DRAFT

Public Health Goal for
MOLINATE
In Drinking Water

Prepared by

Pesticide and Environmental Toxicology Branch
Office of Environmental Health Hazard Assessment
California Environmental Protection Agency

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PREFACE

Drinking Water Public Health Goals
Pesticide and Environmental Toxicology Branch
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This Public Health Goal (PHG) technical support document provides information on health effects from contaminants in drinking water. PHGs are developed for chemical contaminants based on the best available toxicological data in the scientific literature. These documents and the analyses contained in them provide estimates of the levels of contaminants in drinking water that would pose no significant health risk to individuals consuming the water on a daily basis over a lifetime.

The California Safe Drinking Water Act of 1996 (Health and Safety Code, Section 116365) requires the Office of Environmental Health Hazard Assessment (OEHHA) to perform risk assessments and adopt PHGs for contaminants in drinking water based exclusively on public health considerations. The Act requires that PHGs be set in accordance with the following criteria:

1. PHGs for acutely toxic substances shall be set at levels at which no known or anticipated adverse effects on health will occur, with an adequate margin of safety.
2. PHGs for carcinogens or other substances that may cause chronic disease shall be based solely on health effects and shall be set at levels that OEHHA has determined do not pose any significant risk to health.
3. To the extent the information is available, OEHHA shall consider possible synergistic effects resulting from exposure to two or more contaminants.
4. OEHHA shall consider potential adverse effects on members of subgroups that comprise a meaningful proportion of the population, including but not limited to infants, children, pregnant women, the elderly, and individuals with a history of serious illness.
5. OEHHA shall consider the contaminant exposure and body burden levels that alter physiological function or structure in a manner that may significantly increase the risk of illness.
6. OEHHA shall consider additive effects of exposure to contaminants in media other than drinking water, including food and air, and the resulting body burden.
7. In risk assessments that involve infants and children, OEHHA shall specifically assess exposure patterns, special susceptibility, multiple contaminants with toxic mechanisms in common, and the interactions of such contaminants.

8. In cases of insufficient data for OEHHA to determine a level that creates no significant risk, OEHHA shall set the PHG at a level that is protective of public health with an adequate margin of safety.
9. In cases where scientific evidence demonstrates that a safe dose response threshold for a contaminant exists, then the PHG should be set at that threshold.
10. The PHG may be set at zero if necessary to satisfy the requirements listed above in items seven and eight.
11. PHGs adopted by OEHHA shall be reviewed at least once every five years and revised as necessary based on the availability of new scientific data.

PHGs adopted by OEHHA are for use by the California Department of Health Services (DHS) in establishing primary drinking water standards (State Maximum Contaminant Levels, or MCLs). Whereas PHGs are to be based solely on scientific and public health considerations without regard to economic cost considerations or technical feasibility, drinking water standards adopted by DHS are to consider economic factors and technical feasibility. Each primary drinking water standard adopted by DHS shall be set at a level that is as close as feasible to the corresponding PHG, placing emphasis on the protection of public health. PHGs established by OEHHA are not regulatory in nature and represent only non-mandatory goals. By state and federal law, MCLs established by DHS must be at least as stringent as the federal MCL, if one exists.

PHG documents are used to provide technical assistance to DHS, and they are also informative reference materials for federal, state and local public health officials and the public. While the PHGs are calculated for single chemicals only, they may, if the information is available, address hazards associated with the interactions of contaminants in mixtures. Further, PHGs are derived for drinking water only and are not intended to be utilized as target levels for the contamination of other environmental media.

Additional information on PHGs can be obtained at the OEHHA web site at www.oehha.ca.gov.

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PUBLIC HEALTH GOAL FOR MOLINATE IN DRINKING WATER

SUMMARY

Molinate is a herbicide primarily used in California to control grass in rice. It is directly applied in granular form into the water in rice paddies. Exposure to molinate has occurred through volatilization of the chemical and through release of the water from rice paddies into rivers and streams, becoming incorporated into municipal drinking water supplies.

OEHHA proposes a Public Health Goal (PHG) of 1.0 μg/L (1 ppb) for molinate in drinking water. The proposed PHG is based on a study conducted by Pettersen and Richter (1990), in which a significant increase was observed in the incidence of cortical adenomas and carcinomas (combined) in kidneys of male rats administered molinate in the diet at 300 ppm (13 mg/kg-day) for two years. For the proposed PHG, OEHHA estimated a cancer potency of 0.036 $(mg/kg\text{-}day)^{-1}$ and used a *de minimis* theoretical excess individual cancer risk level of 10^{-6}. OEHHA also calculated a level protective against non-cancer effects of 2 μg/L, or 2 ppb, based on children's exposure factors. This number was based on an increased incidence of neurological effects in male and female rats administered 7, 40, or 300 ppm (0.3, 1.8, or 13 mg/kg-day, respectively) molinate in the diet daily for two years (Pettersen and Richter, 1990). The lowest-observed-adverse effect level (LOAEL) of 0.3 mg/kg-day and an uncertainty factor of 1,000 were used in the calculation of a protective level for the non-cancer effects.

