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

OFFICE OF
PREVENTION, PESTICIDES AND
TOXIC SUBSTANCES

DATE:

MEMORANDUM

SUBJECT: **MOLINATE: TOXICOLOGY CHAPTER FOR RED**

FROM: Linda L. Taylor, Ph.D. 1/4/99
Reregistration Branch I
Health Effects Division (7509C)

THRU: Whang Phang, Ph.D., Branch Senior Scientist 1/7/99
Reregistration Branch I
Health Effects Division (7509C)
and
Michael Metzger, Branch Chief 1/11/99
Reregistration Branch I
Health Effects Division (7509C)

TO: Christine L. Olinger
Reregistration Branch I
Health Effects Division (7509C)

PC Code: 041402
DP Barcode: D249717
Submission: S549038

Attached is the Toxicology Chapter for Molinate for the RED.

MOLINATE: TOXICOLOGY CHAPTER FOR RED

DP Barcode No. D 249717
Submission No. S549038
Tox. Chem. No. 444

Reregistration Case No. 818845
P.C. Code No. 041402

Prepared by: Linda L. Taylor, Ph.D.
Reregistration Branch I
Health Effects Division (7509C)

Reviewed by: Whang Phang, Ph.D., Branch Senior Scientist
Reregistration Branch I
Health Effects Division (7509C)

Branch Chief: Michael Metzger, Branch Chief,
Reregistration Branch I
Health Effects Division (7509C)

MOLINATE: TOXICOLOGY CHAPTER FOR RED

1. TOXICOLOGY DATA BASE

HAZARD PROFILE

The toxicological data base on Molinate is complete and supports reregistration eligibility. In general, Molinate is not acutely toxic *via* the oral, dermal, and inhalation routes of exposure in the acute studies required for labeling; is a mild skin and a moderate eye irritant; and is not a dermal sensitizer. Molinate produces delayed neurotoxicity in the hen [axonal degeneration]. Acute and subchronic neurotoxicity studies in the rat demonstrate adverse effects of Molinate on motor activity and various functional observational battery [FOB] measurements, in addition to cholinesterase and neurotoxic esterase [NTE] activity inhibition. The subchronic and chronic toxicity studies demonstrate that Molinate inhibits cholinesterase activity in plasma, red blood cell [RBC], and brain in rats, dogs, monkeys, and rabbits. Clinical signs associated with cholinesterase activity inhibition were observed and included ataxia, tremors, salivation, reduced motor activity, splayed/adducted hindlimbs, and abnormal gait. Molinate was classified as a Group C, possible human carcinogen, and the unit risk [q^*_1] is 1.1×10^{-1} $(mg/kg/day)^{-1}$, based on male rat kidney tumors.

Delayed fetal development was observed in the rabbit at the same dose level where maternal toxicity was observed. In the rat, developmental toxicity/developmental neurotoxicity were observed [increase in runting/reduction in startle amplitude] at dose levels below the maternal NOAEL. Molinate is a reproductive toxicant, and the rat is the most sensitive species for this effect. Abnormal sperm, decreased percent motile sperm, decreased sperm numbers, decreased litter size, decreased % born live, decreased pup viability, increased incidence of microscopic lesions in the ovary, testes, and adrenal, delayed vaginal opening, reproductive organ weight effects, and decreased brain weight are consistent findings in studies in the rat. There is also evidence of increased sensitivity of offspring of rats exposed to Molinate *in utero*.

Molinate was negative in a *Salmonella tymphimurium* assay and for aberrations in cultured human lymphocytes. Because suggestive increases were found for mutations, aberrations, and sister chromatid exchange [SCE] in mouse lymphoma cells, and there was conflicting data in two mouse micronucleus assays, a dominant lethal test was requested. Subsequently, Molinate was shown to be negative in the dominant lethal test.

The metabolism data indicate that Molinate is well absorbed and extensively metabolized following both oral and i.v. exposure and is rapidly excreted, mainly in the urine. The data also indicated that the metabolism of Molinate involved s-oxidation to form the intermediate Molinate sulfoxide, which was either hydrolyzed to hexamethyleneimine or conjugated with glutathionine, ultimately forming Molinate mercapturic acid; ring hydroxylation at the 3 and 4 positions followed by glucurinide conjugation was also a significant route of metabolism. More recent information indicates that the metabolism of Molinate in mammals is primarily *via* three routes: carbon oxidation, sulfur oxidation, and thiocarbamate cleavage, and the proportion of metabolism through each of these pathways varies among the species, including man. The data also suggests that carbon oxidation predominates at low doses of Molinate, and this pathway saturates on increasing dose. Then the metabolism switches to sulfur oxidation. It is not known at what dose level this pathway becomes saturated.

A. Acute Toxicity

Table 1 summarizes the acute toxicity data for Molinate. Molinate is not acutely toxic *via* the oral [rats], dermal [rabbits], or inhalation [rats] routes of exposure in the studies required for labeling. In rats, Molinate is mildly irritating to the skin, is not a dermal sensitizer, and is a moderate eye irritant in the rabbit. Molinate was shown to be a delayed neurotoxicant in the hen, producing axonal degeneration in the brain and cervical spinal cord. Neurotoxic effects were also observed in the rat following acute oral exposure [acute neurotoxicity study].

Table 1. Acute Toxicity of Molinate

Guideline No.	Study Type	MRID #	Results	Toxicity Category
§81-1 870.1100	Acute Oral - rat	40593301	LD_{50} = 730 mg/kg (679-785) Males = 700 mg/kg (620-791) Females	III
§81-2 870.1200	Acute Dermal -rabbit	40593301	LD_{50} > 2000 mg/kg	III
§81-3 870.1300	Acute Inhalation - rat	00245675	LC_{50} = 2.9 mg/L (2.5-3.3) Males = 2.4 mg/L (2.2-2.6) Females	IV
§81-4 870.2400	Primary Eye Irritation	40593301	moderate irritant	II
§81-5 870.2500	Primary Skin Irritation	00247547	mild dermal irritant	IV
§81-6 870.2600	Dermal Sensitization	40593302	not a sensitizer	N/A
§81-7 870.6100	Acute Delayed Neurotoxicity (Hen)	00133562 43136601	NOAEL = 0.2 g/kg, based on axonal degeneration in brain and cervical spinal cord; delayed neurotoxicant.	N/A
§81-8 870.6200	Acute Neurotoxicity - rat	43188001	no NOAEL [LOAEL 25 mg/kg]; ↓ motor activity, ↑ time to tail flick; NTE, ChE, GFAP activities were not assessed at appropriate times	N/A Unacceptable

The above studies satisfy the acute toxicity data requirements (OPPTS 870.1100-870.1300, 870.2400-870.2600, 870.6100-870.6200; formerly §81-1 through §81-8) for Molinate.

B. Subchronic Toxicity

Available studies are adequate to satisfy subchronic testing requirements for Molinate. Although there are no 90-day feeding studies in either the rat or dog, there are chronic studies in both species. The data base provides evidence that Molinate has anticholinesterase activity [ChE], but clinical signs were not observed at dose levels where ChE activity inhibition was observed in rats, mice, or dogs following subchronic oral exposure, except in dogs at a dose level that was toxic and discontinued after 14 weeks of exposure. Decreased serum ChE activity was observed in rats at the 3-month interval in the chronic oral toxicity study, and decreased RBC, plasma, and brain ChE activities were observed in rats following inhalation exposure after 8 and 13 weeks. Male fertility and reproductive effects were observed following 4 weeks and 13 weeks of exposure of males *via* the inhalation route, and testicular degeneration was observed in the 13-week inhalation study.

Subchronic toxicity in rats *via* inhalation

In a **13-week inhalation toxicity study** [Accession No. 241965], 10 Sprague-Dawley CD® rats/sex/group were administered Molinate [% a.i. not provided] *via* the inhalation route [6 hours/day, 5 days/week for 3 months] at concentrations of 0, 2, 10, and 50 mg/m^3 [analytical cumulative mean exposure was 0, 2.2, 11.1, and 42 mg/m^3; MMAD=GSD: 0.96μm ± 1.9].

