 4 weeks during the treatment period and analyzed for clinical chemistry, hematology, clotting time, and LH and FSH values. Sperm samples were collected by electro-ejaculation and analyzed weekly during the treatment period.

There were no treatment related changes in body weight or clinical signs, and no mortalities. There was a treatment related decrease in plasma cholinesterase activity in the 50 mg/kg group. Hematology and clotting time were not affected by treatment. There were no treatment related changes in LH or FSH. The percentage of motile sperm in the 0.2 mg/kg group was

significantly lower than the control value during the ninth week of treatment; however, it was not significantly different from the pretreatment value. The percentage of abnormal sperm in the 0.2 mg/kg group was significantly higher than the control value during the second week of treatment. However, this value was not significantly different from the pretreatment value. The sperm cell concentration was consistently lower in the 0.2 mg/kg group (including the pretreatment period). Treatment of non human primates at doses up to 50 mg/kg for 12 weeks did not have a significant dose-related effect on sperm count, ejaculate volume, motility, morphology, or the reproductive hormones which were assayed.

10. "Epidemiology Assessment of Fertility in Male Workers Exposed to Ordram at the Stauffer Chemical Company", D.T. Taves, A.T.K. Cockett, C. Cox and J. McCusker, Vol. 3, Report Number 415892-01, April 20, 1984.

Men at molinate manufacturing facilities in California (n=62), Alabama (n=77) and Arkansas (n=77) were assayed for sperm measurements and reproductive histories. No effects were reported on either parameters at cumulative dose levels of 0-1 mg, 1-20 mg or 20 to 1500 mg. However, the number of workers at the highest dose level was very small. A number of shortcomings prevented meaningful interpretation of this study.

III. Additional Toxicology Data

A. Acute, Subchronic and Chronic Toxicity Data

The acute oral LD_{50} for Ordram technical in rats was approximately 550 mg/kg (toxicity category 3 (caution)).

A 13 week feeding study in rats with dose levels of 0, 8, 16, and 32 mg/kg, found ovarian vacuolation at 16 mg/kg (LEL) and increased organ weights at 32 mg/kg. The NOEL for this study was 8 mg/kg/day. A thirteen week study in dogs with dose levels of 0, 450, 900, and 1800 ppm, found increased thyroid weight at 1800 ppm (LEL). The NOEL was 900 ppm.

A 3-week dermal study with dose levels of 0, 10, 25, and 50 mg/kg found no significant systemic toxicity. A three month inhalation study testing 0, 2, 10, and 50 mg/m^3 found testicular degeneration and abnormal spermatozoa at 2 mg/m^3 (see Section B. 3 for a discussion of this study).

A one year dog study was performed at concentrations of 0, 1, 10, 50, or 100 mg/kg/day in gel atin capsules. The 100 mg/kg animals were only dosed for 14 weeks due to toxicity. A variety of indications of toxicity were observed at the middose level including decreases in sperm ejaculate, a reduction in the percentage of motile sperm, and testicular atrophy. The NOEL was 10 mg/kg/day.

A two year rat feeding/carcinogenicity study tested dietary concentrations of 0, 7, 40, 300, or 600 ppm. Decreased body weight, body weight gain, food consumption, RBC cholinesterase,

absolute organ weights, and an increase in the number and severity of lesions found in nervous, muscle, and reproductive tissue lesions were found. There was an increase in testicular degeneration with atrophy grades 3, 4 & 5 at the highest dose level (6, 8, 7, 13 for the control, 7, 40 and 300 groups, respectively), a increase in benign interstitial cell tumor (3, 5, 5, and 7, respectively), and at 300 mg/kg there was a increase in ovarian vacuolation/hypertrophy grades 1 & 2, and 3.

An 18 month carcinogenicity study of Ordram in mice at 0, 10, 100, 1000, or 2000 ppm found decreased survival, body weight gain, food consumption, and increases in several clinical observations indicative of neurological involvement in both sexes at the HDT (i.e. hindlimb muscle weakness, adducted hindlimbs, ataxia, and splayed hindlimbs). The incidence of several nonneoplastic lesions (demyelination & Schwann cell hyperplasia of sciatic nerve, eosinophilic bodies in spinal cord and brain) were increased at the two highest dose levels. These observations correlate with clinical signs. The incidence of testicular degeneration was increased in a dose related manner at 100, 1000, and 2000 ppm. The incidence of ovarian hyperplasia was also increased in a dose related manner at 1000 and 2000 ppm.