Based on an earlier health assessment of molinate (DHS, 1987), a Maximum Contaminant Level (MCL) of 20 μg/L (20 ppb) for molinate in drinking water was established in 1988 by the California Department of Health Services (DHS, 1988). There is no U.S. EPA MCL or MCLG for molinate.

INTRODUCTION

Molinate is a thiocarbamate herbicide which has been extensively used to control grasses and other weeds in rice in California. Release of water from molinate-treated rice paddies has resulted in surface water and drinking water contamination in past years, and the volatility of molinate has also resulted in some concern about health hazards from exposure to its vapors.

U.S. EPA announced in a Federal Register notice published on April 7, 2004, its approval of requests by Syngenta Crop Protection, Inc. and Helm Agro US, Inc. to voluntarily cancel the registrations of all of their products containing molinate, and to gradually reduce the amount of molinate that may be sold until the cancellation becomes effective on June 30, 2008. In addition to the voluntary cancellations, Syngenta and Helm requested modifications to their molinate registrations to limit the maximum amount of

molinate that may be sold during 2004, 2005, and 2006 to the 2002 sales level, and decrease the amount to 75% of the 2002 sales level in 2007 and to 50% in 2008. No use of molinate will be permitted after the 2009 growing season ending August 31, 2009 (U.S. EPA, 2004).

The purpose of the present document is to develop a proposed PHG for molinate. In this document, we evaluated the available data on the toxicity of molinate, primarily by the oral route, which are the most appropriate for establishing a PHG for drinking water. We also included new information made available since the previous assessment done by OEHHA (formerly the Hazard Evaluation Section in DHS) (DHS, 1987), and identified and considered sensitive human subpopulations in the calculation of the PHG. The results of this evaluation are described below.

CHEMICAL PROFILE

Chemical Identity

Molinate is a thiocarbamate herbicide. The chemical formula, synonyms, and the CAS Registry number are listed in Table 1. The chemical structure of molinate is provided in Figure 1.

Table 1. Chemical Identity of Molinate

Chemical name	*S*-ethyl hexahydro-1H-azepine-1-carbothioate
Synonyms	*S*-ethyl *N,N*-hexamethylene thiocarbamate; R-4572
Registered trade names	Ordram®
Chemical formula	$C_9H_{17}NOS$
CAS Registry number	2212-67-1

From: Hayes and Laws, 1991; Meister, 1999

Figure 1. Chemical structure of molinate

Physical and Chemical Properties

Molinate is moderately soluble in water, which aids in its distribution to plant roots when applied to rice paddies in granular form (either pre- or post-planting). It also has a relatively high vapor pressure of 5.6×10^{-3} mm Hg at 25°C, which could result in vapor phase losses during application. The combination of water solubility and vapor pressure results in a moderate Henry's constant, so it can be lost by evaporation from flooded fields. Relevant and important physical and chemical properties of molinate are shown in Table 2.

Table 2. Physical and Chemical Properties of Molinate

Property	Value or Information
Molecular weight	187.3 g/mol
Color	Clear to amber
Physical state	Liquid
Odor	Aromatic or spicy
Boiling point	137-202°C at 10 mm Hg
Solubility Water Organic solvents	 970 mg/L at 25°C Soluble in acetone, benzene, methanol, xylene
Specific Gravity	1.0626-1.0669 at 20°/20°C
Partition coefficients K_{ow}, Octanol-water Log K_{ow} K_{oc}, Organic matter (soil)-water	 1,628 3.21 80-89; 190
Vapor pressure	5.6×10^{-3} mm Hg at 25°C
Henry's law constant	4.1×10^{-6} atm-m^3/mole at 20°C

From: Meister, 1999; Hayes and Laws, 1991; HSDB, 2002; Martin *et al.*, 1992; Ruiz and Marzin, 1997