All rats survived until study termination. At the high-dose level in both sexes, there was a decrease in body weight [males 81%/females 84% of control at termination] and body-weight gain [males 69%/females 77% of control overall] throughout the study. Clinical signs [increased incidences of mucoid nasal discharge, excessive lacrimation, labored and rapid breathing, and aggressive behavior] were observed at the high-dose level and were considered related to treatment. Hematology [increased erythrocyte and decreased leukocyte values] and clinical chemistry values [decreased potassium and increased blood urea nitrogen] were noted at the mid- and/or high-dose levels and may be related to treatment. Decreased RBC cholinesterase activity [≈30% of control] was observed in both sexes at the high-dose level at weeks 8 and 13, and decreased plasma cholinesterase activity [≈50% of control] was observed in the high-dose females at weeks 8 and 13. Brain cholinesterase was decreased in the mid-dose males [80% of control] and in both sexes at the high-dose level [males 55%/females 50% of control]. At study termination, decreased brain weight was observed in the high-dose females, decreased pituitary weight was observed in both sexes at the high dose, and decreased testes weight was observed in the high-dose males. Decreased heart, spleen, and lung weights were observed in both sexes at the high-dose level, which may be attributed to the decreased body weight. Increased adrenal weights were observed in the mid-dose males and in both sexes at the high-dose level, and increased thyroid weights were observed in the mid-dose males and the high-dose females. The testes of 3 high-dose males were smaller than normal. Testicular degeneration was observed at all dose levels in the males but not in any of the controls, and a significant number of abnormal spermatozoa were observed in the epididymides at all dose levels. Both findings are considered treatment-related.

No NOEL was determined, based on testicular degeneration and abnormal spermatozoa, which were observed at all dose levels. These results are consistent with those in a 4-week inhalation study [MRID 41589203], in which detached sperm heads and sperm with broken membranes between the head and midpiece or between midpiece and tail sections were seen in treated male rats at dose levels as low as 0.64 mg/m^3 [0.00064 mg/L].

This guideline study is classified Acceptable, and it satisfies the guideline [§82-4] requirement for a subchronic inhalation toxicity study in the rat. Although the % a.i. is not known for this study, other studies performed on Molinate during the time interval when this study was performed indicate a purity ranging from 97.6% to 98.2%.

In a **4-week inhalation toxicity study** [MRID 41589203], 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/m^3 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.**

This nonguideline [OPPTS 870.3465; §82-1] subchronic inhalation toxicity study in the rat is **Acceptable.** It is nonguideline because the duration of exposure was 28 instead of 90 days, only males were exposed to Molinate, and only fertility parameters were assessed.

Dermal toxicity in rabbits [21-day]

In a 21-day dermal toxicity study [MRID 40990601], 5 SPF Wistar-derived albino [Alpk:APfSD] rats/sex/group were administered Molinate [97.6% a.i.] *via* the skin at dose levels of 0, 10, 25, and 50 mg/kg/day for 21 days [6-hour application/day; ≈50 cm² of the dorsal-lumbar region].

There was a slight to moderate skin irritation observed grossly, and minimal or slight acanthosis, unaccompanied by inflammatory cell infiltration, was observed histologically at the 25 and 50 mg/kg/day dose levels in both sexes, which is consistent with low-grade chronic irritation. A statistically-significant decrease in RBC cholinesterase activity was observed at all dose levels, although a dose response was not evident [♂ 11%, 9%, 16%/♀ 20%, 15%, 17% at the low-, mid-, and high-dose levels, respectively]. No other significant systemic toxicity was observed at any dose level. **For RBC cholinesterase inhibition, no NOAEL could be established. The NOAEL for skin irritation is 10 mg/kg/day, and the LOAEL is 25 mg/kg/day, based on skin irritation and acanthosis.** In a preliminary study on 2 rats/sex/dose, up to 5 applications of undiluted Molinate at dose levels of 10, 100, and 1000 mg/kg/day were used, and deaths occurred at the high-dose level [dosing stopped after 4 days in one rat] and severe skin irritation occurred at the mid-dose level.

This guideline 21-day dermal toxicity study in the rat is classified Unacceptable, due to the lack of data with respect to plasma and brain cholinesterase activity. The study does not satisfy the guideline requirement [870.3200; §82-2] for a 21-day dermal toxicity study, and since these measurements were not performed during the study, this study cannot be upgraded.

C. Chronic Toxicity [feeding]

Available studies are adequate to satisfy chronic toxicity and carcinogenicity testing requirements for Molinate. The toxicology data base provides evidence that Molinate has anticholinesterase activity, as evidenced by clinical signs [ataxia, tremors, salivation, reduced motor activity, splayed/adducted hindlimbs, and abnormal gait] and decreased cholinesterase activity in plasma, RBC, and brain. There was evidence of neuropathology in the chronic studies in rats, mice, and dogs, as evidenced by the degeneration/demyelination of the sciatic nerve. Molinate is classified as Group C [Q_1^*], possible human carcinogen, based on an increase in kidney tumors in male rats [Document No. 009761; CPRC memo dated September 14, 1998].

Chronic toxicity in dogs

In a **chronic toxicity study in dogs** [MRID 41781101], the oral administration of Molinate [97.6%] via the diet for one year to Beagle dogs [4/sex/group] at dose levels of 0, 1, 10, 50, and 100 mg/kg/day [the 100 mg/kg dose group was dosed with test material for only 14 weeks] resulted in decreased body-weight gain [47% ♂♂/55% ♀♀ of control], adverse effects on the nervous system [ataxia, reduced locomotor activity, splayed hindlimbs, tremors, eosinophilic bodies/vacuolation of the medulla/pons, and spinal cord and sciatic nerve demyelination], decreased serum cholinesterase in both sexes at 50 mg/kg/day [73%-79% of control], decreased brain weight at 10 mg/kg/day in females and at 50 mg/kg/day in both sexes, increased adrenal weight in both sexes at 50 mg/kg/day, anemia, and decreased ejaculate volume and percent mobile sperm at the 50 mg/kg/day dose level. Neurological abnormalities were observed in one dog/sex at 10 mg/kg/day and in all dogs of both sexes at the 50 mg/kg/day and 100 mg/kg/day dose levels. Demyelination of the sciatic nerve was observed at all dose levels in males and in the lumbar, sacral, and thoracic regions of the spinal cord at all dose levels in both sexes compared to no instances in either sex of the control. **No NOAEL for neurotoxic effects was observed in this study, based on the occurrence of demyelination of the sciatic nerve at all dose levels in males and in the lumbar, sacral, and thoracic regions of the spinal cord at all dose levels in both sexes compared to no instances in either sex of the control.**

Although there is no NOAEL overall, based on the microscopic findings in the sciatic nerve and spinal cord, this guideline chronic toxicity study in the dog is classified Acceptable, and it satisfies the guideline requirement [OPPTS 870.4100; §83-1(b)] for a chronic toxicity study in the non-rodent.

Chronic toxicity/carcinogenicity in rats [feeding]

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], 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 [♂ 29/♀ 35 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. In females at the 300 ppm and 600 ppm dose levels, there was an increased incidence of eosinophilic bodies in the lumbar and sacral areas of the spinal cord 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. NOTE: Results of a 10-day oral dosing study in rats [MRID 42224901] suggest that the mechanism of kidney tumor induction in the rat by Molinate is not related to α-2μ-globulin accumulation.

No NOAEL for neurotoxic effects was determined. The LOAEL for neurotoxic effects 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.

This guideline combined chronic toxicity/carcinogenicity study in rats is classified Acceptable, and it satisfies the guideline requirement [OPPTS 870.4300; §83-5] for the combined chronic toxicity/carcinogenicity study in rats.