B. Mutagenicity

Molinate was nonmutagenic in strains TA98, TA100, TA1535, TA1537, and TA1538 with and without metabolic activation when tested at 1.6, 8, 40, 200, 1000 or 5000 μg/plate. Molinate was not clastogenic in cultured human lymphocytes under the nonactivated and S9 activated systems at 0, 24, 95, or 190 μg/mL. Molinate was found to be mutagenic in the L5178Y +/- mouse lymphoma mutagenesis assay with metabolic activation by both rat and mouse S9 activation systems at 0.01 - 0.1 μL/mL. Additional testing for potential heritable mutations is recommended by the PRC.

C. Metabolism/Pharmacokinetic Data

Doses of unlabeled Molinate were absorbed by the rat and extensively metabolized after both oral and i.v. administration. The main route of elimination after 14 consecutive doses of 10 mg/kg was via the urine (79% females and 83% males). Ninety percent was excreted in 24 hours. Only a small percentage (3-10%) was excreted in the feces and expired air (1-1.5%). The highest tissue levels were found in the blood cells. The total amount retained by the rat at 96 hours post dose was approximately 3.5%. Single doses (10 & 100 mg/kg) of radiolabeled Molinate were similarly handled, i.e.- 70-72% was excreted via the urine; 8-11% in males, or 5% in females, by way of feces, and approximately 1% by expired air. Blood displayed the highest concentration of the radiolabel. A single i.v. dose of 1 mg/kg showed a similar pattern of elimination.

Metabolism of Molinate involves S-oxidation to form the intermediate Molinate sulfoxide, which was either hydrolyzed to hexamethyleneimine or conjugated with glutathione, ultimately

forming mercapturic acid. The ring was hydroxylated at the 3 and 4 positions followed by glucuronide conjugation as another significant route of metabolism. Ten of 22 metabolites were isolated in the urine and identified. These metabolites accounted for 74-86% of the urinary radioactivity. A lower percentage of mercapturic acid metabolites was excreted following i.v. exposure than following oral exposure. No significant sex or dose related differences were noted.

E. Structure Activity Relationships

Molinate is structurally related to eptam, butylate, ethiolate, vernolate, pebulate and thiobencarb. The developmental and reproductive toxicity of these chemicals is summarized in the following table.

TABLE 3: Comparison of Carbothioate Structures for Adverse Developmental/Reproductive Effects

CHEMICAL	TEST/SPECIES	ENDPOINT AFFECTED	EFFECTIVE DOSE
Sutan	Dev. Tox - rat	↓ fetal bw ↑ malaligned sternebrae delayed ossif.	LOEL = 400 mg/kg
		↑ early resorptions	1000 mg/kg (HDT)
	Dev. Tox - rabbit	↑ abs & rel ovarian wt, no develop. effects	500 mg/kg
	Repro. - rat	↓ litter size F_{1a}, F_{1b}, & F_{2b} ↓# live pups day 21 F_{1a} & F_{1b}	4000 ppm (HDT)
	1-yr feeding - rat	uterine & testicular changes w focal hemorrhage	180 mg/kg (HDT)
Eptam (EPTC)	Dev. Tox - rat	↓ fetal bw & ↑ fetal retard., ↑ resorptions	300 mg/kg (HDT)
	Dev. Tox - rabbit	↓ fetal wt	300 mg/kg
	Repro. - rat	↓ fetal wt	800 ppm (HDT)
	1-yr feeding - dog	↓ testes wt	1800 ppm (HDT)
Vernam	Dev. Tox - rat	bw, skeletal	300 mg/kg (HDT)

CHEMICAL	TEST/SPECIES	ENDPOINT AFFECTED	EFFECTIVE DOSE
	Dev. Tox - rabbit	no effects	200 mg/kg (HDT)
	Repro. - rat	↓ maternal bw	Maternal LEL Repro NOEL > HDT
Tillam	Dev. Tox - rat	no data	
	Dev. Tox - rabbit	no data	
	Repro. - rat (1 generation)	↓ pup survival & growth & s.c. hemorrhage in ♂ & ♀	1000 ppm (HDT)
Ethio-late	Dev. Tox - rat	no data	
	Dev. Tox - rabbit	no data	
	Repro. rat	no data	
Thioben-carb	Dev. Tox - rat	↑ runting ↓ fetal bw	150 mg/kg (HDT)
	Dev. Tox - rabbit	fetal deaths & ↓ fetal bw	150 mg/kg (HDT)
	Multigen Repro-rat	+ve trend ↑ in spermhead abnormalities but #/grp is small	effects most striking at 10 (MDT) and 40 (HDT) mg/kg.
Ordram	Dev. Tox - rat	↑ postimp. loss, incidence of runts, S-T & ske. variants, and ↓ bw	140 mg/kg (HDT)
	Dev. Tox - rabbit	↓ oss of sternebrae, % of live fetuses	200 mg/kg (HDT)
	numerous repro/antifert studies	CNS effects, ↓ fecundity, ovarian vacuolation, impaired fertility, test. degeneration, impaired spermatogenesis, etc.	

