Linda Taylor

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

012944

OFFICE OF
PREVENTION, PESTICIDES AND
TOXIC SUBSTANCES

DATE: **October 30, 1998**

MEMORANDUM

SUBJECT: *MOLINATE*: - Report of the Hazard Identification Assessment Review Committee.

FROM: Jess Rowland, Executive Secretary,
Hazard Identification Assessment Review Committee
Health Effects Division (7509C)

and

Linda Taylor, Toxicologist
Reregistration Branch 1
Health Effects Division (7509C)

THROUGH: K. Clark Swentzel, Chairman, 11/2/98
Hazard Identification Assessment Review Committee
Health Effects Division (7509C)

TO: Whang Phang, Branch Senior Scientist
Reregistration Branch 1
Health Effects Division (7509C)

PC Code: 041402

On October 1 and 7, 1998, the Health Effects Division's Hazard Identification Assessment Review Committee (HIARC) evaluated the toxicology database for Molinate, re-assessed the existing Reference dose, and selected the doses and toxicological endpoints for dietary and non-dietary exposure risk assessments. The HIARC also addressed the potential for enhanced sensitivity of infants and children from exposure to Molinate as required by the Food Quality Protection Act (FQPA) of 1996. The Committee's conclusions are presented in this report.

Committee Members in Attendance

Members present were Karl Baetcke, William Burnam, Robert Fricke, Karen Hamernik, Susan Makris, Melba Morrow, John Redden, Jess Rowland (Executive Secretary) and Clark Swentzel (Chairman). Data was presented by Linda Taylor of Reregistration Branch 1.

Other HED members present at the meeting were Jeff Dawson, Sanju Diwan, Paula Deschamp, Wilkelmena Livingston, Mike Metzger, Chris Olinger, Whang Phang, Brenda Tarplee, and Yung Yang.

Data Presentation: _____
Linda Taylor
Toxicologist

Report Preparation: _____
Jess Rowland.
Executive Secretary

I. INTRODUCTION

On October 1 and 7, 1998, the Health Effects Division's Hazard Identification Assessment Review Committee (HIARC) evaluated the toxicology database for Molinate, reassessed the existing Reference dose, and selected the doses and toxicological endpoints for dietary and non-dietary exposure risk assessments. The HIARC also addressed the potential for enhanced sensitivity of infants and children from exposure to Molinate as required by the Food Quality Protection Act (FQPA) of 1996. The Committee's conclusions are presented in this report.

II. HAZARD IDENTIFICATION

A. Acute Reference Dose [RfD]

Study Selected: Developmental neurotoxicity study -Rat §83-6
Guideline #: OPPTS 870.6300

MRID No. 44079201

EXECUTIVE SUMMARY: In a developmental neurotoxicity study (MRID 44079201), Molinate [96.8% a.i.] was administered to 30 female Alpk:AP$_f$SD rats/group in the diet at dose levels of 0, 20, 75, and 300 ppm (0, 1.8, 6.9, and 26.1 mg/kg/day, respectively) from gestation day 7 through lactation day 11.

MATERNAL TOXICITY: There was no evidence of a treatment-related effect on maternal survival or clinical signs of toxicity. Mean maternal body weight values for the 300 ppm group were decreased slightly [93%-94% of control] from day 10 of gestation and throughout lactation [89%-95% of control] compared to the controls. Mean body weight gain at the 300 ppm dose level was decreased prior to dosing [days 1-4 of gestation (88% of control)] and during gestation days 7-22 [76% of control] and 1-22 [80% of control]. During the first 3 days of dosing, dams at the 300 ppm dose level displayed a negative body-weight gain. During lactation, the 300 ppm dose group displayed a negative body-weight gain during days 1-7, and the overall body-weight gain for both the mid- and high-dose groups was decreased [70% and 73% of control, respectively] compared to the control. A statistically significant reduction in group mean food consumption was noted in the 300 ppm group throughout gestation [73%-94%-of control] and lactation [75%-87% of control] compared to the control group.

Litter size and the number of pups born live/dead were comparable among the groups, and the mean number of total pups born and live birth index were unaffected by treatment. The mid- and high-dose groups displayed the lowest percent of litters with all pups born live compared to the controls. At 300 ppm, there was an increase in the number of litters with small female pups, a slightly higher mortality rate during days 1 to 5 *post partum*, and the number of missing and presumed dead pups [both sexes] was increased compared to the controls. Whole litter losses occurred at the control [2 litters] and high-dose [4 litters] levels only.

There were no treatment-related findings observed in the dams at necropsy [brain weights were not measured].

SELECTED F1 OFFSPRING: At the high-dose level [300 ppm], there was an increase in mortality, and a higher number of 300 ppm pups were reported missing/presumed dead compared to the controls. There was an increase in the number of small pups of both sexes at 300 ppm compared to the control group. There was no effect on the sex ratio [percent males]. Decreased body weight was observed at the 300 ppm dose level for both sexes [males 73%-84%/females 72%-82% of control] from days 5-29 of lactation, and the decrease continued post weaning [days 29-63], although the magnitude of the decrease in both sexes [males 81%-88%/females 84%-91% of control] decreased with time. Decreased body-weight gains were observed mainly during the preweaning period in both sexes [64%-84% of control] at 300 ppm. There was a delay in both preputial separation and vaginal opening at 300 ppm compared to the control groups.

On day 23 *post partum*, there was a significant decrease in the startle amplitude for both sexes at 300 ppm at all 5 intervals, and the females at this time point displayed a dose-related decrease in the startle amplitude, which was statistically significant at all dose levels in 3 of 5 intervals. Males at all dose levels and females at the low- and mid-dose levels displayed comparable responses to those of the controls on day 61, but the high-dose females continued to display a decrease in startle amplitude on day 61. Time to maximum amplitude was increased on day 23 in the high-dose males only and only during the second interval. On day 61, females at 300 ppm displayed an increase in the time to maximum amplitude during 4 of the 5 intervals.

Motor activity was comparable among the female groups, but an effect on this parameter cannot be ruled out for males at the 300 ppm dose level because of the initial [day 14] decrease and subsequent, sustained [days 22 and 60 *post partum*], increase in motor activity observed.

Straight-channel swimming time was increased in both sexes on day 21 *post partum* compared to the controls but comparable among the groups at all other time points. In both the initial learning [day 21] and memory [day 64] phases of the Y-shaped water maze test, both sexes at 300 ppm had a lower percentage of successful trials compared to the controls throughout the test. In the subsequent learning [day 59] and memory [day 62] phases of the Y-shaped water maze test, comparable successes were observed among the groups [both sexes].

There was a treatment-related decrease in absolute brain weight in both sexes at 300 ppm at both the day 12 and day 63 sacrifice times. Brain length was decreased in both sexes at 300 ppm on day 12, and the females of this group also displayed a decrease in brain width. At day 63, slight decreases in both length and width were observed in both sexes at 300 ppm, but statistical significance was not attained.

There were no treatment-related findings at necropsy on either day 12 or day 63, no microscopic abnormalities in the brains of any pups on day 12, and there were no changes in the central or peripheral nervous systems on day 63 that could be attributed to treatment. With respect to morphometric measurements, treatment–related changes in the cortex and/or cerebellum of the brain [decreased structural measurements and decreased thickness of cellular layers] were observed at the mid- and high-dose levels on day 12, and treatment-related changes in the cortex, hippocampus, and/or cerebellum were observed at the 300 ppm dose level on day 63.

The NOAEL for maternal toxicity is 75 ppm [6.9 mg/kg/day], and the LOAEL for maternal toxicity is 300 ppm [26.1 mg/kg/day], based on decreased body weight/gain and food consumption.

The NOAEL for developmental neurotoxicity was not determined, based on a reduction in startle amplitude in the auditory startle test in females [day 23] at all dose levels. The developmental LOAEL is 20 ppm [1.8 mg/kg/day]. At 75 ppm [6.9 mg/kg/day], in addition to the reduction in startle amplitude in the auditory startle test, there were treatment-related reductions in some morphometric measurements in areas of the cerebellum of the brain [day 12] in both sexes.

At 300 ppm [26.1 mg/kg/day], (1) increased mortality, (2) decreased body weight, (3) a delay in the appearance of developmental landmarks [preputial separation and vaginal opening], (4) an increase in swimming time in the straight channel test at day 21 and reduced performance in the learning and memory tests on days 21 and 24, respectively, (5) a reduction in startle amplitude, (6) an increase in the time to maximum amplitude [days 23 and/or 61], (7) a possible increase [slight] in mean motor activity level in males, (8) reduced brain weight [both sexes on days 12 and 63], brain length [both sexes on day 12], and brain width [females on day 12], and (9) reductions in several morphometric measurements in areas of the cortex, hippocampus, and cerebellum of the brain were observed.