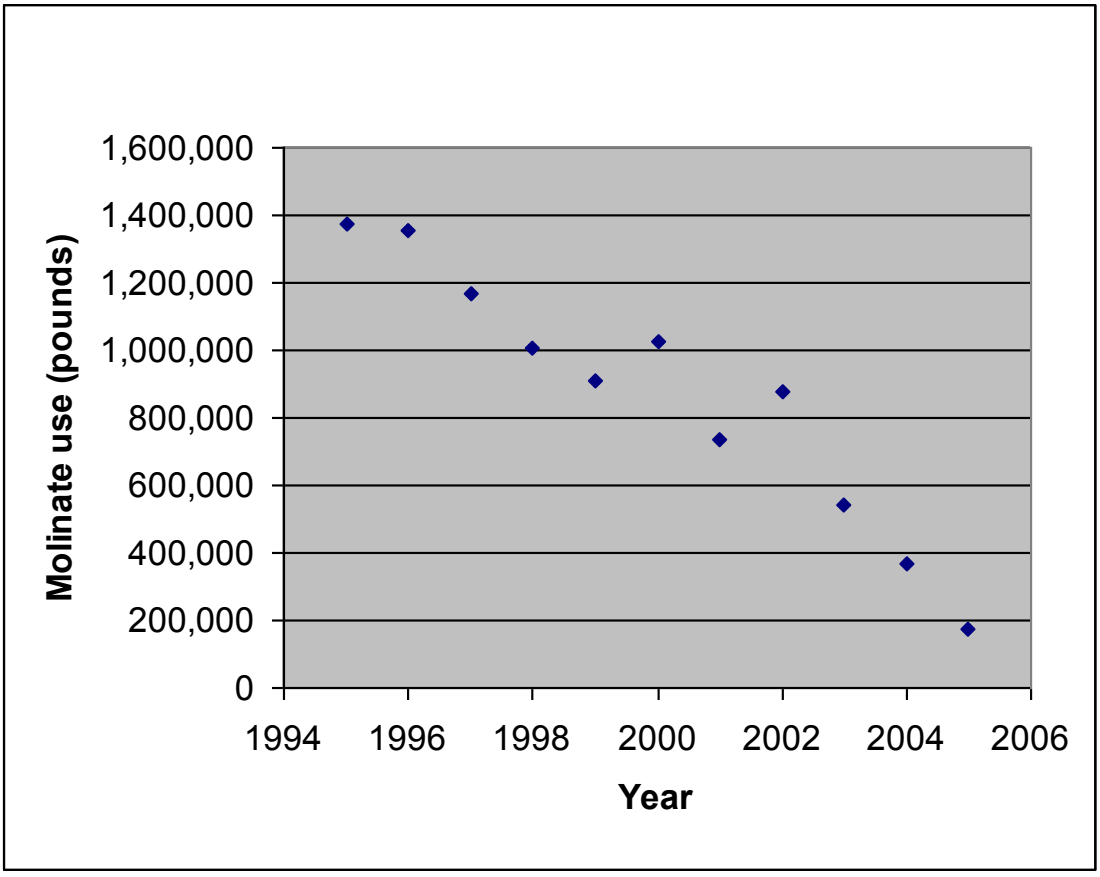
Production and Uses

Molinate was introduced in 1945 by Stauffer Chemical Company for the control of weeds in rice paddies (Hayes and Laws, 1991). It is produced from ethyl chlorothiolformate and hexamethyleneimine (HSDB, 2002).

Molinate has been used in the Sacramento Valley since 1966 (Dawson, 2001). The molinate products currently registered for use in California are Ordram 8-E (emulsifiable concentrate), which contains 8 pounds of active ingredient per gallon (Zeneca Ag Products, 2000a), and Ordram 15-GM (granules), which contains 15 pounds of active ingredient per 100 pounds (Zeneca Ag Products, 1999). Ordram 15-GM may be used for water-seeded rice by preplant, preflood, soil incorporation. It is applied by air or ground equipment, incorporated into the soil mechanically, and then the field is flooded for rice seeding. The other major application technique is by air to flooded, preseeded fields when the rice is in the seedling stage. For Ordram 8-E, application may be by ground for preplant, preflood, water-seeded rice, or postflood, postemergence for water-seeded or drilled rice, after the rice is in the seedling stage.

During the years 1991 to 1997, an average of 1.35 million pounds of molinate was used yearly in California (DPR, 1999b). Plans for voluntary cancellation were announced in 2004, but California use had been declining over the last 10 years (to less than 200,000 pounds in 2005), as shown in Figure 2.

Figure 2. Molinate use in California (DPR 2007)



ENVIRONMENTAL OCCURRENCE AND HUMAN EXPOSURE

Volatilization is the main mechanism of molinate dissipation from shallow water and soil surfaces (HSDB, 2002; Soderquist *et al*., 1977). The rate of volatilization is relatively high when the air and field water temperature increases in the late afternoon and under windy conditions, leading to diurnal variations in volatilization rates (Seiber *et al*., 1989). Other routes of molinate dissipation (in order of importance) are: reversible partitioning to soil; indirect photolysis of molinate to 4-keto-molinate, formed when molinate desorbs into the rice paddy water; bio- and photodegradation; plant uptake, and release of rice water from treated fields into surface water (Mastrota and Breithaupt, 2001; HSDB, 2002). Molinate is stable to hydrolysis and direct photolysis, however, indirect photolysis may be occurring in the field (Mastrota and Breithaupt, 2001).

Because of previous releases of molinate contaminated tailwater into the Sacramento River, California state regulations now prevent rice farmers from discharging tailwater from rice fields for at least 28 days after molinate application (DPR, 2001b).