Chronic toxicity/carcinogenicity in mice [feeding]

In an 18-month dietary mouse carcinogenicity study [MRID 41809201], exposure of Crl:CD®-1 (ICR)BR mice [50/sex/group] to Molinate [97.6%] at dose levels of 10 [1.0 ♂♂/1.3 ♀♀ mg/kg/day], 100 [10.4 ♂♂/13.9 ♀♀ mg/kg/day], 1000 [105 ♂♂/133 ♀♀ mg/kg/day], and 2000 [200 ♂♂/249 ♀♀ mg/kg/day] ppm in the diet for 18 months resulted in decreased survival [62% ♂♂/58% ♀♀ vs 76% in control ♂&♀], lower body weight [85% ♂♂/73% ♀♀ of control]/body-weight gain [64% ♂♂/63% ♀♀ of control], decreased food consumption [89% ♂♂/83% ♀♀ of control], and an increase in the incidence of several clinical observations indicative of neurological involvement [hindlimb muscle weakness, adducted hindlimbs, ataxia, splayed hindlimbs] in both sexes at the highest dose level. Absolute brain weight was decreased in the high-dose females, adrenal weight (absolute and relative to body and brain weight) was increased in the high-dose females, and testes weight (absolute and relative to brain) was decreased in males at the highest dose level. There was a treatment-related increase in the incidence of several nonneoplastic lesions [(1) demyelination and Schwann cell hyperplasia of the sciatic nerve [both sexes], eosinophilic bodies in the spinal cord and brain and (2) thecal/interstitial cell hyperplasia of the ovaries were increased at the two highest dose levels, (3) degeneration of the testes was increased at the three highest dose levels, (4) atrophy of the uterus and mammary gland was increased in females at the highest dose level, and (5) degeneration and mineralization of the adrenal gland was increased in both sexes at the two highest dose levels]. There was no treatment-related increase in neoplastic lesions. **The NOAEL for all effects except those on the testes can be set at 100 ppm (males 10.4/females 13.9 mg/kg/day), the LOAEL at 1000 ppm (males 105/females 133 mg/kg/day), based on decreased survival [62% ♂♂/58% ♀♀ vs 76% in control ♂&♀], decreased body weight [85% ♂♂/73% ♀♀ of control]/gain [64% ♂♂/63% ♀♀ of control], food consumption [89% ♂♂/83% of control], and increased incidence of nonneoplastic lesion of the brain, spinal cord, sciatic nerve, adrenals, and ovaries. A NOAEL of 10 ppm (1.0 mg/kg/day) can be set for effects on the testes, the LOAEL at 100 ppm (10.4 mg/kg/day), based on testicular degeneration.**

This guideline study is classified Acceptable, and it satisfies the guideline [OPPTS 870.4300; §83-2] requirement for a carcinogenicity study in the mouse.

D. Developmental/Reproductive Toxicity

Available developmental toxicity studies are adequate to satisfy guideline requirements. There is evidence of increased sensitivity of offspring in the rat, but no evidence of increased sensitivity of offspring in rabbits after prenatal exposure. There was no evidence of increased sensitivity of offspring in the two-generation reproduction study in rats. Developmental toxicity [increased runting] was observed in rats at the maternal NOAEL and delayed fetal development was observed in rabbits at the same dose that produced maternal toxicity [increased abortions]. Increased sensitivity was also noted in the developmental neurotoxicity study in rats [see below under Neurotoxicity]. In the 2-generation reproduction study, no NOAEL was determined for decreased brain weight for either sex, and the effect is considered both a reproductive/developmental effect and a systemic effect. Also observed was an increase in the incidence of testicular and ovarian lesions.

Developmental toxicity in rats

In a **developmental toxicity study** [MRID 41473401], 26 sperm-positive Crl:CD(SD) BR VAF/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 [memo dated 9/22/92; Document No. 009731] 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.**

This guideline developmental toxicity study in the rat is classified **Acceptable**, and it satisfies the guideline requirement for a developmental toxicity study in the rodent [OPPTS 870.3700; §83-3(a)].

Developmental toxicity in rabbits

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. Although there was a decrease in supernumery ribs at the high-dose level, the HED Developmental and Reproductive Toxicity Peer Review Committee (PRC) [memo dated 9/22/92; Document No. 009731] 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.

Reproductive toxicity in rats

(1) 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 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 NOAEL 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, F0 females displayed decreased uterus weight, F0 and F1 males displayed decreased epididymis weight, and pup survival was the lowest among the groups in all litters.

This guideline 2-generation reproduction study in the rat is classified Acceptable, and it satisfies the guideline requirement [§83-4; OPPTS 870.3800] for a 2-generation reproduction study.

(2) In a non-guideline 2-generation reproduction study [MRID 41333402], female Crl:CD®(SD) BR VAF/Plus™ rats [25/group] were administered Molinate [97.6% a.i.] *via* the diet for 60 [P0]/63 [P1] days of treatment 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.

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 450 ppm dose level [28 mg/kg/day], 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. The reproductive NOAEL is 6 ppm [0.34 mg/kg/day], and the reproductive LOAEL is 50 ppm [2.9 mg/kg/day], based on the occurrence of vacuolation/ hypertrophy of the ovary. At the 450 ppm dose level [28 mg/kg/day], 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 [0.34 mg/kg/day, and the neonatal LOAEL is 50 ppm [2.9 mg/kg/day], based on ovarian lesions. At the 450 ppm dose level [28 mg/kg/day], decreased brain weight and increased adrenal weights were observed, in addition to the ovarian lesions.

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

(3) In a reproduction/fertility inhalation study [Accession No. 241965], 10 Sprague-Dawley CD® male rats/group were administered Molinate [% a.i. not provided] *via* the inhalation route [6 hours/day, 5 days/week for 3 months] at mean cumulative/achieved concentrations of 0, 2, 10, and 50 mg/m³ [analytical cumulative mean exposure was 0, 2.2, 11.1, and 42 mg/m³; MMAD was 0.96μm ± 1.9.] . Following (a) a one-month period, (b) a 3-month exposure period, © a 3-month exposure period followed by a 1-month recovery period, and (d) a 3-month exposure period followed by a 3-month recovery period, each male was caged with 2 untreated female Sprague-Dawley rats nightly for 10 consecutive days [during last 10 treatment days for (b)].

There were no treatment-related deaths, and clinical signs were comparable among the groups with the exception of an increase in the incidence of closed eyes in the high-dose males during the last 4 weeks of the treatment period. Body weight [87%-93% of control] and body-weight gain [9% of control] were decreased at the high-dose level compared to the control during the exposure phase, decreased body weight was also observed during the recovery

phase [84%-96% of control] at the high-dose level, but body-weight gain was increased compared to the control at the mid- and high-dose levels during recovery. Terminal body weights were comparable among the groups. At the high-dose level, testes weights were decreased [76% of control] compared to the control.

One-Month Exposure: Pregnancy indices were decreased at the mid- and high-dose levels compared to the control, and male fertility indices were lower than control at these 2 dose levels, although only the high-dose group attained statistical significance. There was a dose-related decrease in the mean number of implantations, and the mean number of fetuses was significantly decreased at the low- and mid-dose levels. At the high-dose level, all implantations were resorptions; no viable fetuses were observed. Three-Month Exposure: None of the mated females was pregnant at the high-dose level. Male mating indices were comparable among the groups, but the pregnancy rates and male fertility indices were decreased at the mid-dose level compared to the control. The mean number of implantations and fetuses at the low- and mid-dose levels were significantly lower than the control, but the mean number of resorptions was comparable. One-Month Recovery: Mating indices were comparable among the groups, but the pregnancy rate was decreased at the high-dose level compared to the control. Male fertility index was slightly lower at the high-dose level compared to the control. The mean numbers of implantations, resorptions, and fetuses were comparable among the groups. Three-Month Recovery Period: Mating, pregnancy, and fertility indices were comparable among the groups. The mean numbers of implantations, resorptions, and fetuses were comparable among the groups.

There was a dose-related decrease in the mean number of implantations and the mean number of fetuses, both of which were statistically significant at all dose levels. No NOAEL was determined in this study.

This nonguideline study is classified Acceptable, but it does not satisfy any guideline requirement [it is part of a subchronic inhalation toxicity study (cited above under B. Subchronic Toxicity)]. Only male rats were administered Molinate.

(4) In a 4-part study [Accession No. 245675], 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].

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. It should be noted that the source of rats in Part IV was different than the source of rats in the other three parts of the study. 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.

In summary, 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 .**

The HED Developmental and Reproductive Toxicity Peer Review Committee [Document No. 009731] considered the NOEL 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."

This nonguideline study is classified Acceptable.