Dose and Endpoint for Risk Assessment: Developmental LOAEL = 1.8 mg/kg/day based on neurotoxic effects at the lowest dose tested; a NOAEL was not achieved.

Uncertainty Factor(s): 300 which includes 10x for inter-species extrapolation, 10x for intra-species variation, and 3x for the use of a LOAEL (i.e, lack of a NOAEL in the critical study).

Comments about Study and Endpoint: The neurotoxic effects observed in pups are appropriate for acute risk assessment because: 1) increased susceptibility was demonstrated in offspring when compared to maternal animals; 2) reduction in startle amplitude in the auditory startle test was seen on *post partum* day 23 and reductions in some morphometric measurements in areas of the cerebellum of the brain in both sexes of offspring was seen on *post partum* day 12; 3) neurotoxicity and reproductive effects were observed at higher doses in the database; 4) male reproductive effects are observed at comparable doses following short-term exposure in another study; and 5).the acute neurotoxicity study is unacceptable due to technical deficiencies and can not be used for regulatory purposes. Typically, when a developmental endpoint is selected it is applicable for risk assessment of Females 13+ only (since the effects occur *in utero)*. Therefore, another endpoint would be selected for the general population including adult males as well as for infants and children. However, for instance, the HIARC concluded that the dose/endpoint selected here can also be used for acute dietary risk assessment for all population since the effects were seen in the offsprings during *post partum* days (i.e., not *in utero*), and therefore, would be protective of all populations including adult males and females as well as infants and children.

Uncertainty Factor(s): 300 which includes 10 for inter-species extrapolation, 10x for intra-species variation and 3x for the use of a LOAEL (i.e, lack of a NOAEL in the critical study).

$$\text{Acute RfD} = \frac{1.8 \text{ mg/kg}}{300} = 0.006 \text{ mg/kg}$$

This risk assessment is required.

B. Chronic Dietary [Reference Dose (RfD)]

Study Selected: 2-year chronic toxicity - Rat §83-5
Guideline #: OPPTS 870.4300

MRID No.: 41815101

Executive Summary: In a 2-year chronic toxicity/carcinogenicity study in rats [MRID 41815101], Crl:CD®(SD)BR rats [50 rats/sex/ treatment group] were administered Molinate [97.6%] via the diet at dose levels of 0 ppm, 7 ppm [♂♂ 0.3/♀♀ 0.4 mg/kg/day; standard conversion factor], 40 ppm [♂♂ 1.8/♀♀ 2.0 mg/kg/day], and 300 ppm [♂♂ 13/♀♀ 15 mg/kg/day] for 24 months. A satellite group of rats [20 rats/sex] was administered Molinate via the diet for 12 months at a dose level of 600 ppm [≈30 mg/kg/day] to evaluate pathology other than neoplasia. An additional 20 rats/sex of the control group and 10 rats/sex/group of the Molinate rats were sacrificed at 12 months.

Survival was not adversely affected by treatment. Neurological signs [adducted hindlimbs, ataxia, atrophied hindlimb, atrophied sacral region, atrophied thigh], which were first noted during the 21st month, were observed at the high-dose level in both sexes, although the males were affected more than the females. Decreased body weight, body-weight gain, and food consumption were observed at the 300 ppm [BW 88% ♂♂/87% ♀♀ of control at 54 weeks; 92% ♂♂/95% ♀♀ of control at 12 weeks]/BWG ♂♂ 85%/♀♀ 83% of control for 0-13 week interval; ♂♂ 79%♀♀ 70% overall] and 600 ppm [♂♂ 78%/♀♀ 71% of control for 0-13 week interval] dose levels in both sexes. The decrease in body weight in males was observed throughout the study, but the decrease in females at the 300 ppm dose level was not observed until the ≈12 weeks due probably to the fact that this group weighed ≈6% more than the control group initially.

RBC cholinesterase was decreased in both sexes at the 300 ppm [♂♂ 61%-86%/♀♀ 67%-85% of control] and 600 ppm]♂♂ 68%-72%/♀♀ 58%-63% of control] dose levels throughout the study. Brain cholinesterase was decreased in females at the 300 ppm [87% of control] and 600 ppm [84% of control] dose levels at the 12-month sacrifice.

After 12 months of treatment, there was an increased incidence of muscle thinness, especially in males at the 300 ppm and 600 ppm dose levels. Decreased brain weight was observed in males at the 300 ppm dose level at 12 months, in both sexes at the 600 ppm dose level at the 12-month sacrifice, and in both sexes at 300 ppm at study termination. Increased adrenal weight was observed in males at the 600 ppm dose level at 12 months.

The decrease in testes weight and the slight increase in ovarian weight observed at the 12-month sacrifice at 600 ppm and that observed at 300 ppm at study termination are considered treatment-related, and this is supported by the microscopic findings in these organs.

The incidence of degeneration/demyelination of the sciatic nerve was increased in a dose-related manner in both sexes, the severity was increased with dose, and the increase was noted at all dose levels. There was a dose-related increase in the incidence of muscle atrophy/reserve cell hyperplasia in both sexes in the main study rats. In males at the 300 ppm dose level, there was an increased incidence of spinal cord degeneration and eosinophilic bodies in the sacral area compared to the controls. There was a dose-related increase in the incidence of thecal/interstitial cell vacuolation/hypertrophy in the ovary, and males at 300 ppm displayed an increase in the incidence of degeneration with atrophy of the testes.

Kidney tumors [2 benign cortical adenomas and 3 carcinomas] were observed in males at 300 ppm, with none found in any of the other groups. There was a slight increase in the incidence of interstitial cell tumors in treated males compared to the control males, and there were 2 males [3.3%] at the 300 ppm dose level with mesothelioma in the testes.

The LOAEL is 7 ppm [♂♂ 0.3/♀♀ 0.4 mg/kg/day], based on the increased incidence of degeneration/demyelination in the sciatic nerve and atrophy/reserve cell hyperplasia in the muscle. No NOAEL for these effects was observed in this study.

Dose and Endpoint for Establishing RfD: LOAEL = 0.3 mg/kg/day based on degeneration/demyelination in the sciatic nerve and atrophy/reserve cell hyperplasia in the muscle at the lowest dose tested; a NOAEL was not achieved.

Comments about Study and Endpoint: The existing RfD is based on a NOAEL of 0.2 mg/kg/day established in a 5-week fertility study (MRID No. 000245675) in which males only were dosed and then mated to untreated females. Reproductive effects characterized as decreased fertility, decreased number of viable fetuses/litter, increased number of sperm abnormalities were observed at 4 mg/kg/day and above. The HIARC determined that this study is not appropriate because of the short-duration of exposure (5 weeks) and therefore, selected the 2-year rat study with chronic exposure regimen which is appropriate for chronic dietary risk assessment. In addition, the neuropathology observed in the 2-year study is consistent with the neurotoxicity seen in other studies (short- and long-term) in the database.

Uncertainty Factor(s): 300 which includes 10 for inter-species extrapolation, 10x for intra-species variation and 3x for the use of a LOAEL (i.e, lack of a NOAEL in the critical study).

$$\textbf{Chronic RfD} = \frac{\textbf{0.3 mg/kg/day}}{\textbf{300}} = \textbf{0.001 mg/kg/day}$$

This risk assessment is required.

C. Occupational/Residential Exposure

There are no residential uses. Therefore, doses and toxicology endpoints were selected only for occupational exposure risk assessments.

1. Dermal Absorption

Study Selected: Dermal absorption study 85-3 Guideline #: OPPTS 870.7600

MRID No.: 43284101

Executive Summary: In a dermal absorption study [MRID 43284101], radiolabeled Molinate [99% a.i.] was applied dermally to male Crl:CD(SD)BR rats [4/dose/exposure period] on the dorso-lumbar region [≈100 mm x 75 mm] at dose levels of 0.1, 1.0, 10.0 mg/rat [aqueous formulation] and 1.0 mg/rat [kaolin clay formulation] for 4, 10, and 24 hours, and one group at each dose level was dosed for 10 hours followed by a wash and assessment after 120 hours.