Air

Recent monitoring data for molinate levels in ambient air or near application sites were not available. Baker *et al.* (1996) and ARB (1993) reported the results of ambient air monitoring for molinate that was conducted by the California Air Resources Board (ARB) in May 1992. Samples were collected for five days in Williams and Maxwell in Colusa County. The mean 24-hour average air concentration was 0.57 $\mu g/m^3$, and the maximum value was 1.2 $\mu g/m^3$. The maximum air concentration of molinate sampled near a rice field on the day of application (2.5-hour sample) was 23 $\mu g/m^3$ (Baker *et al*., 1996; ARB, 1993). DPR estimated the annual average daily dosage (AADD) for children and adults from inhalation exposure using these ambient air monitoring data (DPR, 2000b). The values were 0.013 µg/kg-day (child) and 0.006 µg/kg-day (adult) in Williams and 0.026 µg/kg-day (child) and 0.013 µg/kg-day (adult) in Maxwell. The AADD equals $1/12^{th}$ the seasonal average daily dose because the annual use occurs over a 30-day period each year.

Soil

Few data were available on the levels of molinate in soils. Imai and Kuwatsuka (1988) measured the amount of radiolabeled molinate and its degradates after application to clay paddy soil planted with rice seedlings under glasshouse conditions. At harvest, 21 percent of the total radioactivity was detected in the soil, with 14 percent in the bottom 7 to 14 cm. Molinate concentration was approximately 0.06 ppm, whereas the degradates 2-oxo-molinate, 4-oxo-molinate, molinate acid, and hexamethyleneimine were present at approximately 0.02 ppm.

Based on batch equilibrium studies, molinate and the degradates, hexamethyleneimine and molinate sulfoxide, were found to be moderately mobile in a clay loam aquatic sediment from the Colusa Canal in California (Mastrota and Breithaupt, 2001). In the

field, applied molinate that did not volatilize tended to partition reversibly to sediment within three days. The half-life of molinate in most loam soils (21 to 27 °C) is about three weeks (HSDB, 2002).

Water

Molinate has been detected in surface water and groundwater in the rice growing areas and downstream of use areas in California. Drainage water from most rice fields eventually ends up in the Sacramento River, which is the source of drinking water for the cities of West Sacramento and Sacramento. The intakes for the drinking water utilities are located downstream of the molinate use area. Newhart (2002) reported that from late-April to mid-July in 2002, molinate levels ranged from <0.10 µg/L (4 of 13 samples) to 1.7 µg/L at the Sacramento River Water Treatment Plant intake (9 detects in 13 samples) and from <0.10 µg/L (3 of 13 samples) to 4.2 µg/L at the West Sacramento Water Treatment Plant intake (10 detects in 13 samples). Molinate levels in the Sacramento River at the Village Marina ranged from 1.06 to 3.21 µg/L (12 detects in 22 samples taken May 14 to July 9, 2002) (Newhart, 2002). While these high molinate levels have been attributed in part to a late May rainstorm, the monitoring data indicate that early releases of tailwater before the end of the 30-day holding period may have contributed to the higher levels (Newhart, 2002; CRC, 2002).

Molinate also was monitored in the Sacramento River at Freeport; results were reported from April 28, 1997 to August 13, 1998 (DPR, 2002). The range was 0.006 to 1.57 µg/L, with the highest concentrations being detected in May and June of those years. There were 10 non detections out of 25 samples.

Rice fields generally are located on fine-textured, poorly drained soils of clay and silt with impervious hardpans or claypans (Dawson, 2001). Estimated soil permeabilities in the Sacramento Valley rice-growing area generally are less than 6 meters/day, with most areas being less than 0.6 meters/day (Dawson, 2001). These characteristics reduce the potential for molinate-treated paddy water to leach into deep groundwater wells. Therefore, it is not surprising that the most recent data for California ground water sampling indicate that molinate was not detected in any of the wells sampled in California from July 1, 1997 through June 30, 2000 (DPR, 1999a, 2000d,e).

Nevertheless, molinate has been measured in shallow ground water wells. As part of the U.S. Geological Survey's National Water-Quality Assessment Program, in 1997 molinate and other pesticides were measured in 28 shallow observation wells drilled in the rice-growing region of the Sacramento Valley (Dawson, 2001; Domagalski *et al*., 2000). The wells were between 8.8 and 15.2 meters deep, and water levels were between 0.4 and 8.0 meters below land surface. Samples were collected during the months of August through September, which is after the molinate use season of April through June. Molinate was detected in 4 of the 28 wells. The levels ranged from 0.005 to 0.056 µg/L (method reporting limit = 0.004 µg/L). In 3 of the 28 wells, molinate was detected but at levels less than the reporting limit; the estimated levels were between 0.002 and 0.003 µg/L.

Molinate was not detected (method detection limit = 0.004 μg/L) in various shallow groundwater aquifers in the San Joaquin-Tulare Basin of California monitored between 1993 and 1995 (Kolpin *et al*., 1998). However, molinate was measured in Orestimba Creek and the San Joaquin River at concentrations ranging from 0.005 to 0.220 μg/L and 0.005 to 0.018 μg/L, respectively, during the months of May to July 1992 (Domagalski, 1997).