Special Studies

(1) In a special study [MRID 44373601], 40 Sprague-Dawley Crl:CD(SD)BR female rats [10-12 weeks old] were exposed to Molinate [96.8%] *via* the diet at dose levels of 0 ppm and 300 ppm from day 7 of gestation until the weaning of their pups [day 22 *post partum*]. All selected F1 pups received the appropriate test diet from the day of weaning until study termination. On *post partum* day 22, two control groups and two Molinate groups were created, each consisting of 40 F1 pups/sex. On day 28 *post partum*, the female pups in one of the control groups and one of the Molinate groups were injected with estradiol benzoate. Beginning on day 28 *post partum*, the female F1 rats were examined daily for vaginal opening.

There were no treatment-related deaths. In general, comparable body weights were observed during gestation between the control and Molinate F0 dams. Body-weight gain was decreased during the 7-15 day interval [≈82% of control] compared to the control. There was a significant decrease [93%-94% of control] in body weight in the Molinate F0 group compared to the control from day 8 to 22 of lactation, and body-weight gains were decreased during the 1-15 day interval of lactation [56%-88% of control] compared to the control also. The number of whole litter losses, the number of dams that failed to litter, pup survival, sex ratio, and litter size were comparable between the groups.

Decreased body weight was observed in the Molinate F1 males on days 1 [94% of control], 15 [90% of control], and 22 [91% of control], in the Molinate F1 females on day 22 [91% of control], and in the Molinate F1 litters on days 11 [89% of control], 15 [87% of control], and 22 [90% of control] compared to the control. After day 8 *post partum*, both sexes of the Molinate group displayed lower body-weight gains than the controls. Total litter weight was significantly lower than the control from day 8.

Administration of Molinate delayed vaginal opening in both the hormone-treated [32.9 days] and the untreated groups [39.5 days] compared to the control [31.8 days and 35.2 days, respectively] groups. Hormone treatment accelerated vaginal opening from 35.2 days to 31.8 days in the control females and from 39.5 days to 32.9 days in the Molinate females. The study author concluded that the results demonstrated that the delay in vaginal opening associated with Molinate exposure was due to the lack of estrogen at this sensitive time in development. This reviewer points out that the administration of estradiol on day 28 post partum had a similar effect on the control female pups in that there was a shortening of the time required for vaginal opening, under conditions of presumed normal hormone levels. No data were provided to demonstrate either the lack of estrogen in the Molinate-treated females or lower hormone levels compared to the control. There was no discussion regarding any mechanism by which lower hormone levels might result; e.g., blocking of the hormone receptor, interference with hormone synthesis, or on changes in thyroid or growth hormone, which can also cause a delay in development.

Molinate has been shown to delay vaginal opening in female rats exposed to Molinate *in utero* and during lactation, but no definitive mechanism/mode of action has been demonstrated. Administration of estradiol benzoate on day 28 *post partum* resulted in an earlier occurrence of vaginal opening in both the control [from 35.2 days to 31.8 days] and Molinate-treated [from 39.5 days to 32.9 days] rats.

This nonguideline special study on vaginal opening in rats is classified Acceptable.

(2) In a special study [MRID 43158202], **male** Crl:CD(SD)BR rats [12/group] were exposed to Molinate [96.8% a.i.] *via* gavage for 35 consecutive days at dose levels of 0, 0.5, 1, 2, 3, 4, and 8 mg/kg/day.

There were no deaths or clinical signs that could be attributed to treatment, and body weights and body-weight gains were comparable among the groups. The objective of the study was to define more precisely the NOEL for changes in sperm morphology observed in the rat following exposure to Molinate. All rats displayed headless sperm, but the percent abnormal was greater at all dose levels of Molinate [not explicitly dose-related] compared to the controls. There was a dose-related increase in the incidence of sperm midpiece abnormalities, and the number of sperm affected was increased with increasing dose. None of the concurrent controls displayed this type of abnormality. **No NOEL for sperm morphology was determined.**

This nonguideline study is classified Unacceptable, based on the lack of individual data with respect to total sperm counts and the number of sperm that displayed the different abnormalities, among other deficiencies.

(3) In a mechanistic study [MRID 42361308], 10 sperm-positive female Crl:CD(SD)BR Sprague-Dawley rats per group were administered Molinate [98.1% a.i.] *via* gavage at dose levels of 0 [corn oil], 75, 135, and 200 mg/kg/day on gestation days 7 through 9. The stated objective of the study was to investigate the potential histopathological ovarian changes following a short-term [days 7-10 of gestation], high-dose regime to pregnant rats.

There were 14 treatment-related deaths during the study [5 mid-dose sacrificed on day 10, 9 high-dose {sacrificed on days 8 (1), 9 (1), and 10 (3); 4 found dead on day 10}]. Clinical signs observed prior to death included piloerection, signs of urinary incontinence, sides pinched in, subdued behavior, hunched posture, salivation, abnormal gait [high-stepping and splayed], perinasal and perioral staining. Clinical signs displayed by survivors at the mid-dose level included head held twisted to one side [3 dams] and rolling gait [1 dam]. One dam at the high-dose level displayed head held twisted to one side. There was a dose-related decrease in body weight, with a statistically-significant decrease at all dose levels on day 10 [95%, 87%, and 85% of control]. Negative body-weight gains were observed at the mid- and high-dose levels during the dosing period [days 7-9] and after [days 9-10], and cumulative body-weight gains [days 2-10] were significantly decreased at all dose levels [82%, 51%, and 26% of control] compared to the controls. Although lower progesterone levels were observed at the mid- [74% of control]

and high-dose [67% of control] levels, but too few rats were available for any definitive conclusion regarding this parameter. Comparable numbers of corpora lutea and implantations were observed among the groups. There was a dose-related increase in adrenal weights, with statistical significance being attained at all dose levels [121%, 125%, and 168% of control]. There was a dose-related increase in the incidence and severity of cellular swelling and vacuolation in the zonae fasciculata and reticularis of the adrenal cortex in the mid- and high-dose dams. One mid- and 5 high-dose dams also displayed minimal or slight multifocal degeneration with necrosis and loss of cells in the zona fasciculata. There was a dose-related increase in the neutral lipid content of cells in these two regions of the adrenal cortex at all dose levels. In the ovary, there was a dose-related increase in the incidence and/or severity of fatty vacuolation of corpora luteal cells. There was no evidence of an adverse effect on the ability to maintain a pregnancy. **No NOEL was demonstrated for microscopic lesions in the adrenal cortex and ovary. There was no evidence of an effect on the ability to maintain a pregnancy. Additionally, there was no discussion as to a possible cause of the deaths observed in this study at dose levels that had not been shown previously to result in death in this strain/sex of rat.**

This mechanistic [non-guideline] study is classified Unacceptable, but it can be upgraded with the submission of the individual animal data.

E. Mutagenicity

Available mutagenicity studies are adequate to satisfy the guideline requirements. Because there was an indication of activity for three endpoints in the mouse lymphoma assays with activation, the observed germ cell interaction of Molinate, and the positive response in a bone marrow micronucleus test, a dominant lethal study in the rat was performed. There was no indication of a positive dominant lethal effect in male germinal cells.

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 870.5100.

Molinate was negative for clastogenic activity in cultured human lymphocytes with and without metabolic activation [MRID 40946701; Document No. 8549]. OPPTS 870.5375.

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

Molinate was negative in the *in vitro* Unscheduled DNA Synthesis assay [MRIDs 41052701 and 43192301; Document Nos. 008549 and 011187]. OPPTS 870.5500.

In a dominant lethal assay, there was no evidence that Molinate technical induced a dominant lethal effect in male rat germinal cells treated over the entire period of spermatogenesis [MRID 43986701/44562201; Document No. 013017]. OPPTS 870.5450.

F. Metabolism

Available metabolism data are adequate to satisfy the guideline requirements. Doses of Molinate were well absorbed and extensively metabolized after both oral and i.v. administration. Molinate was rapidly excreted, mainly in the urine, and of that excreted in the urine, the majority was excreted within 24 hours. A small percent of the dose was eliminated *via* the feces and expired air. The highest tissue levels were found in the blood cells, with the total

amount retained by the rat at 96 hours post dose being ≈3.5% of the dose. Metabolism of Molinate involved s-oxidation to form the intermediate Molinate sulfoxide, which was either hydrolyzed to hexamethyleneimine or conjugated with glutathionine, ultimately forming Molinate mercapturic acid; ring hydroxylation at the 3 and 4 positions followed by glucurinide conjugation was also a significant route of metabolism.