The percent on/in the treated skin and that absorbed were similar for each dose and duration of exposure [volatile (40%-58%), on/in skin (1%-9%), absorbed (17%-47%)]. Whole blood/plasma concentrations were dose dependent and indicated that the majority of the material at 10, 24, and 120 hours is in the erythrocytes.

This study is classified Acceptable, and it satisfies the guideline [OPPTS 870.7600; §85-2] requirement for a dermal absorption study.

Percentage (%) Dermal Absorption: 40%

2. Short-Term Dermal (1 - 7 days)

Study Selected: Developmental neurotoxicity study - Rat §83-6

MRID No.: 44079201

EXECUTIVE SUMMARY: See summary cited under Acute Reference Dose.

Dose and Endpoint for Risk Assessment: Developmental LOAEL = 1.8 mg/kg/day based on neurotoxic effects seen in offspring during *post partum* days at the lowest dose.

Comments about Study and Endpoint: A developmental LOAEL was selected because 1) of the concern for the neurotoxicity seen in the developmental neurotoxicity study, which demonstrated increased susceptibility, 2) neurotoxicity is a consistent findings in studies with Molinate, 3) the 21-day dermal toxicity study is unacceptable; and 4) although these effects were seen in the offspring, neurotoxicity was also seen in adult animals in other studies with Molinate and thus appropriate for female worker exposure risk assessment. Since an oral LOAEL was identified, a dermal absorption factor of 40% should be used for this risk assessment. A Margin of Exposure of 300 (i.e, conventional 100 and 3 for the use of a LOAEL) is required for occupational exposure risk assessments.

This risk assessment is required.

3. Intermediate-Term Dermal (1-Week to Several Months)

Study Selected: Fertility study - Rat Guideline #: §none

MRID No.: (accession) 00245675

Executive Summary: In a four-part fertility study, male Charles River Sprague-Dawley rats [Part I : 12/group; Part II: 20/group; Part III: 12/group; Part IV: 12 /group] were administered Molinate [98.2% a.i.] *via* gavage [corn oil vehicle] at dose levels of 0, 12, and 60 mg/kg/day for 5 consecutive days [Part I]; 0 and 12 mg/kg/day for 10 weeks [Part II]; 0, 12, and 30 mg/kg/day of 5 weeks [Part III]; and 0, 0.2, and 4.0 mg/kg/day for 5 weeks [Part IV]. Part IV results were chosen for this risk assessment.

At the end of the dosing period, [Part I] each male [9-10 weeks old] was cohabited with a new female [10-12 weeks old] each week for 10 consecutive weeks [fertility of each male assessed prior to the start of dosing]. The females were sacrificed after 9-10 days following cohabitation, and the number of corpora lutea, implants, viable fetuses, and resorptions were determined. The males were sacrificed after the mating period; [Part II] each male was cohabited with two females [10-12 weeks old] per week for 2 consecutive weeks. Nine to ten days after cohabitation, the females were sacrificed and the number of corpora lutea, implants, viable fetuses, and resorptions were determined. Following the second cohabitation period, the males were sacrificed. Blood was collected for serum hormone assessment, the adrenals and testes plus epididymides were weighed, sperm samples were analyzed, the testes and epididymides were examined microscopically; during the last week of dosing [Part III], each male [9-11 weeks old] was cohabited with two females [10-12 weeks old] for 15 days, after which the females were sacrificed, and the reproductive tract was examined as in Part II above . The males were sacrificed following cohabitation, and blood and sperm samples were collected and analyzed as in Part II above. [Part IV] is the same as [Part II] at lower dose levels.

Part I: This phase was designed to determine which phase(s) of spermatogenesis was(ere) affected by Molinate [based on a reduction in fertility]. There were no apparent treatment-related clinical signs and no deaths. No adverse effects were observed on body weight, but body-weight gains during weeks 2-3 [35% of control] and weeks 7-8 [71% of control] were decreased at the high-dose level [60 mg/kg/day] compared to the control. There was a statistically-significant reduction in the number of pregnancies in females mated to males dosed at 60 mg/kg/day during the third week. There was a reduction in the number of implants and viable fetuses per litter after the third mating and a significant reduction in the number of implants per litter during the fourth mating. There was a statistically significant reduction in the implantation index at 60 mg/kg/day during the third mating. This part of the study suggests that at **60 mg/kg/day,** the mid to late stages of the spermatogenic cycle were affected by treatment; the major effect being on the late spermatid stage.

Part II: This phase was designed to evaluate the effect of Molinate on male fertility after 10 weeks of exposure. One control [dosing accident] and two 12 mg/kg/day males [hematopoietic system neoplasia and esophageal impaction] died on test. Body weights were comparable between the control and Molinate groups, but decreased body-weight gains were observed at various intervals during the study [overall (weeks 1-12) 78% of control]. There was a significant reduction in the female fertility index at the 12

mg/kg/day dose level during the second mating [reduction in the number of pregnancies]. At 12 mg/kg/day, there were significant reductions in the number of corpora lutea in the second mating, the number of implants and viable fetuses per litter in both the first and second matings, and the number of total resorptions in the second mating There was a significant reduction in the implantations indices for both matings and a significant increase in the implant viability index in the second mating, both of which indicate that there was a significant increase in preimplantation loss but no increase in postimplantation loss in females mated to males treated with Molinate at 12 mg/kg/ day for a period of 10 weeks. Reduced male fertility was observed following 10 weeks of exposure to Molinate at 12 mg/kg/day. There was no apparent effect on serum hormone levels following 10 weeks of exposure to 12 mg/kg/day. There were decreases in the percent of viable sperm, the percent of motile sperm, and sperm concentration, and an increase in the percent of abnormal sperm following Molinate exposure for 10 weeks. There was a good correlation between the decreased number of implants and the increase in abnormal sperm, the decrease in viable sperm, the decrease in motile sperm, and the decrease in sperm concentration. No apparent difference was observed in testes/epididymides weight or adrenal weight. There was a treatment-related increase in the number of seminiferous tubules containing degenerating spermatids/spermatocytes per testis; i.e., between 3 and 10 tubules were affected in the control compared to between 11 and 20 tubules being affected in the Molinate group.

Part III: Male fertility was assessed following exposure for 5 weeks. One 12 mg/kg/day male died due to a dosing accident. There was a dose-related decrease in body-weight gain [overall 89% and 78% of control for the 12 and 30 mg/kg/day males, respectively]. Male and female fertility were both reduced following exposure to Molinate at 30 mg/kg/day. There were significant reductions in the number of implants and viable fetuses per litter at both the 12 and 30 mg/kg/day dose levels. There was also a significant reduction in the number of resorptions per litter at 30 mg/kg/day. Increases were observed in FSH, testosterone, and T4 levels at the 30 mg/kg/day dose level and in testosterone and T3 at the 12 mg/kg/day dose level after 5 weeks of exposure, but there was no apparent dose response. There was a dose-related decrease in the % viable sperm, the % motile sperm, sperm cell concentration, and in the # of implants/female, and a dose-related increase in the % abnormal sperm. No apparent differences were observed in testicular/ epididymal weight. There was a dose-related increase in the number of seminiferous tubules containing degenerating spermatids/spermatocytes per testis.

Part IV: This phase was designed to determine a no-effect level after 5 weeks of treatment. One 4 mg/kg/day male died due to a dosing accident. A slight decrease in body-weight gain [96% of control] was observed at the 4 mg/kg/day dose level. There were no significant reductions in male or female fertility indices, although at 4 mg/kg/day, the male fertility index was 73% compared to the control and low dose groups [100%]. At the 4 mg/kg/day dose level, there was a significant reduction in the number of viable fetuses per litter in females mated to males at this level. There was a significant increase in the number of resorptions per litter at 0.2 mg/kg/day, and a significant decrease in the implant viability index at 0.2 mg/kg/day. The author stated that the control value for the number of resorptions per litter in this phase of the study was very low compared to the other control groups and the implant viability index was very high, and since the results at the 0.2 mg/kg/day dose level were well within the control range, the increase in postimplantation loss was not considered biologically significant at 0.2 mg/kg/day. There were no apparent effects observed on serum hormone levels. At the 4

mg/kg/day dose level, there were decreases in the % viable sperm, the % motile sperm, sperm cell concentration, and the # of implants/female, and an increase in the % of abnormal sperm. There were no apparent effects on testicular/epididymides weight at either dose level. There was a slight increase in the number of seminiferous tubules containing degenerating spermatids/spermatocytes at the 4 mg/kg/day dose level compared to the control and low-dose [0.2 mg/kg/day] groups. The HED Developmental and Reproductive Toxicity Peer Review Committee [12/12/91; memo dated 7/15/92] considered the NOAEL to be 0.2 mg/kg/day also. The apparent increase in the number of resorptions/litter in the 0.2 mg/kg/day group was considered by the committee 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."