F. Issues and Recommendations

1. The NOEL for developmental toxicity in the rat appears to be 2.2 mg/kg/day based upon runting observed at higher dose levels. Pointed snout, runting and increases in variations at the low dose level were not considered by the majority of the PRC to be due to molinate exposure. Other forms of developmental toxicity, such as dilated cerebral ventricles, were observed at the highest dose tested (140 mg/kg/day). The NOEL for maternal toxicity in the rat is 35 mg/kg/day. A developmental neurotoxicity study in the rat was recommended based upon the induction of neurotoxicity in other studies.

2. The NOEL for developmental toxicity in the rabbit study was 20 mg/kg based upon a decrease in the percentage of pregnant does with live fetuses and an increased incidence of unossified 5th sternebrae at 200 mg/kg. The NOEL for maternal toxicity was also considered to be 20 mg/kg. Although the bones of the skull were not stained and examined, the study was considered to be acceptable by the majority of the PRC based upon the lack of any indication of alterations of cranial bones in the external and soft tissue examinations and in the trunk. It was also noted that no alterations of cranial bones were observed in the rat developmental toxicity study. The PRC could not determine whether the decrease in supernumerary ribs at 200 mg/kg was related to treatment. However, other developmental effects were determined to be compound-related at that dose level.

3. A number of studies examining reproductive toxicity in male and female rats were available to the PRC. Although the multigeneration reproduction study was considered to be unacceptable, effects on reproduction were observed after treatment of either the male or female in other studies. Decreases in fertility were observed in both genders (with NOELs of 0.2 mg/kg and 2.5 mg/kg for males and females, respectively) accompanied by alterations in ovarian histology in females and sperm measurements in males. The alterations in ovarian histology are considered to be biologically significant and the NOEL was 6 ppm (equivalent to 0.3 mg/kg) and the LOEL was 50 ppm. No acceptable study was available in which both males and females were treated and mated or in which males were treated continuously from conception until mating.

4. The PRC noted that the NOELs for reproductive toxicity varied between species. Although reproductive effects have been observed in rats, mice (NOEL of 1.5 mg/kg) and dogs (NOEL of 10 mg/kg/day), the rat appears to be the most sensitive species with alterations in sperm and fertility observed at dose levels of 2-4 mg/kg/day. Effects on male fertility have not been observed at relatively high doses in rabbits (200 mg/kg) and primates (50 mg/kg). Because information (such as comparative metabolism and pharmacokinetic data) is not available to determine the most appropriate species for extrapolation to man, the PRC recommends that the rat be used for risk assessment purposes.

5. The PRC recommended that further reproductive testing in the rat be undertaken to examine the effects of molinate resulting from in utero exposure through mating and potential effects on fertility which might result from the mating of treated males and females. Because molinate is

distributed to the testes and causes alterations in sperm, and because there is some suggestion of genotoxic potential, testing to determine the potential for heritable mutations is also recommended.

6. The PRC recommended that the rationale for a 100 fold uncertainty factor in the calculation of the RfD be re-examined due to limitations in the data base from which the NOEL was derived (see #3, above).

G. Conclusions

Developmental toxicity (runting) was observed in the absence of maternal toxicity in the rat. The majority of the PRC concluded that the NOEL was 2.2 mg/kg/day. Some PRC members recommended that the study be considered to have no NOEL, based primarily on the increase in runting observed at each dose level. In the rabbit, developmental toxicity was induced only at a level associated with maternal toxicity (NOEL= 20 mg/kg/day). Both studies were considered to be acceptable by the majority of the PRC. The conduct and submission of a developmental neurotoxicity study in the rat was recommended.

Clear evidence for reproductive toxicity was found in the mouse, rat and dog. The most extensive testing is available in what appears to be the most sensitive species, the rat. A NOEL for male reproductive toxicity was established at 0.2 mg/kg/day based upon effects on sperm measures and fertility at dose levels of 2-4 mg/kg/day and greater. The NOEL for histological changes in the ovary is 0.3 mg/kg/day. A NOEL for reproductive effects in rats for the inhalation route of exposure could not be established (LOEL= 2 mg/m^3). No evidence of reproductive toxicity was found in rabbits or monkeys. An epidemiological study of workers exposed during the manufacture of molinate, although reporting no effect, was considered to be inconclusive. However, because no acceptable study is available in which both males and females were treated and mated or in which males where exposed from conception until mating, further study of the reproductive toxicity of molinate in rats is recommended. A protocol should be submitted by the registrant prior to study initiation for an investigation which will address these concerns. Based upon the information available to the PRC, it was recommended that the NOEL of 0.2 mg/kg bw/day found in the most sensitive species (the rat) be used for comparison to human exposure to assess risk.