In a metabolism study [MRID 41781801-41781805], groups of 5 Crl:CD®(SD)BR rats/sex were administered radiolabeled Molinate [99.5%] *via* gavage as a (1) single low [10 mg/kg] dose; (2) single high [100 mg/kg] dose; (3) single i.v. [1 mg/kg] dose into tail vein; or (4) 14 repeat low doses [10 mg/kg/day] of unlabeled Molinate followed by a single low [10 mg/kg] dose of radiolabeled Molinate on day 15.

Doses of Molinate were well absorbed and extensively metabolized after both oral and i.v. administration. After 14 consecutive doses of 10 mg/kg/day, a single dose of radiolabeled Molinate [10 mg/kg] was rapidly excreted, mainly in the urine [79% for females and 83% for males], and of that excreted in the urine, 90% was excreted within 24 hours. A small percent of the dose was eliminated *via* the feces [3%-10%] and expired air [1%-1.5%]. The highest tissue levels were found in the blood cells, with the total amount retained by the rat at 96 hours post dose being ≈3.5% of the dose. Greater that 90% of the dose was recovered. Single doses of 10 mg/kg and 100 mg/kg were handled similarly [70%-72% of the dose was excreted *via* the urine; 8%-11% (males)/5% (females) *via* the feces; ≈1% *via* expired air; tissue concentration was dose-related and represented 2%-3% of the administered dose; ≈85% of the dose was recovered]. Single i.v. doses showed similar excretion pattern as the oral dose, and the major route of excretion was the urine, with 90% of that excreted in the urine being excreted within 72 hours of dosing; 5%-7% (males)/2%-6% (females) *via* the feces; 0.9%-1.3% *via* expired air. The highest tissue concentration was found in the liver; the mean % of the dose after 168 hours was 2.6% (males)/2.8% (females); total recovery of radiolabel was 89% (males)/85% (females). Biliary excretion would account for the slower elimination following i.v. dosing.

Metabolism of Molinate involved s-oxidation to form the intermediate Molinate sulfoxide, which was either hydrolyzed to hexamethyleneimine or conjugated with glutathionine, ultimately forming Molinate mercapturic acid; ring hydroxylation at the 3 and 4 positions followed by glucurinide conjugation was also a significant route of metabolism. Ten of 22 metabolites isolated in the urine were identified, and these accounted for 74%-86% of the urinary radiolabel. A lower percentage of mercapturic acid metabolites was excreted following i.v. exposure than following oral exposure. There were no sex- or dose-related differences noted.

These metabolism studies, when considered together, are classified Acceptable, and the guideline [OPPTS 870.7485; §85-1] requirement for a metabolism study in the rodent is satisfied.

G. Neurotoxicity

Available neurotoxicity studies are adequate to satisfy the guideline requirements. Neurotoxic effects are consistent findings in studies on Molinate. Molinate caused axonal degeneration in the brain and cervical spinal cord in a delayed neurotoxicity study in the hen, and neurotoxic effects [characterized by decreased motor activity, a decrease in brain ChE activity, a reduction in startle amplitude] were observed in the rat following both acute and subchronic oral exposures.

Acute neurotoxicity in hens

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 incoordination, 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.**

This guideline [OPPTS 870.6100; §81-7] acute delayed neurotoxicity study in the hen is classified Acceptable.

Acute neurotoxicity in rats

In an acute neurotoxicity study [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.

This guideline [OPPTS 870.6200; §81-8] acute neurotoxicity study in rats is classified Acceptable.

Subchronic neurotoxicity in rats

In a 90-day neurotoxicity study [MRID 43270701 and 43965901], Molinate [96.8%] was administered 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.

Survival and clinical signs were comparable among the groups. There was 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.

This guideline [OPPTS 870.6200; §82-7] subchronic neurotoxicity study in rats is classified Acceptable.

Developmental neurotoxicity in rats

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 24] 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 neurotoxicity 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.

Recommendation for a developmental neurotoxicity study

There is a developmental neurotoxicity study on Molinate [see above].

II. Uncertainty Factor/FQPA Considerations

The following evaluation of the chemical Molinate is provided to address FQPA considerations on the sensitivity of infants and children.

The application of a FQPA Safety Factor to ensure the protection of infants and children from exposure to Molinate, as required by FQPA, was determined by the HED FQPA Safety Factor Assessment Review Committee [see below]. The HIARC recommended to the FQPA Safety Factor Committee that the FQPA 10X Safety Factor be retained due to the increased sensitivity of offspring observed in both the developmental toxicity study in rats and the developmental neurotoxicity study in rats. Molinate was presented to the HED FQPA Safety Factor Committee on November 30, 1998.

In the prenatal developmental toxicity study in rats, developmental toxicity [increased runting] was observed at 35 mg/kg/day [LOAEL] while maternal toxicity was observed at 140 mg/kg/day [LOAEL]. The developmental toxicity NOAEL [2.2 mg/kg/day] was lower than the maternal toxicity NOAEL [35 mg/kg/day], indicating increases in sensitivity in the rat fetuses following *in utero* exposures to Molinate. Additionally, in the prenatal developmental neurotoxicity study in rats, developmental ~~neu~~rotoxicity [a reduction in startle amplitude in the auditory startle test] was observed at the lowest dose tested, 1.8 mg/kg/day [LOAEL] while maternal toxicity was observed at 26.1 mg/kg/day [LOAEL]. The maternal NOAEL was 6.9 mg/kg/day, and the developmental neurotoxicity NOAEL could not be determined, indicating increases in sensitivity in the rat fetuses. The data provided no evidence of increased susceptibility/sensitivity of rabbits to *in utero* exposure to Molinate or of rats following pre- and/or postnatal exposure in the 2-generation reproduction study.

The FQPA Safety Factor Committee determined that the 10x FQPA safety factor should be retained, based on, among other factors, the following: (1) increased susceptibility observed in the prenatal developmental toxicity study in rats; (2) increased susceptibility observed in the developmental neurotoxicity study in rats; and (3) reproductive effects *were observed* in mice [anti-fertility study] and rats [sperm morphology study] following oral administration, although there was no evidence of increased susceptibility in the 2-generation reproduction study in rats. A copy of the FQPA Safety Factor Committee Report, dated November 30, 1998 [HED Document No. 013026] is appended.

III. Toxicity End-Point Selection

On October 1 and 7, 1998, the Hazard Identification Assessment Review Committee [HIARC] evaluated the entire toxicological database on Molinate and selected the relevant toxicity endpoints, taking into consideration the use patterns and exposure information on this chemical. The selected toxicological endpoints and the doses for risk assessment are summarized in Table 2, and additional relevant details for each endpoint are presented. A copy of the HIARC Report, dated October 30, 1998 [HED Document No. 012944] is appended.

Table 2. Doses and Toxicological Endpoints Selected for Various Exposure Scenarios			
EXPOSURE SCENARIO	DOSE(mg/kg/day)	ENDPOINT	STUDY
Acute Dietary ♪	LOAEL = 1.8 (UF = 300) [FQPA UF = 10]	neurotoxic effects	Developmental Neurotoxicity
		Acute RfD = 0.006 mg/kg FQPA adjusted acute dose - 0.0006 mg/kg	
Chronic Dietary ♪ non-carcinogenic effects	LOAEL=0.3 (UF=300) [FQPA UF = 10]	degeneration/demyelination	Rat Chronic Toxicity/ Carcinogenicity
		Chronic RfD = 0.001 mg/kg/day FQPA adjusted chronic dose = 0.0001 mg/kg/day	
Carcinogenic effects	$Q^*_1 = 1.1x10^{-1}$ $(mg/kg/day)^{-1}$	male rat kidney tumors	
Short-Term* (Dermal)	LOAEL = 1.8 UF = 300	neurotoxic effects	Developmental Neurotoxicity
Intermediate-Term* (Dermal)	NOAEL = 0.2 UF = 100	↓ % viable sperm. % motile sperm, % normal sperm, sperm counts, # implants, # viable fetuses; ↑ pre-implantation loss	5-week rat fertility
Chronic Dermal non-carcinogenic effects	N/A	N/A	N/A
Inhalation Short-Term	NOAEL = 0.12 mg/L UF = 100	hindleg muscle weakness and testicular effects	acute inhalation - rat
Inhalation Intermediate-Term	NOAEL = 0.0003 mg/mL UF = 100	decreased # implants and increased % abnormal sperm	4-week inhalation - rat
Chronic Inhalation non-carcinogenic effects	N/A	N/A	N/A

♪ FQPA Safety Factor retained for acute and chronic dietary risk assessments for All Populations, which include Infants and Children; * appropriate route-to-route extrapolation should be performed for these risk assessments. Exposure values using a dermal absorption factor of 40% should be converted to equivalent oral doses and compared to the oral NOAEL.