Molinate exposure to male rats resulted in a decrease in male fertility at dose levels of 4, 12, 30, and 60 mg/kg/day for periods from 5 days to 5 and 10 weeks. Sperm abnormalities were observed following 5 and 10 weeks of treatment at dose levels of 4 mg/kg/day and above and included detached sperm heads and tails, heads and tails bent at abnormal angles, and rupture of sperm membranes at head-midpiece and midpiece-tail junctions. The NOAEL is 0.2 mg/kg/day, and the LOAEL is 4 mg/kg/day, based on decreases in the % viable sperm, % motile sperm, % normal sperm, sperm counts, numbers of implants, number of viable fetuses, and increased pre-implantation loss.

Dose and Endpoint for Risk Assessment: NOAEL= 0.2 mg/kg/day, based on decreases in the % viable sperm, % motile sperm, % normal sperm, sperm counts, numbers of implants, number of viable fetuses, and increased pre-implantation loss at 4 mg/kg/day (LOAEL).

Comments about Study and Endpoint: The endpoint (male reproductive effects) is appropriate for this risk assessment since reproductive effects were also seen in oral studies with mice (anti-fertility study) and rats (sperm morphology and 2-generation reproduction) and the inhalation toxicity study in rats; this endpoint is a concern for male workers, and the effects were seen after 5 weeks of treatment which is appropriate for the exposure period of concern (7 days to 90 days).

Since an oral NOAEL was identified, a dermal absorption factor of 40% should be used for this risk assessment. A MOE of 100 is adequate for occupational exposure risk assessment.

The Registrant has submitted arguments against the use of the rat fertility effects as an endpoint for risk assessment and these were presented to the Committee. Only abstracts and published papers have been submitted to date. Based on the lack of an Agency assessment of the referenced studies [no individual data have been submitted for review], the Committee concluded that these arguments could not be considered in its assessment. The Committee further concluded that the rat fertility is an appropriate endpoint for risk assessment.

This risk assessment is required.

4. Long-Term Dermal (Several Months to Lifetime)

Based on the use pattern (1-2 aerial applications per season to rice), there is minimal concern for potential long-term dermal exposure.

This risk assessment is NOT required.

5. Short-Term Inhalation (1-7 days)

Study Selected: Acute inhalation - Rat §81-3
Guideline #: OPPTS 870.1300

MRID No.: 00245675

Executive Summary: In an acute inhalation study, rat exposed at 0.06, 0.12, 0.28, 0.83, 0.9, 1.6, 2.2, 2.4, 2.8, 4.0, and 4.9 mg/L of chamber air for a 4-hour period and were observed for 14 days following exposure. All rats at the 4.9 mg/L dose died [males between days 2-7; females by day 2], 8 males [days 2-6] and all females [by day 2] died at 4.0 mg/L, 7 males [days 2-7] and 7 females [by day 2] died at 2.8 mg/L, 2 males [days 3-4] and 4 females [days 2-4] died at 2.4 mg/L, 3 females [days 2-3] died at 2.2 mg/kg, and 1 males [day 6] died at 0.83 mg/L. Toxic signs included depression [all treatment groups; severity related to dose], prostration, ataxia, shallow/audible breathing, salivation, brown/red stains on face, hindleg weakness [dose levels of 0.28 mg/L and above], aggression &/or hyperexcitability , and abnormal-appearing eyes [opague cornea or dark-appearing pupils, cloudy &/or protruding cornea]. These treatment-related clinical signs disappeared in those rats surviving the 14-day observation period. Decreased body weight was observed in all treated groups compared to the controls. At necropsy, dark red lungs, black areas &/or red patches on the lungs were observed at the 2-highest dose level, and the testes had a generalized purple appearance with occasional white &/or red mottling, apparent atrophy at dose levels of 0.83 and above. Histological examination of the testes at the 4.9 mg/L dose revealed moderately severe lesions [vasculitis, edema, congestion, interstitial hemorrhage, necrosis of the germinal cells, and necrosis of the interstitial cells] and # moderate reduction of spermatozoa in the seminiferous tubules [only one rat examined (died day 2); autolysis of others]. Four males at 4.0 mg/L [died day 2] displayed testicular lesions that were similar to those of the high dose. Testicular atrophy was observed at dose levels of 0.83 mg/L and above. Microscopic lesions in the testes of those males sacrificed at day 14 included capsular thickening, vascular congestion, focal necrotizing vasculitis [phlebitis], germinal cell necrosis, hyperplasia of the interstitial cells. No microscopic lesions were reported in the testes of males dosed at 0.28 mg/L and below. The acute LC50 of Molinate was determined to be 2.9 [2.5-3.3] mg/L in male and 2.4 [2.2-2.6] mg/L in female rats.

The NOAEL for male rats is 0.12 mg/L, and the LOAEL is 0.28 mg/L, based on hindleg weakness and testicular effects.

Dose and Endpoint for Risk Assessment: NOAEL= 0.12 mg/L, based on hindleg weakness and testicular effects at 0.28 mg/L (LOAEL).

Comments about Study/Endpoint/Uncertainty Factor(s): The endpoint is consistent following short- and long-term exposure. [10x for intraspecies variation, 10x for interspecies variation].

This risk assessment is required

6. Intermediate-Term Inhalation (1 Week to Several Months)

Study Selected: 4-week inhalation - rat Guideline #: §none

MRID No.: 41589203

Executive Summary: In a 4-week inhalation toxicity study, male Sprague-Dawley rats [12/group] were exposed to Molinate [98.2% a.i.] *via* the inhalation route at proposed exposure levels of 0, 0.1, 0.2, 0.4, 0.8, and 1.6 mg/m³ for 6 hours per day, 5 days per week for a total of 20 exposure days, prior to mating with unexposed Sprague-Dawley female rats. During week 5 of the study, the treated males were housed with two females for a maximum of 7 nights or until each male mated with both females. Examination of vaginal smears occurred on the first 3 mornings following initial cohabitation. Prior to the end of the 7-night mating period, epididymal sperm samples were collected from selected males, and these males were subjected to a gross necropsy and the testes plus epididymides were removed, weighed, and sperm collected from the cauda epididymides and analyzed for sperm concentration, motility, morphology, and viability. The females were sacrificed on projected gestation days 10-18, and the reproductive tract was examined to determine the number of corpora lutea, implants, and viable fetuses.

There were no deaths, and clinical signs were comparable among the groups. There was a dose-related increase in the percentage of abnormal sperm, with the values at the 2 highest dose levels showing statistical significance [18% and 29.1% *vs* 9.4% in the control]. The major sperm abnormalities observed were detached heads and sperm with broken membranes between the head and midpiece or between the midpiece and tail sections. The percent motile sperm was decreased significantly at the highest dose level [57.8%] compared to the control [72.8%]. The number of corresponding implants per female was decreased significantly at the highest dose level [8.4] compared to the control [14.0]; the percent of females that were pregnant was slightly decreased [81% *vs* 89%] at the highest dose level compared to the control; and males at this dose level displayed the lowest percent fertility [92% *vs* 96%]. The mean number of implants and the percent of implants per corpora lutea were decreased at the two highest dose levels [dose-related]. The mean number of viable implants was decreased significantly at the highest dose level [10.4 *vs* 13.7] compared to the control. The NOAEL for effects on male fertility is 0.30 mg/m³, and the LOAEL is 0.64 mg/m³, based on decreased number of implants and increased percentage of abnormal sperm.

Dose and Endpoint for Risk Assessment: NOAEL=~~-~~0.0003 mg/L; decreased number of implants and increased percentage of abnormal sperm observed at 0.00064 mg/L (LOAEL).

Comments about Study and /Endpoint: The male reproductive effects observed via this route were also seen following oral exposure in mice and rats. The duration of exposure (4-weeks) in this study is appropriate for the exposure period of concern (7 days to several months).

This risk assessment is required.

7. Long-Term Inhalation (Several Months to Lifetime)

Based on the use pattern (1-2 applications per season to rice), there is minimal concern for potential long-term inhalation exposure.

This risk assessment is not required.