In May and June of 1981 and 1982, the City of Sacramento received an increase in telephone complaints related to a bitter taste in drinking water supplied by the local water treatment plants (DHS, 1987). These complaints coincided with release of molinate- and thiobencarb-treated water from the agricultural drains upstream into the Sacramento River. A taste test conducted by the City of Sacramento with water spiked with molinate or thiobencarb indicated that the off-taste was from thiobencarb rather than molinate (DHS, 1987, citing Young, 1982). The concentration of herbicide that produced the off-flavor was not provided. From field and laboratory studies, Martin *et al*. (1992) estimated the organoleptic threshold concentration for molinate in water to be 0.25 to 0.50 μg/L. Molinate has not been reported in California drinking water at concentrations above the MCL in recent years (DHS, 2006).

Food

Molinate was not detected (<0.01 μg or 0.4 ppb equivalent) in rice grain from plants grown under glasshouse conditions and treated with [^{14}C]molinate (Imai and Kuwatsuka, 1988). Molinate metabolites, including 4-hydroxymolinate, 2-oxo-molinate, 4-oxo-molinate, *S*-ethyl *N*-carboxymethylthiocarbamate, and molinate acid were detected at levels less than 3 ppb in the grain. The authors hypothesized that [^{14}C]molinate was metabolized in the rice plants and converted to carbon dioxide or assimilated into amino acids, organic plant acids, proteins and cellulose as plant constituents. The tolerance level for molinate in or on raw rice grain and straw is 0.1 ppm (CFR, 2000).

Molinate residues accumulated in bluegill fish exposed to 0.1 ppm molinate for 25 days (Mastrota and Breithaupt, 2001). Maximum mean bioconcentration factors for edible tissue (muscle, skin, skeleton), nonedible tissue (fins, head, internal organs), and whole fish tissues were 29, 140, and 72, respectively. Molinate was metabolized to molinate sulfoxide, carboxymolinate, molinate sulfone, 4-ketomolinate, and 4-hydroxymolinate, but none of the metabolite concentrations exceeded 10 percent of the total residues. Seventy-five to 90 percent of the accumulated [^{14}C] residues were eliminated from the fish tissues by day 14 of the depuration period.

Molinate concentrations in pale chub caught in the Ezura River in Japan ranged from <2 ng/g (detection limit) to 465 ng/g (Tsuda *et al*., 1998). The field bioconcentration factors for molinate in pale chub (n=5) and ayu sweetfish (n=4) were 16 and 13, respectively. 2-Ketomolinate, an oxidation product, was detected in pale chub at a concentration of about 6 ng/g (detection limit = 5 ng/g), and its field bioconcentration factor in chub was 55 (Tsuda *et al*., 1998).

Molinate was measured in channel catfish from commercial fishponds in Mississippi (Martin *et al*., 1992). The fish were sampled after off-flavor was detected by processing plant personnel, presumably as a result of molinate applications to adjacent rice fields and around the pond levees. The herbicide concentrations in catfish and water samples from one pond were 8.33 ppb and 0.267 ppb, respectively, and from another pond were 33.3 ppb and 1.33 ppb, respectively. Thus, the bioconcentration factor for molinate in channel catfish muscle ranged from 25 to 31 (Martin *et al*., 1992). Tjeerdema and Crosby (1988) reported similar bioconcentration factors for molinate in white sturgeon (19.7) and common carp (30.5).

Under laboratory conditions, Martin *et al*. (1992) exposed catfish for 24 hours to molinate in inoculated water for sensory analysis by a trained industrial panel. The aroma of the cooked (microwaved) fish samples was noted and the flavor was rated on a scale of 0 (no off-flavor) to 5 (severe off-flavor). Control fish had no off-flavor, fish exposed to 0.5 ppb molinate were off-flavor 2 to 3, fish exposed to 1 ppb molinate were off-flavor 5, and fish exposed to 5 ppb molinate had a very characteristic molinate odor, were not tasted, and were designated an off-flavor of 5+, based on the odor characteristics of the cooked fillet.

METABOLISM AND PHARMACOKINETICS

Absorption

Molinate is rapidly absorbed via oral, respiratory, and dermal exposure routes (Batten *et al*., 1992; DeBaun *et al*., 1978a; Hext *et al*., 1992; Lythgoe *et al*., 1992; Macpherson, 1998). In vitro absorption of technical grade molinate (97.4 percent purity) through rat epidermis was as much as 60 times higher than through human epidermis, depending on the type of application (neat versus dissolved in solvent; occluded versus unoccluded) (Ward and Scott, 1990). For example, neat molinate (100 $\mu l/cm^2$) was absorbed through occluded human epidermis at a rate of 34.1 $\mu g/cm^2$-hour during a 1 to 31-hour time period, whereas the absorption rate through occluded rat epidermis was 473 $\mu g/cm^2$-hour during the 1 to 10-hour time period and 1100 $\mu g/cm^2$-hour during the 8 to 24-hour time period. About 50 percent less molinate was absorbed through unoccluded versus occluded human skin (18.7 $\mu g/cm^2$-hour versus 34.1 $\mu g/cm^2$-hour), probably from volatilization of molinate from the skin surface (Ward and Scott, 1990).