A. DERMAL ABSORPTION FACTOR

A dermal absorption study [MRID 43284101] is available. A 40% absorption relative to an oral dose should be used.

B. ACUTE DIETARY ASSESSMENT [ONE DAY]

For acute dietary exposure, the developmental neurotoxicity study in the rat [MRID 44079201] was selected, based on neurotoxic effects at the LOAEL of 20 ppm [1.8 mg/kg/day]. There was no NOAEL. The Reference Dose [RfD] for acute oral exposure is 0.006 mg/kg/day, based on a LOAEL of 1.8 mg/kg/day for neurotoxic effects and an Uncertainty Factor of 300 [10 for intraspecies, 10 for interspecies, 3 for the use of the LOAEL]. The FQPA Safety Factor [10x] is retained for acute dietary risk assessment for **All Populations, which include Infants and Children**]. The FQPA Safety Factor is retained because increased susceptibility was demonstrated in both the prenatal developmental toxicity and developmental neurotoxicity studies.

C. CHRONIC DIETARY ASSESSMENT

The Reference Dose [RfD] for chronic oral exposure is 0.001 mg/kg/day, based on a LOAEL of 0.3 mg/kg/day for degeneration/demyelination in the sciatic nerve and atrophy/reserve cell hyperplasia in the muscle and an Uncertainty Factor of 300 [10 for intraspecies, 10 for interspecies, 3 for the use of the LOAEL]. For this risk assessment, the FQPA Safety Factor is retained [10x]for **All Populations, which include Infants and Children**]. The FQPA Safety Factor is retained because of the concern for the severe reproductive effects observed following repeated oral exposures in studies with rats and mice.

D. SHORT TERM OCCUPATIONAL OR RESIDENTIAL EXPOSURE [1 to 7 days] **DERMAL**

For short term occupational ~~exposure~~, the developmental neurotoxicity study in the rat [MRID 44079201] was selected, based on neurotoxic effects at the LOAEL of 20 ppm [1.8 mg/kg/day]. There was no NOAEL. An UF of 300 will be used, which includes an additional UF of 3, due to the use of the LOAEL, in addition to the traditional UF of 100. Although there is a 21-day dermal toxicity study available on Molinate in rats, it was determined that it was not appropriate for use in this risk assessment because no NOAEL was determined in that study due to the lack of data with respect to plasma and brain cholinesterase. Since an oral NOAEL was identified, a dermal absorption factor of 40% should be used for this risk assessment.

E. INTERMEDIATE TERM OCCUPATIONAL OR RESIDENTIAL EXPOSURE [1 week to several months] **DERMAL**

For intermediate term occupational exposure, the four-part fertility study [Accession No. 245675] in rats was selected, based on decreases in the % viable sperm, % motile sperm, % normal sperm, sperm counts, number of implants, number of viable fetuses, and increased preimplantation loss at the LOAEL of 4 mg/kg/day. The NOAEL is 0.2 mg/kg/day. An UF of 100 will be used. Since an oral NOAEL was identified, a dermal absorption factor of 40% should be used for this risk assessment.

G. CHRONIC OCCUPATIONAL OR RESIDENTIAL EXPOSURE [several months to lifetime] **DERMAL**

Based on the use pattern, long-term exposure risk assessment is not required.

H. SHORT TERM OCCUPATIONAL OR RESIDENTIAL EXPOSURE [1 to 7 days] **INHALATION**

For short term inhalation exposure, the acute inhalation study in the rat was selected, based on hindleg weakness and testicular effects at the LOAEL of 0.28 mg/L. The NOAEL is 0.12 mg/L. The traditional UF of 100 will be used.

I. INTERMEDIATE TERM OCCUPATIONAL OR RESIDENTIAL EXPOSURE [1 week to several months] **INHALATION**

For intermediate term inhalation exposure, a subchronic [4-week] inhalation study [MRID 41589203] in rats was selected, based on a decreased number of implants and an increased percentage of abnormal sperm at the LOAEL of 0.00064 mg/L. The NOAEL was 0.0003 mg/L. The traditional UF of 100 will be used.

J. CHRONIC OCCUPATIONAL OR RESIDENTIAL EXPOSURE [several months to lifetime] **INHALATION**

Based on the use pattern, a long-term exposure risk assessment is not required.

SUMMARY OF TOXICOLOGY ENDPOINT SELECTION

III. **DATA GAPS**

There are no data gaps *per se*. However, the 21-day dermal toxicity study and the acute neurotoxicity study are both Unacceptable. Since both lack data with respect to cholinesterase activity and/or NTE activity at appropriate time points during the studies, neither is upgradeable.

The toxicology profile for Molinate is summarized in Table below. The toxicology database on Molinate is complete and will support reregistration eligibility.

Table 1. Toxicology Profile

Guideline [§/OPPTS]	Study Type	MRID #	Required	Satisfied
81-1/870.1100	acute oral - rats	40593301	yes	yes
81-2/870.1200	acute dermal - rabbits	40593301	yes	yes
81-3/870.1300	acute inhalation - rats	00245675	yes	yes
81-4/870.2400	primary eye irritation	40593301	no	yes
81-5/870.2500	primary dermal irritation	00247547	no	yes
81-6/870.2600	dermal sensitization	40593302	no	yes
81-7/870.6100	acute delayed neurotoxicity - hen	00133562 43136601	no	yes✖
81-8/870.6200	acute neurotoxicity - rat	43188001	yes	no☜
82-1/870.3100	subchronic feeding - rats	-	yes	yes♪
82-1/870.3150	subchronic feeding - dog	-	yes	yes♪
82-2/870.3200	21-day dermal - rabbits	40990601	yes	no♬
82-4/870.3465	90-day inhalation - rodent 4-week inhalation	00241965 41589203	no no	yes N/A
82-5/870.6200	subchronic neurotoxicity - rats	43270701 43965901	yes	yes
83-1(a)/870.4100	chronic toxicity - rats	41815101	yes	yes
83-1(b)/870.4100	chronic toxicity - dog	41781101	yes	yes
83-2/870.4200	carcinogenicity - mice	41809201	yes	yes
83-3(a)/870.3700	developmental toxicity - rat	41473401	yes	yes
83-3(b)/870.3700	developmental toxicity - rabbits	14021015	yes	yes
83-4/870.3800	2-generation reproduction - rats	44403201 41333402	yes	yes no√
83-5/870.4300	chronic toxicity/carcinogenicity - rat	41815101	yes	yes
83-6/870.6300	developmental neurotoxicity - rat	44079201	yes	yes
84-2/870.5100	gene mutation	40918301	yes	yes
84-2/870.5375	chromosomal aberration	40946701	yes	yes
84-2/870.5300	*in vitro* mammalian cell gene mutation	00163790	yes	yes

Table 1. Toxicology Profile				
Guideline [§/OPPTS]	Study Type	MRID #	Required	Satisfied
84-2/870.5550	unscheduled DNA synthesis	41052701 43192301	yes	yes
84-2/870.5450	dominant lethal assay	43986701 44562201	yes	yes
85-1/870.7485	metabolism	41781801- 41781805	yes	yes
85-2/870.7600	dermal penetration	43284101	no	yes
86-1\870.7200	domestic animal safety	-	no	-
none	other/fertility studies	00241965 00245675 44373601 43158202 42361308	no	N/A

♪there is a chronic study available; ♬ ChEI was not monitored; √ only females were administered Molinate;
✖ a 28-day hen study is not available; ☜ NTE, ChE, GFAP activities were not assessed at appropriate times

HED DOC. NO. 012944

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,
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, 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.