E. Recommendation for Aggregate (Food, Water and Dermal) Exposure Risk Assessments

There are no registered residential home owner uses or registered uses that will result in post-application residential exposure; therefore, aggregate exposure risk assessment will be limited to Food + Water only.

F. Margins of Exposures for Occupational Exposure Risk Assessments

A MOE of 300 is required for Short-term dermal exposure risk assessment since a LOAEL was selected. A MOE of 100 is adequate for Intermediate-term dermal as well as Short-and Intermediate-term inhalation exposure risk assessments. The use pattern does not show potential Long-term exposure via the dermal or inhalation routes.

III. CLASSIFICATION OF CARCINOGENIC POTENTIAL

1. Combined Chronic Toxicity/Carcinogenicity Study in Rats

MRID No. 41815101

Discussion of Tumor Data: There was a statistically-significant increase in combined kidney adenomas and/or carcinomas at the high-dose level in male Crl:CD®(SD)BR rats and a statistically-significant positive trend for kidney carcinomas and combined adenomas and/or carcinomas. The increased incidence of kidney tumors in the high-dose male rats exceeded the available historical data for both adenomas and carcinomas. With respect to the testes, there was a statistically-significant positive trend for mesotheliomas in the testes of males rats, testicular interstitial cell tumor incidence exceeded the historical control incidences, and the increase was observed at all dose levels. The CPRC concluded that the evidence is equivocal, since there was no increase in trend or pairwise comparisons. Supportive evidence that the testes is a target organ for Molinate is the finding of adverse reproductive effects. In an incompletely reported Japanese study, there is an indication that the testes is a site for tumors caused by Molinate, which lends support to the presumption that the tumors are compound-related. There was no increase in tumors in the female rat.

Adequacy of the Dose Levels Tested: The dose levels were considered adequate for assessing the carcinogenic potential of Molinate [BWG 83%-85% of control at 90 days; dose-related decrease in brain weight in both sexes at 12 months and significantly decreased in both sexes at the HDT at termination; RBC cholinesterase decreased in both sexes (males 61%-86%/females 67%-85% of control); decreased brain cholinesterase in females at 300 ppm [87% of control] and 600 ppm [84% of control].

2. Carcinogenicity Study in Mice

MRID No.: 41809201

Discussion of Tumor Data: There was no evidence of carcinogenicity in male or female mice.

Adequacy of the Dose Levels Tested: [Doses of 10, 100, 1000, 2000 ppm (males 1, 10.4, 105, 200/females 1.3, 13.9, 133, 249 mg/kg/day]. There was a decrease in survival in both sexes at the HDT, but there were adequate numbers of mice surviving to termination in all groups for both sexes for an adequate assessment of the carcinogenic potential in the mouse. [Body weight [males 85%/females 73% of control during 0-13 weeks]/body weight gain [63%-82% of control] at two highest dose levels for both sexes].

3. Classification of Carcinogenic Potential

At the June 17, 1992 meeting , the HED Carcinogenicity Peer Review Committee classified Molinate as a **Group C - possible human carcinogen** and recommended that for the purpose of risk characterization, a low dose extrapolation model applied to the experimental animal tumor data should be used for the quantification of human risk (q_1^*). The **unit risk, q_1^* (mg/kg/day)$^{-1}$ of Molinate, based on male rat kidney (cortical adenomas and/or carcinomas) tumors is 1.1 x 10^{-1} (mg/kg/day) in human equivalents.** The HIARC concurred with the previous classification.

IV. MUTAGENICITY DATA

Molinate was negative for mutagenic activity, with and without metabolic activation in Salmonella typhimurium [strains TA1535, TA1537, TA1538, TA98, and TA100] (MRID 40918301). [Document No. 008549]; OPPTS 870.5265 (MRID Nos. 00163789, 00163790, 00163791 40918301, 40946701, 41052701, 43986701/44562201.

Molinate was negative for clastogenic activity in cultured human lymphocytes with and without metabolic activation [MRID 40946701] at concentrations of 24, 95, and 190 µg/mL [Document No. 8549]. OPPTS 870.5375

Molinate was mutagenic [weakly] in the L5178Y TL+/- mouse lymphoma mutagenesis assay [MRID 00163790] with metabolic activation by both rat and mouse S9 activation systems over the concentrations [0.01-0.1 µL/mL] tested [Document No. 008549]. OPPTS 870.5300

Molinate was negative in the *in vitro* Unscheduled DNA Synthesis assay [MRID 41052701] (Document No. 011187). OPPTS 870.5500.

A positive response was reported in a published mouse bone marrow micronucleus test [Mutation Research 242:279-283 (1990)]. OPPTS 870.5395

In a dominant lethal assay [MRID 43986701/44562201], there was no evidence that Molinate technical induced a dominant lethal effect in male germinal cells treated over the entire period of spermatogenesis. OPPTS 870.5450.

Molinate was negative in a mouse micronucleus assay [MRID 00163789].

An aberration and sister chromatid exchange study in mouse lymphoma cells [MRID 00163791] indicated suggestive, but not reproducible increase with activation.

V. FQPA CONSIDERATIONS

1. Adequacy of the Data Base

All required guideline studies are available. Adequate neurotoxicity studies have been performed on Molinate, including an acute delayed neurotoxicity study in hens, a subchronic neurotoxicity study in rats, and a developmental neurotoxicity study in rats. The acute neurotoxicity study in rats is classified Unacceptable [see below]. There are adequate rat and rabbit developmental toxicity studies and an adequate rat reproductive toxicity study on Molinate.

2. Neurotoxicity Data

Neurotoxic effects are consistent findings in studies on Molinate. Molinate was positive in a delayed neurotoxicity study in the hen, and neurotoxic effects were observed in rat following both acute and subchronic oral exposures.

In an acute delayed neurotoxicity study [MRID 00133562], white Leghorn hens were administered Molinate [98.6% a.i. in corn oil] *via* gavage [single dose] after a 17-20 hour fast. The study consisted of two parts. In Part I, designed to test the acute delayed neurotoxic potential of Molinate, 26 hens received corn oil [control], 10 hens received a dose level of 20 mg/kg, and 25 hens received a dose level of 2000 mg/kg. In Part II, designed to determine whether the effects observed in Part I were reproducible, dose-related, or reversible, 10 hens received 63 mg/kg, 10 hens received 200 mg/kg, 15 hens received 630 mg/kg, 30 hens received 2000 mg/kg, and 15 each received the negative [corn oil] and positive [TOCP] control.

In the LD50 phase of the study, mortality was delayed, with most deaths occurring within 2-11 days of treatment. One hen died on day 22 and one on day 26. Diarrhea, motor in coordination, and loss of body weight [10%-30%] were the primary effects observed. There was a dose-related inhibition of plasma cholinesterase following single doses of 3500 mg/kg and greater. No inhibition of cholinesterase was reported at dose levels of 2800 mg/kg or below. **The oral LD50 for Molinate in unprotected hens is 1930 mg/kg; in protected hens, the LD50 is 2300 mg/kg.** In Phase I, 14 of the 25 hens at the 2000 mg/kg dose level survived to day 43 [termination]. In phase II, 23 of the 30 hens at

2000 mg/kg died prior to day 43. Clinical signs included unsteady standing [at 200 mg/kg and greater], sitting on hocks and unable to stand [630 mg/kg and greater]. Axonal degeneration in the brain and upper spinal cord were observed at dose levels of 630 mg/kg and 2000 mg/kg, and these appeared to involve predominantly ascending [i.e., sensory] pathways, probably the spinocerebellar and vestibulospinal tracts. **The NOAEL is 200 mg/kg, and the LOAEL is 630 mg/kg, based on axonal degeneration in the brain and cervical spinal cord.**

In an acute neurotoxicity study in rats [MRID 43188001], a single dose of Molinate [96.8%] was administered via gavage to Alpk:APfSD rats [12/sex/group] at dose levels of 25, 100, and 350 mg/kg. Several clinical signs suggestive of general systemic toxicity and neurological involvement were observed in all dosed groups. The clinical signs included decreased body weight (93%-95% of control)/gain (83%-85% of control) and food consumption, decreased activity, decreased cholinesterase activity [brain (males 91% and 84%/females 93% and 77% of control at mid- and high dose, respectively) and erythrocyte (males 79% and females 89% of control at high dose)], increased landing foot splay, and increased time to tail flick. Decreased brain weight and brain length were observed in males at the high dose. **No no-effect level (NOAEL) was determined for either decreased motor activity or increased time to tail flick for either sex, and NTE, GFAP, and cholinesterase activities were not assessed at appropriate times immediately after dosing; i.e., at 4 hours post dose and/or within 72 hours post dose. No definitive conclusion regarding a NOAEL for acute neurotoxicity can be made.**