Distribution

Molinate appears to distribute rapidly to highly perfused tissues. The distribution of radioactivity after oral administration of [^{14}C]molinate to rats was predominantly to whole blood (cellular components, not plasma), liver, small intestine, kidney, lung, and spleen (DeBaun *et al*., 1978a; Lloyd, 1997). Whole-body tissue residues of ^{14}C decreased from approximately 13.8 percent to 3.7 percent of the administered dose over a 7-day period after oral administration of [^{14}C]molinate to rats (DeBaun *et al*., 1978a).

Metabolism

Molinate is metabolized through two major pathways: 1) via oxidation to the corresponding sulfoxide (and sulfone), followed by conjugation with glutathione to form the mercapturate, and 2) via hydroxylation of the hexamethyleneimine (HMI) ring, predominantly at position 4, followed by conjugation with glucuronic acid (DeBaun *et al*., 1978b; Lythgoe *et al*., 1992; Lloyd, 1997; Macpherson, 1998). Another pathway is thiocarbamate cleavage to HMI and corresponding ring hydroxylated and conjugated products.

Rats orally dosed with 72 mg/kg [^{14}C]Ordram (99.1 percent molinate), excreted 35.4 percent of the ^{14}C in the 0 to 48-hour urine as molinate mercapturate (DeBaun *et al*. 1978b). Other urinary metabolites included 3- and 4-hydroxymolinate (0.8 percent) and corresponding O-glucuronide conjugates (26.1 percent), HMI (14.6 percent) and 3- and 4-hydroxy HMI (10.3 percent). There were no significant qualitative differences observed in urinary metabolites from male and female rats (DeBaun, 1978b). Jewell *et al*. (1998) found that the primary metabolite of molinate when incubated with rat liver or testes microsomes was molinate sulfoxide. Testes microsomes produced only slightly less molinate sulfoxide than liver microsomes. A minor metabolite was hydroxymolinate, with testes microsomes producing substantially less than liver microsomes.

An average of 40 percent of a single oral dose (5 mg) administered to six male human volunteers (60 to 90 kg body weight) was excreted in the urine, 39 percent (range 22 to 49 percent) as the glucuronide conjugate of 4-hydroxymolinate and approximately 1 percent (range 0.5 to 1.5 percent) as molinate mercapturate (Batten *et al*., 1992; Wilkes *et al*., 1993). The remainder may have been unabsorbed or converted into other metabolites. The average dose used in Batten *et al*. (1992) was similar to that used by Krieger *et al*. (1992), where workers excreted 1 to 2 percent of an oral molinate dose (0.03 to 0.1 mg/kg) as molinate mercapturate.

Studies using rats and monkeys (Lloyd, 1997; Macpherson, 1998) suggest that molinate metabolism is dose-dependent: at lower doses the hydroxylation and glucuronidation pathway is predominant. At higher doses, this pathway becomes saturated, resulting in a higher proportion of the administered dose being metabolized by the glutathione pathway to the cysteine and mercapturate conjugates. For example, 4- or 3-hydroxymolinate glucuronide accounted for approximately 30 percent of a single oral dose of 0.1, 2.0. or 40 mg [^{14}C]molinate/kg body weight administered to male cynomolgus monkeys, whereas cysteine conjugates plus mercapturates accounted for about 5.6 percent, 13 percent, and 20 percent of the respective doses (Macpherson, 1998). In rats, the percent of urinary radiolabel as molinate mercapturate increased with increasing dose: 11, 22, 29, and 32 percent with oral molinate doses of 1, 16, 40, and 200 mg/kg, respectively (Lloyd, 1997). The only human data suggestive of a dose-dependent metabolism were reported as unpublished results by Krieger *et al*. in Batten *et al*. (1992). An oral dose of 0.03, 0.05, 0.1, 0.6, or 0.7 mg/kg of molinate resulted in 1.1, 1.6, 1.8, 5.5, and 6.2 percent, respectively, of the dose recovered in urine as the mercapturate metabolite. These results are consistent with those reported in monkeys by Macpherson (1998).

Jewell and Miller (1999) found that in rat and human liver microsomes and slices, sulfoxidation is the preferred high-dose pathway for molinate metabolism, whereas hydroxylation predominates at low molinate doses in both species. Molinate metabolism in liver slices did reveal a greater capacity for detoxification of molinate sulfoxide by glutathione conjugation in humans compared with rats.