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 [75%-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 24] 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], ~~b~~rain 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 molinate, the HIARC concluded that the dose/endpoint selected here can also be used for acute dietary risk assessment for all populations since the effects were seen in the offspring 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
Guideline #: OPPTS 870.4300 §83-5

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 (10%-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)

Guideline #: §none

Study Selected: Fertility study - Rat

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

37

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 a 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/m^3 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/m^3, and the LOAEL is 0.64 mg/m^3, 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 the 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

97

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 1. Acute Reference Dose) Molinate [96.8% a.i.] was administered to 30 female Alpk:AP,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. 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.

56

REPRODUCTIVE TOXICITY: Clear evidence of reproductive toxicity has been found in rats, dogs, and mice. The most extensive testing has been performed in the male rat, which appears to be the most sensitive species/sex.

Rats: There is no question that Molinate is a reproductive toxin in the rat. Numerous studies have been performed in the rat, mainly in the male rat, and a wide range of parameters [testes weight, testicular lesions, sperm, fertility] are affected. Following acute inhalation exposure, microscopic lesions in the testes were observed. The number of implants was decreased and the percentage of abnormal sperm was increased following 4-week inhalation exposure of males. No NOAEL was determined in a 13-week inhalation exposure study [only males exposed] in which decreased testes weight, testicular degeneration, and abnormal spermatozoa in epididymides were observed at all dose levels. Following chronic oral exposure, decreased testes weight, increased ovarian weight, testicular degeneration with atrophy, increased incidence of thecal/interstitial cell vacuolation/hypertrophy of the ovary, and increased adrenal weights were observed. Increased incidence of vacuolation/hypertrophy of the ovary, decreased testes, right cauda, and adrenal weights, increased incidence of abnormal sperm, increased incidence/severity of diffuse fine cortical fat vacuolation of the adrenal, and delayed vaginal opening were observed in a 2-generation reproduction study. In the developmental toxicity study, decreases were observed in the number of live fetuses per dam and fetal body weight, and increases were observed in resorptions, post-implantation loss, number of runts, number of litters with runts, and the incidence of external/soft tissue [head]/skeletal variants. In the developmental neurotoxicity study [in addition to neurotoxic effects], there was an increase in the number of litters with small pups, a slightly higher pup mortality rate initially, an increased number of missing/presumed dead pups, and a delay in both preputial separation and vaginal opening following exposure to Molinate. In a fertility study in males, decreases were observed in the number of pregnancies, corpora lutea, implants, viable fetuses, sperm viability, sperm motility, sperm counts, and increases were observed in pre-implantation loss, abnormal sperm, number of seminiferous tubules with generating spermatids/spermatocytes per testis, and post-implantation loss. ___.

Dogs: In a one-year dog study, there were decreases in sperm ejaculate, a reduction in the percentage of motile sperm, testicular atrophy, and increased adrenal weight.

Mice: Testicular degeneration in males, increased incidence of ovarian hyperplasia and increased adrenal weight in females, and increased incidence of degeneration, ceriod/lipofuscin and mineralization of the adrenal in both sexes were observed following long-term exposure. Following acute inhalation exposure, decreased testes weight and germinal cell necrosis of the testes were observed. In a 7-week gavage study, there were decreases in the number of pregnancies, implants, and viable fetuses, and the incidence of mild testicular germinal cell degeneration of seminiferous tubules was increased in the treated mice compared to the controls.

Proposed Mode of Action [Fertility Effects]: The Registrant contends that the use of the data from the rat with respect to reproductive effects is not appropriate for the assessment of risk to man. The information submitted by the Registrant on a proposed mode of action with respect to the fertility/reproductive effects in the rat includes abstracts, published articles, and summary reports, but no individual data for review. The hypothesis

submitted is that (1) the effect of Molinate in inducing the male reproductive impairment is *via* a block in the production of testosterone, and (2) Molinate is likely inhibiting one of the esterases responsible for hydrolysis of cholesterol esters in the Leydig cells, which would profoundly affect testicular function through the inhibition of testosterone synthesis. In the rodent, a major source of cholesterol is from high-density lipoproteins that are hydrolyzed in the cell cytosol, which the Registrant states is unique to the rodent. It is also hypothesized that this impairment may be attributed to Molinate or a metabolite and, consequently, the species differences in the potency of Molinate can be accounted for by differences in the rate and route of metabolism, in addition to biochemical and physiological differences.

VII. DATA GAPS

Acute Neurotoxicity Study (§81-7).
21-Day Dermal Toxicity Study (§82-1).

VIII. ACUTE TOXICITY ENDPOINTS:

Acute Toxicity of Molinate

Guideline No.	Study Type	MRIDs #	Results	Toxicity Category
81-1 870.1100	Acute Oral - rat	40593301	LD_{50} = 730 mg/kg (679-785) Males = 700 mg/kg (620-791) Females	III
81-2 870.1200	Acute Dermal - rabbit	40593301	LD_{50} > 2000 mg/kg	III
81-3 870.1300	Acute Inhalation - rat	00245675	LC_{50} = 2.9 mg/L (2.5-3.3) Males = 2.4 mg/L (2.2-2.6) Females	IV
81-4 870.2400	Primary Eye Irritation	40593301	moderate irritant	II
81-5 870.2500	Primary Skin Irritation	00247547	mild dermal irritant	IV
81-6 870.2600	Dermal Sensitization	40593302		N/A
81-7 870.6100	Acute Delayed Neurotoxicity (Hen)	00133562 43136601	NOAEL = 0.2 g/kg, based on axonal degeneration in brain and cervical spinal cord; delayed neurotoxicant.	N/A

81-8 870.6200	Acute Neurotoxicity - rat	43188001	↓ motor activity, ↑ time to tail flick; NTE, ChE, GFAP activities were not assessed at appropriate times	N/A Unacceptable

IX. SUMMARY OF TOXICOLOGY ENDPOINT SELECTION

The doses and toxicological endpoints selected for various exposure scenarios are summarized below.

EXPOSURE SCENARIO	DOSE (mg/kg/day)	ENDPOINT	STUDY
Acute Dietary	LOAEL = 1.8	Neurotoxic effects	Developmental Neurotoxicity
	UF = 300	**Acute RfD = 0.006 mg/kg**	
Chronic Dietary non-carcinogenic effects	LOAEL=0.3	Degeneration/demyelination	Rat Chronic Toxicity/Carcinogenicity
	UF=300	**Chronic RfD = 0.001 mg/kg/day**	
Carcinogenic effects Dietary/Dermal	$Q^*_1 = 1.1 \times 10^{-1}$ $(mg/kg/day)^{-1}$	Male rat kidney tumors	——
Short-Term* (Dermal)	Oral LOAEL = 1.8	Neurotoxic effects	Developmental Neurotoxicity
Intermediate-Term* (Dermal)	Oral NOAEL = 0.2	Male reproductive effects	5-week rat fertility
Long-Term (Dermal / Non-cancer)	None	The use pattern (1-2 applications per season to rice) does not indicate potential long-term dermal exposure; risk assessment is NOT required.	
Short-Term (Inhalation)	NOAEL = 0.12 mg/L	Hindleg muscle weakness and testicular effects	Acute inhalation - rat
Intermediate-Term (Inhalation)	NOAEL = 0.0003 mg/mL	Male reproductive effects	4-week inhalation - rat
Long-Term (Inhalation)	None	The use pattern (1-2 applications per season to rice) does not indicate potential long-term dermal exposure; risk assessment is NOT required.	

* = Since an oral LOAEL was selected a dermal absorption factor of 40% should be used for dermal risk assessments.

NOTE: For Short-term dermal risk assessments, MOE of 300 is required; MOE of 100 is adequate for all other exposure (dermal and inhalation) risks.

HED DOC. NO. 013026

17-DEC-1998

MEMORANDUM

SUBJECT: *MOLINATE* - Report of the FQPA Safety Factor Committee.

FROM: Brenda Tarplee, Executive Secretary
FQPA Safety Factor Committee
Health Effects Division (7509C)

THROUGH: Ed Zager, Chairman
FQPA Safety Factor Committee
Health Effects Division (7509C)

TO: Christine Olinger, Risk Assessor
Reregistration Action Branch 1
Health Effects Division (7509C)

PC Code: 041402

The Health Effects Division (HED) FQPA Safety Factor Committee met on October 30, 1998 to evaluate the hazard and exposure data for molinate and recommended that the FQPA safety factor (as required by Food Quality Protection Act of August 3, 1996) be retained in assessing the risks posed by this chemical.