In a 90-day neurotoxicity study in rats [MRID 43270701 and 43965901], administration of Molinate [96.8%] to Alpk:APfSD rats of both sexes [12/sex/group] *via* the diet at dose levels of 50 [♂ 4.0/♀ 4.5 mg/kg/day], 150 [♂ 11.7/♀ 13.9 mg/kg/day], and 450 ppm [♂ 35.5/♀ 41.0 mg/kg/day] for at least 90 days resulted in a dose-related decrease in body weight [♂ 86%/♀♀ 84% of control at the high dose; ♀ 93% of control at the mid dose; ♀ 94% of control at the low dose], body-weight gain [♂ 78%/♀ 65% of control at the high dose; ♀ 84% of control at the mid dose; ♀ 88% of control at the low dose], food consumption, and food utilization. At the high-dose level, (1) females displayed an increase in landing foot splay at week 5; (2) both sexes displayed an increase in the time to tail flick at week 14; (3) both sexes displayed a decrease in forelimb grip strength during week 9; (4) females displayed a decrease in hindlimb grip strength during week 5 and males displayed a decrease during week 14. Additionally, a decrease in the time to tail flick was observed at week 5 in males at all dose levels, but there was no dose response. No apparent effect was demonstrated on overall motor activity in males, but when compared to pretest values, high-dose females displayed increased motor activity following treatment. There was a dose-related decrease in both brain [males 16% and 42% at the mid- and high-dose levels; females 7%, 23%, and 47% at the low-, mid-, and high-dose levels, respectively] and erythrocyte [high-dose males 27%; mid- (22%) and high-dose (32%) females] cholinesterase activities in both sexes, and a dose-related decrease in NTE activity at all dose levels in both sexes [males 80%, 63%, 41% of control; females 75%, 59%, 39% of control] compared to the control values. Although the decrease in brain cholinesterase activity was observed in females at all dose levels, the decrease at the low dose in both sexes is considered minimal [93% of control]. Absolute brain weight was decreased significantly in both sexes at the high-dose level. Microscopically, nerve fiber degeneration in the sciatic nerve and the sural nerve was increased in the high-dose males

compared to the control incidence. **No NOAEL was attained in this study, based on the decrease in brain cholinesterase activity and the decrease in NTE activity in both sexes at all dose levels.**

Evidence of neurotoxicity observed in the other studies from the database are summarized below:

In the 2-year rat chronic toxicity study, (1) adducted hindlimb, ataxia, atrophied hindlimb (sacral region/thigh) were observed at 300 ppm [21st month]; (2) decreased brain weight was observed in both sexes at 600 ppm [dose given for only 1 year] and in males at 300 ppm at 12 months and in both sexes at 300 ppm at 24 months; (3) dose-related increase in the incidence and severity of degeneration/ demyelination of the sciatic nerve; and (4) males at 300 ppm displayed an increase in spinal cord degeneration.

In the mouse carcinogenicity study, (1) hindlimb muscle weakness, adducted hindlimbs, ataxia, and splayed hindlimbs were observed in both sexes at the high-dose level [2000 ppm]; (2) decreased brain weight was observed in females at 2000 ppm; and (3) there were microscopic lesions in the brain and spinal cord of both sexes at the 1000 ppm and 2000 ppm dose levels.

In the chronic toxicity study in dogs, (1) ataxia, reduced locomotor activity, splayed hindlimbs were observed in both sexes at 50 mg/kg/day and 100 mg/kg/day (dosed for only 14 weeks); (2) tremors in both sexes at 10/50/100 mg/kg/day; (3) eosinophilic bodies/vacuolation of the medulla/pons (both sexes); (4) spinal cord demyelination at all dose levels in both sexes; (5) sciatic nerve demyelination at all dose levels in males; and (6) decreased brain weight in females at 10 mg/kg/day and in both sexes at 50 mg/kg/day. Serum cholinesterase was decreased [≈21%-27%] in both sexes at 50 mg/kg/day, although not statistically significant in males. Brain cholinesterase was not measured.

In the 2-generation reproduction study in rats, brain weight was decreased in both the adults and offspring of all generations/litterings, and the decrease was observed at all dose levels in the F1 males, the F0 females, and the F1 females.

In the rat developmental toxicity study, RBC cholinesterase was decreased [56%] at the high-dose level, and increased salivation was observed only at this dose level. Brain weight was not measured.

In a mechanistic study in rats, clinical signs suggestive of neurotoxicity [subdued behavior, hunched posture, abnormal gait (high-stepping and splayed), head twisted to one side, rolling gait] in addition to salivation were observed. Plasma, RBC, and brain cholinesterase activity were not measured.

In two range-finding developmental neurotoxicity studies in rats, aggression was observed at the time of dosing in the high-dose rats, and in one study difficulty in restraining the high-dose female rats was observed.

In a subchronic toxicity inhalation study in rats, aggressive behavior was observed at the high-dose level, decreased RBC cholinesterase was observed in both sexes at the high dose, decreased plasma cholinesterase was observed in the high-dose males, and brain cholinesterase was observed in the mid-dose males and in both sexes at the high dose.

3. Developmental Toxicity

In a developmental toxicity study [MRID 41473401], 26 sperm-positive Crl:CD(SD) BRVAF/PLUS female rats per group were administered *[via* gavage] Molinate [97.6% a.i.] at dose levels of 0 [corn oil], 2.2, 35, and 140 mg/kg/day from gestation days 6 through 15 [dose based on day 6 body weight]. For study details, see under proposed acute dietary endpoints.

The NOAEL for maternal toxicity is 35 mg/kg/day, and the maternal toxicity LOAEL is 140 mg/kg/day, based on decreased body weight, body-weight gain, and food consumption, increased salivation and dehydration, and RBC cholinesterase inhibition. **NOTE: The original DER set the developmental toxicity NOAEL at 35 mg/kg/day, and the developmental toxicity LOAEL is 140 mg/kg/day, based on an increased postimplantation loss, decreased fetal body weight, increased incidence of runts, and external/soft tissue [head]/skeletal variants. The HED Developmental and Reproductive Toxicity Peer Review Committee concluded that, with respect to runting, the control value was unusually low and that the increase in runting was not biologically significant at the low dose. The committee concluded that the developmental toxicity NOAEL was 2.2 mg/kg/day, based on an increase in runting at 35 mg/kg/day and 140 mg/kg/day.**

In a developmental toxicity study [MRID 14021015], 16 sperm-positive female New Zealand White [D1a:(NZW) SPF] rabbits/group [17 at high-dose] were administered Molinate [98.8% a.i.] *via* gavage at dose levels of 0 [corn oil], 2, 20, and 200 mg/kg/day [dosing based on gestation day 7 body weight] on gestation days 7 through 19. There were no treatment-related deaths. Maternal toxicity was observed at the high-dose level as evidenced by an increased incidence of abortions, a significant reduction in maternal body-weight gain [loss of body weight] during gestation days 14-21 [when all does are considered] with an accompanying decrease in food consumption during days 14-21, and increased liver weights. Corrected body-weight gains were comparable among the groups.

There was a decrease in the percent of does with live fetuses at the high-dose level [71% *vs* 94% in the control], an increase in the percent of does aborting at this dose level [24% *vs* 6% in the control], and one high-dose doe had a litter of 10 that was totally resorbed. There were no adverse effects on the mean number of corpora lutea, implants, resorptions, live and dead fetuses per litter at any dose level. The incidence of unossified 5th sternebrae in the 200 mg/kg/day group was not significantly increased but was greater than that observed in the concurrent and historical controls. 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 high-dose level, but these findings were not considered biologically significant by the PRC. Although there was a decrease in supernumery ribs at the high-dose level, the PRC determined that it was not possible to conclude that the decrease was associated with Molinate exposure. The reduction in extra paired ribs at 200 mg/kg/day was considered by the author to be indicative of a delay in fetal development

The NOAEL for maternal toxicity is 20 mg/kg/day, and the LOAEL is 200 mg/kg/day, based on the increase in abortions, decreased [negative] body-weight gain during days 14-21, and increased liver weight. The developmental NOAEL is 20 mg/kg/day, and the

developmental LOAEL is 200 mg/kg/day, based on a delay in fetal development as evidenced by the reduced ossification of sternebrae.