Excretion

Rats dosed orally with 72 mg/kg of ring-labeled [^{14}C]molinate excreted approximately 97 percent of the dose in the first 48 hours: 80 percent in the urine, 10 percent in the feces, <1 percent in expired air, and about 6 percent in the cage wash (DeBaun *et al*., 1978a). Ford and Gray (1964) reported a mean [^{14}C] recovery of 90.4 percent after a single oral dose of 1.122 g or 1.074 g of [^{14}C]R-4572 to male and female rats, respectively. Seventy-two hours after dosing, the respective amount of radiolabel in exhaled carbon dioxide, urine, feces and tissues was 47.4, 28.4, 7.9, and 6.2 percent for males, and 40.4, 25.4, 21.0. and 4.2 percent for females. Male cynomolgus monkeys administered a single oral dose of 0.1, 2, or 40 mg/kg [^{14}C]molinate excreted approximately 90 percent of the administered dose over a 336-hour collection period, with >80 percent excreted in the first 48 hours (Macpherson, 1998). Urinary excretion ranged from 67.5 to 77 percent and fecal excretion ranged from approximately 5 to 8.5 percent. Up to 15.7 percent of the ^{14}C was measured in the cage wash, which could be attributed to urinary excretion (Macpherson, 1998). Lythgoe *et al*. (1992) also studied the excretion of [^{14}C]molinate given orally (6 and 60 mg/kg) and intravenously (6 mg/kg) to cynomolgus monkeys. Total ^{14}C recovery after 8 days was 51.1 percent, 83.1 percent, and 97.5 percent for 6 mg/kg oral, 60 mg/kg oral, and 6 mg/kg intravenous molinate, respectively. The low recovery at 6 mg/kg molinate given orally was thought to be an error in the dosing, as discussed by the authors (Lythgoe *et al*., 1992). An average of 40 percent of a single oral dose (5 mg) administered to six male human volunteers (60 to 90 kg body weight) was excreted in the urine as the glucuronide conjugate of 4-hydroxymolinate (39 percent) and molinate mercapturate (~1 percent) (Batten *et al*., 1992; Wilkes *et al*., 1993). The remainder may have been unabsorbed or converted into other metabolites.

TOXICOLOGY

Toxicological Effects in Animals

Acute/Short-term Toxicity

Acute lethal toxicity values are provided in Table 3 for several laboratory species administered technical grade molinate or Ordram products via different exposure routes.

Horner (1992b) administered molinate (98.1 percent) to five groups of eight Crl:CD(SD)BR male rats via gavage for up to ten consecutive days to study the effects of molinate on accumulation of α-2u-globulin in the kidney. Dose levels were 0 (corn oil), 15, 75, or 150 mg/kg-day molinate or 200 mg/kg-day 2,2,4-trimethylpentane (positive

control). Clinical observations included salivation at 75 and 150 mg/kg-day, and hunched posture, piloerection, and urinary incontinence at 150 mg/kg-day. All molinate-treated animals exhibited a statistically significant dose-related reduction in body weight and/or body weight gain. Three animals receiving 150 mg/kg-day were killed on days 4 or 5 for humane reasons, due to molinate treatment. 2,2,4-Trimethylpentane caused a marked increase in α-2u-globulin in the kidney, but molinate treatment did not. The 15 mg/kg-day molinate dose was the lowest-observed-effect level (LOEL) for body weight decrements and the no-observed-effect level (NOEL) for clinical signs in male rats.

Table 3. Acute Lethality of Molinate

Acute Toxicity Test	Species	Dose	Reference(s)
Technical grade molinate			
Oral LD_{50}	Rat	500 to 720 mg/kg	Hayes and Laws, 1991; HSDB, 2002
	Male rat	369 mg/kg	HSDB, 2002
	Female rat	450 mg/kg	HSDB, 2002
	Mouse	530 to 795 mg/kg	HSDB, 2002
	Hen	1,930 mg/kg	[3]Sprague, 1983
Dermal LD_{50}	Rabbit	>10,000 mg/kg	Hayes and Laws, 1991
Inhalation LC_{50} (4-hour)	Female rat	1.39 mg/L	Zeneca Ag Products, 2000b,c
	Male rat	2.91 mg/L	Zeneca Ag Products, 2000b,c
Ordram 8E			
Oral LD_{50}	Male rat	955 mg/kg	Zeneca Ag Products, 2000b
	Female rat	549 mg/kg	Zeneca Ag Products, 2000b
Dermal LD_{50}	Rabbit	3,536 mg/kg	Zeneca Ag Products, 2000b
Ordram 15 GM			
Oral LD_{50}	Male rat	4,100 mg/kg	Zeneca Ag Products, 2000c
	Female rat	4,198 mg/kg	Zeneca Ag Products, 2000c
Dermal LD_{50}	Rabbit	>2,000 mg/kg	Zeneca Ag Products, 2000c

Ordram 8E produced severe eye irritation and mild skin irritation, whereas Ordram 15GM produced severe eye irritation but no skin irritation in treated rabbits (Zeneca Ag Products, 2000b,c). However, Ramsey (1996) reported that Ordram 15GM produced moderate (Toxicity Category III) eye irritation in the rabbit, with corneal involvement, iritis, and irritation that cleared by day 4.