60

I. HAZARD ASSESSMENT

1. Determination of Susceptibility

The Hazard Identification Assessment Review Committee (HIARC) determined that there was clear evidence of increased susceptibility in rat fetuses following *in utero* exposure to molinate in developmental toxicity study. Increased susceptibility was also demonstrated in the developmental neurotoxicity study in rats.

In the prenatal developmental toxicity study in rats, developmental toxicity manifested as an increase in runting occurred at a dose lower than that which caused maternal toxicity characterized as decreased body weight, body-weight gain, and food consumption, increased salivation and dehydration, and RBC cholinesterase inhibition.

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. 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).

In the prenatal developmental toxicity studies in rabbits, developmental and maternal toxicity were observed at the same dose levels.

There was no evidence of increased susceptibility to offspring in the two-generation reproduction study in rats, since reproductive/offspring toxicity 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.(*Memorandum:* L. Taylor to W. Phang, dated October 30, 1998; HED Doc. No. 012944).

2. Adequacy of Toxicity Database

There are **no data gaps** for the assessment of the effects of molinate following *in utero* and/or postnatal exposure.

3. Neurotoxicity

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 the rat following both acute and subchronic oral exposures. Evidence of neurotoxicity was also observed in the following studies:

2-year chronic toxicity study - rat
Carcinogenicity study - mouse
Chronic toxicity study - dog
Mechanistic study - rat
Range finding developmental studies - rat
Subchronic toxicity inhalation study - rat

Refer to the HIARC report on molinate for the details on findings (HED Doc. No. 012944).

II. EXPOSURE ASSESSMENT AND CHARACTERIZATION

1. Dietary (Food) Exposure Considerations

Molinate is applied early- to mid-season as a pre-plant, post-plant, and post-flood herbicide used on rice. The maximum seasonal rate is 9 lb ai/A in 2 or 3 applications. The parent compound, 4-hydroxy-molinate, and molinate acid are regulated and included in the risk assessment. No Codex MRLs have been established.

There are no monitoring data (FDA, PDP, etc) for molinate, however, fourteen field trials are available reflecting two formulations and four different application scenarios. Residues of molinate and its metabolites were found at less than the limit of quantitation for half of the field trials. Residues detected in six of the remaining trials were less than 0.25 ppm; and in one trial, residues of the hydroxy compound and the molinate acid were detected at 0.56 ppm and 0.12 ppm, respectively (LOQ for each analyte is 0.05 ppm). The metabolites are not considered to be any more toxic than the parent (the parent compound was not detected in any of the trials).

Residues of molinate are distributed throughout the plant with hay/straw exhibiting higher residues than grain. Meat and milk tolerances, however, are not required (Category 3, 40 CFR §180.6).

The HED Dietary Exposure Evaluation Model (DEEM) is used to assess the risk from acute and chronic dietary exposure to residues of molinate in food. The preliminary DEEM analyses, assuming tolerance level residues and 100% crop treated, indicate that refinements will be required for molinate. Additional refinements may include averaging of residues (since rice is a blended commodity), use of a processing factor (residues in polished rice are approximately one-third of raw grain), and incorporation of percent crop treated information.

2. Dietary (Drinking Water) Exposure Considerations

The environmental fate data base for molinate is complete. These data indicate the parent compound is persistent and is expected to reach surface water. This is supported by the available monitoring data.

Surface water monitoring data are available (for the parent) and will be used for risk assessment since there are no standard rice models for conducting drinking water exposure assessments. Data are available from the USGS, State of California, State of Arkansas, and other sources. Not all of these data represent locations where drinking water exposure is possible. Additionally, there is concern for the lack of characterization of these data for localities downstream of rice fields in the Southeast.

3. Residential Exposure Considerations

There are currently no registered residential uses of molinate, therefore, this type of exposure to infants and children is not expected.

III. SAFETY FACTOR RECOMMENDATION AND RATIONALE

1. FQPA Safety Factor Recommendation

The Committee recommended that the **FQPA safety factor** for protection of infants and children (as required by FQPA) be **retained.**

2. Rationale for Retaining the FQPA Safety Factor

The FQPA Safety Factor Committee recommended that the **FQPA safety factor** be **retained** due to:

- Increased susceptibility observed in the prenatal developmental toxicity study in rats.
- Increased susceptibility observed in the developmental neurotoxicity study in rats.
- Reproductive effects *were seen* in mice (anti-fertility study) and rats (sperm morphology study) following oral administration (although there was no evidence of increased susceptibility in the 2-generation reproduction study).
- Uncertainty associated with the lack of characterization for the surface water monitoring data used for drinking water exposure assessments.

3. Population Subgroups for Application of the Safety Factor

The Committee determined that the 10x FQPA safety factor is applicable for the following subpopulations:

Acute Dietary Assessment: The Committee determined that the FQPA Safety Factor should be **retained** (**10x**) for acute dietary risk assessment for **All Populations which**

include **Infants and Children** because the increased susceptibility was demonstrated in both the prenatal developmental toxicity and developmental neurotoxicity studies.

Chronic Dietary Assessment: The Committee determined that the FQPA Safety Factor should be **retained (10x)** for chronic dietary risk assessment for **All Populations which include Infants and Children** because of the concern for the severe reproductive effects seen following repeated oral exposures in studies with rats and mice.

MEMORANDUM

November 18, 1999

SUBJECT: REVISED Molinate™ Quantitative Risk Assessment (Q_1^*) Based On Charles River Crl:CD(SD)BR Rat Dietary Study Using mg/kg b.w.$^{3/4}$'s/day Cross Species Scaling Factor

TO: Virginia Dobozy, Veterinary Medical Officer
Reregistration Branch 1
Health Effects Division (7509C)

P.C. Code 041402

FROM: Lori L. Brunsman, Statistician
Science Analysis Branch
Health Effects Division (7509C)

THROUGH: William L. Bumam, Branch Chief
Science Analysis Branch
Health Effects Division (7509C)

The upper bound estimate of unit risk, Q_1^*(mg/kg/day)$^{-1}$, of Molinate™ based upon male rat kidney cortical adenoma and/or carcinoma combined tumor rates is 4.92×10^{-2} in human equivalents. The dose levels used from the 105-week dietary study were 0, 7, 40, and 300 ppm of Molinate™. The corresponding tumor rates were 0/47, 0/46, 0/49, and 5/48, respectively.

Background

On June 17, 1992, the Cancer 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 be applied to the experimental animal tumor data for quantification of human risk (Q_1^*). A Q1* was generated using mg/kg b.w.$^{2/3}$'s/day cross species scaling factor (Molinate, Quantitative Risk Assessment, Two-Year Charles River Crl:CD(SD)BR Rat Dietary Study, B. Fisher, 12/4/92). This revised memo has been generated to reflect the Agency policy change from use of the $^2/_3$'s to the $^3/_4$'s scaling factor in 1994[1].

65

All unit risks have been converted from animals to humans by use of the ¾'s scaling factor (Tox_Risk program, Version 3.5, K. Crump, 1994)[1]. For the conversion to human equivalents, weights of 0.35 kg for the rat and 70 kg for humans were used.

It is to be noted that the Q_1^* $(mg/kg/day)^{-1}$ is an estimate of the upper bound on risk and that, as stated in the EPA Risk Assessment Guidelines, "the true value of the risk is unknown, and may be as low as zero."

Dose-Response Analysis

The statistical evaluation of mortality (Molinate™ Qualitative Risk Assessment Based On Charles River Crl:CD(SD)BR Rat Dietary Study, L. Brunsman, 4/28/92) indicated a significant decreasing trend with increasing doses of Molinate in male rats. The unit risk, Q_1^*, was obtained by the application of the time-to-tumor Weibull model (Tox_Risk program, Version 3.5, K. Crump, 1994).

Male rats had a significant increasing trend at $p < 0.01$, and a significant difference in the pair-wise comparison of the 300 ppm dose group with the controls at $p < 0.05$, for kidney cortical adenoma and/or carcinoma tumors combined.

[1]See memo - Deriving Q_1^*s Using the Unified Interspecies Scaling Factor, P.A. Fenner-Crisp, Director, HED, 7/1/94.