This guideline rabbit developmental toxicity study is classified Acceptable, and it satisfies the guideline requirement [OPPTS 870.3700; §83-3(b)] for a developmental toxicity study in rabbits. NOTE: There was no skeletal examination of the skulls in this study. One PRC member objected to the conclusion that the study was acceptable, based on the fact that the bones of the skull were not stained and examined.

4. Reproductive Toxicity

In a 2-generation reproduction study [MRID 44403201], Molinate [96.8% a.i.] was administered to 40 Crl:CD(SD)BR rats/sex/dose via the diet at dose levels of 0, 5, 10, and 15 ppm for males/0, 20, 50, and 300 ppm for females [F0 males: 0.4, 0.8, and 1.3 mg/kg/day, respectively; F1 males: 0.5, 1.1, and 1.6 mg/kg/day, respectively; F0 females: 1.9, 4.7, 28.8 mg/kg/day, respectively; F1 females: 2.2, 5.6, and 34.5 mg/kg/day, respectively] during the pre-mating period of 10 weeks; dams through gestation [F0/Litter 1A: 1.6, 4.1, and 23.8 mg/kg/day, respectively; F1/Litter 2A: 1.6, 4.1, and 24.4 mg/kg/day, respectively; F1/Litter 2B: 1.5, 3.6, and 22.0 mg/kg/day, respectively] and lactation [F0/Litter 1A: 5.1, 12.0, and 54.5 mg/kg/day, respectively; F1/Litter 2A: 4.7, 12.2, and 60.4 mg/kg/day, respectively; F1/Litter 2B: 4.4, 11.7, and 49.2 mg/kg/day, respectively]. The F0-generation rats were mated once to produce F1 litters, and F1-generation rats were mated twice to produce F2A and F2B litters. There were no treatment-related deaths. Comparable body weights and body-weight gains were observed in F0 males among the groups during the pre-mating period. F1 males displayed decreased body weight [88% of control] initially during the pre-mating period, due possibly to ingestion of the dams' diet during the latter part of lactation. Decreased body weight [91% of control at week 11] was observed in both the F0 and F1 females during the pre-mating period, and body-weight gains were decreased [F0 82%/F1 86% of control] also compared to the controls. Decreased food consumption was observed in the high-dose F0 females, mid- and high-dose F1 females, and at all dose levels of F1 males initially. Body weights [F0 87%-92%; F1/2A 83%-85%; F1/2B 78%-85% of the control] and body-weight gains [F0 77%-87%; F1/2A 74%-82%; F1/2B 67%-77% of control] were decreased at the high-dose level compared to the controls during each gestation period and at the mid-dose level during the second gestation period of the F1 dams, with the effect increasing with time. During lactation, body weights [F0 89%-95%; F1/2A 82%-88%; F1/2B 78%-85% of control] and body-weight gains [F0 3%-74%; F1/2A 15%-64%; F1/2B 14%-54% of control] were decreased also compared to the controls.

There was no evidence of an adverse effect of Molinate on the number of estrus cycles during the pre-mating period for either the F0 or F1 females, no apparent effect on the numbers of small, growing, or large oocytes at the high-dose level, and no adverse effects were reported on the precoital interval. There was a dose-related increase in abnormal sperm morphology in both the F0 and F1 males, and sperm motility and total sperm count were decreased in the F0 and F1 males at the high-dose level.

Reproductive parameters appear to be somewhat affected by treatment, in that at the high-dose level, a greater number of dams displayed slightly longer gestation times [all three litters], there were fewer successful matings, and a greater number of high-dose dams failed to litter [all litters]. The number of whole litter losses was comparable among the

groups for all litterings, but litter size [all litterings] and the percent live born [F1A and F2B] were decreased at the high-dose level. Pup survival to day 22 was lowest at the high-dose level for all litterings [F1A 72.6% vs 76.5%; F2A 81.7% vs 82.3%; F2B 72.1% vs 83.4% (high dose vs control)] and litter sizes were decreased significantly throughout lactation in the F1A, F2A, and F2B litters at the high-dose level. In each littering, the percent of high-dose males dying on test was greater than any other group [F1A 28% vs 22%; F2A 22% vs 18%; F2B 32% vs 15% (high dose vs control)]. There was no apparent effect observed on the sex ratio for any generation. Decreased pup body weight [♂♂ 83%-90%/♀♀ 80%-90% of control] and body-weight gains [overall: ♂♂ 81%-89%/♀♀ 87%-91% of control] were observed in both the generations/all litterings. The magnitude of the decrease in both body weight and body-weight gain progressively increased with each subsequent littering [both sexes]. There was no apparent effect on the age of the F1 males at which preputial separation occurred, although there was a dose-related increase in the number of male pups requiring greater than 48 days for separation to occur. The age of the high-dose F1 females at which vaginal opening occurred was delayed [36.9 days vs 34 days (high dose *vs* control)].

Decreased brain weight was observed in both the adults and pups of all generations/litterings, and the decrease was observed at all dose levels in the F1 males, the F0 females, and the F1 females. Other treatment-related organ-weight effects observed in both the adults and pups were decreased spleen weight [both sexes], testes weight [males], ovarian weight [females], as well as decreased epididymides, prostate, and right cauda weights in the adult males. With few exceptions, there were no microscopic findings that correlated with these changes in organ weight. In the testes, there was a dose-related increase in the incidence of bilateral focal testicular tubular degeneration in F1 males. In the ovary, there was a slight to marked interstitial cell vacuolation/hypertrophy in both the F0 and F1 females at the high-dose level. In the adrenal gland, a dose-related increase in the incidence and severity of diffuse fine cortical fat vacuolation was observed in both generations of adult females [adrenal weights comparable among groups], with none of the control females displaying this lesion. There were no histopathological changes observed in the adrenals, gonads, or spleen in any of the F1A, F2A, or F2B pups.

A NOEL was not attained for decreased brain weight for either sex [F0 females and F1 rats of both sexes], and this effect is both a reproductive/developmental effect and a systemic effect.

For effects other than decreased brain weight, the NOAEL for paternal toxicity is 5 ppm [0.4 mg/kg/day], and the paternal LOAEL is 10 ppm [0.8 mg/kg/day], based on the increased incidence of abnormal sperm and decreased absolute right cauda weight in F0 males. The maternal NOAEL is 20 ppm [1.9 mg/kg/day], and the maternal LOAEL is 50 ppm [4.7 mg/kg/day], based on microscopic lesions in the adrenal and ovary. At 300 ppm [28.8 mg/kg/day], decreased body weight, body-weight gain and food consumption were observed. The neonatal NOAEL is 5 ppm/20 ppm [0.4 mg/kg/day/1.9 mg/kg/day], and the neonatal LOAEL is 10 ppm/50 ppm [0.8 mg/kg/day/4.7 mg/kg/day], based on decreased brain weight in F2B females, decreased testes and spleen weights in F1A males, and delayed vaginal opening in females. At the high-dose level [15 ppm; 1.3 mg/kg/day/300 ppm; 28.8 mg/kg/day], F1A, F2A, and F2B pup body weights/body-weight gains and F2B pup survival were decreased, decreased spleen and ovarian weights were observed in the F1A, F2A, and F2B females, and decreased thymus weights were observed in both sexes [F1A, F2A, F2B]. The reproductive NOAEL is 5 ppm [males; 0.4

mg/kg/day]/20 ppm [females; 1.9 mg/kg/day], and the reproductive LOAEL is 10 ppm [males; 0.8 mg/kg/day]/50 ppm [females; 4.7 mg/kg/day], based on microscopic lesions in the ovary [vacuolation/hypertrophy and increased interstitial tissue in both generations, cystic follicles in F0 females], increased incidence of abnormal sperm morphology [both generations], decreased absolute right cauda weight in F0 generation males, decreased % pups born live [F1A and F2B], decreased F2B pup survival, and decreased litter size [F1A, F2A, F2B]. Additionally, at the 15 ppm/300 ppm dose level, the proportion of successful matings was lowest for all litters, a greater number of dams failed to litter [all litters] compared to the control and other treatment groups, decreased uterus weight in F0 females, decreased epididymis weight [F0 and F1 males], and pup survival was the lowest among the groups in all litters.

In a non-guideline 2-generation reproduction study, female Crl:CD®(SD) BR VAF/Plus™ rats [25/group] were administered Molinate [97.6% a.i.] *via* the diet for 60 days prior to mating and continued through the second generation at dose levels of 0 [0.1% corn oil], 6 ppm [0.34 mg/kg/day], 50 ppm [2.9 mg/kg/day], and 450 ppm [28 mg/kg/day]. The females were mated [1:1] to untreated, proven, males after 60 [P0]/63 [P1] days of treatment.

The maternal toxicity NOAEL is 6 ppm [0.34 mg/kg/day], and the maternal toxicity LOAEL is 50 ppm [2.9 mg/kg/day], based on decreased fecundity [F1], an increased incidence of vacuolation/hypertrophy of the ovary, and decreased brain weight [F1 females]. At the high-dose level, in addition to this ovarian lesion and decreased brain weight, there was a decrease in body weight [86%-94% of control], body-weight gain [68%-87% of control], food consumption, and fecundity [uterine implants and litter size], an increase in absolute adrenal weight in both generations. Also, both the fertility index and the gestation index were lowest at the high-dose level in both generations [see appended Table 15 from the data summaries presented to the HED Peer Review Committee {PRC} for Developmental and Reproductive Toxicity on 12/12/91]. The reproductive NOAEL is 6 ppm, and the reproductive LOAEL is 50 ppm, based on the occurrence of vacuolation/ hypertrophy of the ovary. At the 450 ppm dose level, in addition to the effects listed above, decreased litter size and decreased pup body weight were observed. With regard to the increased incidence of vacuolation/ hypertrophy of the ovary, all high-dose dams of both generations displayed this lesion, and the mid-dose F0 and F1 dams displayed an increase compared to the control, with the incidence in the F1 dams being quantitatively greater than that in the F0 dams. Since there was only one litter per generation, it is not known whether subsequent pregnancies might display a greater incidence and/or a more severe lesion, and there are no data on possible effects on the aging ovary. The neonatal NOAEL is 6 ppm, and the neonatal LOAEL is 50 ppm, based on ovarian lesions. At the 450 ppm dose level, decreased brain weight and increased adrenal weights were observed, in addition to the ovarian lesions.

The HED Developmental and Reproductive Toxicity Peer Review Committee [memo dated 7/15/92] determined that this 2-generation reproduction study, in which only the females were dosed with Molinate, was not an adequate study [memo dated 9/22/92; Document No. 009731].

5. Developmental Neurotoxicity

In a developmental neurotoxicity study study (discussed in detail in Section I. Acute Reference Dose) Molinate [96.8% a.i.] was administered to 30 female Alpk:APfSD rats/group in the diet at dose levels of 0, 20, 75, and 300 ppm (0, 1.8, 6.9, and 26.1 mg/kg/day, respectively) from gestation day 7 through lactation day 11. The NOAEL for maternal toxicity is 75 ppm [6.9 mg/kg/day], and the LOAEL for maternal toxicity is 300 ppm [26.1 mg/kg/day], based on decreased body weight/gain and food consumption. The reviewer established a NOAEL for developmental neurotoxicity of 1.8 mg/kg/day, and a developmental LOAEL of 6.9 mg/kg/day, based on a reduction in startle amplitude in the auditory startle test in females [day 23] and treatment-related reductions in some morphometric measurements in areas of the cerebellum of the brain [day 12] in both sexes (MRID 44079201). However, during the HED Toxicity Scientific Advisory Committee (Tox SAC) evaluation, it was concluded that the 1.8 mg/kg/day should be a LOAEL since the effects observed at the lowest dose tested cannot be discounted. The HIARC concurred with the Tox SAC and recommended that the DER be revised to reflect this change in LOAEL/NOAEL. Therefore, for developmental neurotoxicity, the LOAEL is 1.8 mg/kg/day and a NOAEL is not established.

6. Determination of Susceptibility

The HIARC concluded that there is clear evidence of increased susceptibility in rat fetuses following *in utero* explore to molinate in the prenatal study. Increased susceptibility was also demonstrated in the developmental neurotoxicity study in rats.

In the prenatal developmental toxicity study in rats, the developmental NOAEL is 2.2 mg/kg/day, based on an increase in runting observed at the LOAEL of 35 mg/kg/day. The maternal NOAEL is 35 mg/kg/day, based on decreased body weight, body-weight gain, and food consumption, increased salivation and dehydration, and RBC cholinesterase inhibition at 140 mg/kg/day (LOAEL).

In the developmental neurotoxicity study in rats, the NOAEL for developmental neurotoxicity was not achieved, based on a reduction in the startle amplitude in the auditory startle test at all dose levels [1.8, 6.9, and 26.1 mg/kg/day]. The maternal NOAEL is 6.9 mg/kg/day, based on decreased body weight/gain and food consumption at 26.1 mg/kg/day (LOAEL). The dose levels at which the developmental toxicity/neurotoxicity NOAELs were established are both less than the maternal NOAELs (2.2 mg/kg/day vs 35 mg/kg/day and $\leq$1.8 mg/kg/day *vs* 6.9 mg/kg/day).

There was no evidence of increased sensitivity to offspring in the two-generation reproduction study in rats, since reproductive/developmental effects in pups (decreased brain, testes, spleen, cauda weights, delayed vaginal opening, microscopic lesions in ovary, increased incidence of sperm abnormality, decreased % pups born live, pup survival and litter size) were observed at the same dietary levels where maternal toxicity effects were observed (increased incidence of abnormal sperm, decreased cauda weight, microscopic lesions in adrenal and ovary) in the parental animals. In the prenatal developmental toxicity studies in rabbits, developmental and maternal toxicity were observed at the same dose levels.

7. Determination of the FQPA Safety Factor

Based on the hazard assessment alone, the HIARC recommends to the FQPA Safety Factor Committee that the 10x additional factor for the protection of infants and children (as required by FQPA) be retained due to the increased susceptibility observed in the prenatal developmental toxicity study in rats and the developmental neurotoxicity study in rats. However, the final recommendation will be made by the FQPA Safety Factor Committee during risk characterization.

VI. HAZARD CHARACTERIZATION

The Molinate toxicology database is complete, although the acute neurotoxicology study is classified Unacceptable.

Molinate is a thiocarbamate herbicide. The findings in multiple studies demonstrate that Molinate is both a neurotoxin and a reproductive toxicant after single and multiple doses *via* the oral, dermal, and inhalation routes of exposure and across species [rat, dog, mouse, monkey, rabbit].

NEUROTOXICITY: Cholinesterase inhibition [plasma, erythrocyte, and/or brain] was observed in all species for which it was monitored [rat, dog, rabbit, monkey, hen], with and without clinical signs. Molinate was positive in the delayed neurotoxicity study in the hen [motor in coordination, behavior depression, axonal degeneration in the brain and upper spinal cord]. In the acute neurotoxicity study in rats, decreased activity, increased landing foot splay, and increased time to tail flick were observed at all dose levels. Decreased erythrocyte cholinesterase activity was observed at 15 days post dose but was not monitored at appropriate earlier time points [study classified Unacceptable for this reason]. In the subchronic neurotoxicity study in rats, increased landing foot splay, increased time to tail flick, decreased forelimb and hindlimb grip strength, increased motor activity, decreased brain weight, and neuropathology [increased nerve fiber degeneration] were observed at the highest dose tested [male 35.5 mg/kg/day and female 41 mg/kg/day], and brain cholinesterase activity and neuropathy target esterase [NTE] activity were decreased [dose-related] at all dose levels [males 4, 11.7, and 35.5 mg/kg/day and females 4.5, 13.9, and 41 mg/kg/day in both sexes]. Following chronic oral exposure to rats, dogs, and mice, clinical signs and pathology indicative of neurotoxicity [ataxia, splayed/adducted hindlimbs, hindlimb muscle weakness, atrophied hindlimbs, decreased brain weight, degeneration/demyelination of the sciatic nerve and spinal cord, lesions in the brain] were observed in both sexes. In the developmental neurotoxicity study in rats, there was a significant [all dose levels], dose-related, decrease in the startle amplitude in the auditory startle test for female F1 pups at day 23 *post partum* and the decrease was significant for both sexes at 26.1 mg/kg/day [HDT]. An increase in swimming time in the straight channel test at day 21, a reduction in performance in the learning and memory tests on days 21 and 24, respectively, an increase in time to maximum amplitude [days 23 and/or 61] in the startle test, a possible increase [slight] in mean motor activity level in males, reduced brain weight [both sexes on days 12 and 63], brain length [both sexes on day 12], and brain width [females on day 12], and reductions in several morphometric measurements in areas of the cortex, hippocampus, and cerebellum of the brain were observed at the HDT.