Neither molinate technical or formulated products containing 15.7 or 10.4 percent molinate produced skin sensitization in the guinea pig (Ramsey, 1996).

The dermal toxicity of molinate was tested in SPF Wistar-derived albino rats (Leah, 1989). Molinate (97.6 percent; w/w) at doses of 0, 10, 25, or 50 mg/kg was applied to the shaved backs of five rats per sex per group for a 6-hour per day contact period for 21 days. The treated areas were covered with a bandage, and cleaned after each exposure period. There were no deaths or significant clinical observations. Skin irritation in the 10 mg/kg group was minimal, with slight desquamation, erythema and thickening of the skin in a few animals. At 25 or 50 mg/kg, the signs were similar, except that the severity increased to slight to moderate, and edema was also observed. In addition, occasional extreme desquamation and edema were noted in the 50 mg/kg group only. Neither body weights nor food consumption were affected by molinate treatment. Relative adrenal weights were significantly increased ($p<0.05$) in males given 25 or 50 mg/kg compared to controls. Plasma cholinesterase activity of treated animals did not differ from control animals. Red blood cell (RBC) cholinesterase activity was statistically significantly inhibited in all treated groups (males and females), but the inhibition was not dose-related and did not exceed 20 percent. Brain cholinesterase activity was not measured. There was a slight dose-related increase in minimal to slight acanthosis in the skin of treated and control males (0 (control), 1/5; 10 mg/kg, 1/5; 25 mg/kg, 3/5; and 50 mg/kg, 4/5). Unilateral hydronephrosis was found in the kidneys of males and females given 25 or 50 mg molinate/kg; the incidence was 2/5 and 3/5 in males and 1/5 and 1/5 in females. The no-observed-adverse-effect level (NOAEL) for acute dermal toxicity and systemic toxicity was 10 mg/kg, based on mild to moderate skin irritation and adrenal, skin, and kidney effects at 25 or 50 mg/kg.

Subchronic Toxicity

R-4572 (molinate, 99.5 percent purity) was administered in the diet of Charles River albino rats (15/sex/group) for 13 weeks (Johnston, 1964). Dose levels were 0, 35, 70, or 140 mg/kg-day. One male rat in the high dose group died during week 3, possibly from a respiratory infection. Body weights and food consumption were decreased in the 70 and 140 mg/kg-day groups from the start of the study. Hemoglobin and hematocrit levels of males given 70 or 140 mg/kg-day were slightly depressed at 8 weeks but not at 13 weeks. Several statistically significant ($p<0.05$) changes in mean relative organ weights were noted: in males, heart (140 mg/kg-day), liver (70, 140 mg/kg-day), kidney (70, 140 mg/kg-day), adrenal (70, 140 mg/kg-day), and thyroid weights (35, 70, 140 mg/kg-day) were increased and testes weights (140 mg/kg-day) were decreased. In females, liver (70, 140 mg/kg-day), kidney (140 mg/kg-day), adrenal (35, 70, 140 mg/kg-day), and thyroid weights (35, 70, 140 mg/kg-day) were significantly increased and ovary weights (140 mg/kg-day) were significantly decreased. Histopathological changes included dose-related upper nephron tubular cell degeneration with cell hypertrophy in kidneys from treated males but not females, degeneration in seminiferous tubules with nearly complete azospermia in males at 140 mg/kg-day, ovarian hypertrophy in females at 140 mg/kg-day, and adrenal cortical cell vacuolation in all males and some females at 140 mg/kg-day and in some males at 70 mg/kg-day. A LOAEL of 35 mg/kg-day was identified, based on significant changes in relative organ weights of both male and female rats.

In a second subchronic rat feeding study, Johnston (1967) administered Ordram (95 percent purity) in the diet to Charles River albino rats (15/sex/group) at dose levels of 0, 8, 16, or 32 mg/kg-day for 13 weeks. One male rat receiving the high dose died during week 5 from bronchopneumonia and pulmonary abscess. Body weights for males and females fed 8 or 16 mg/kg-day were reduced to 92 to 99 percent of controls, whereas body weights for males and females fed 32 mg/kg-day were reduced to 83 and 87 percent of controls, respectively. Hematological parameters in treated animals were not different from controls. Mean absolute adrenal weights in females given 32 mg/kg-day were significantly greater than controls. Statistically significant ($p \leq 0.05$) differences in relative organ weights included an increase in kidney and adrenal weights in females at all dose levels and an increase in adrenal and testis weights in males at 32 mg/kg-day and in thyroid weight in males given 16 and 32 mg/kg-day. In females given 16 or 32 mg/kg-day, foamy vacuolation in ovarian stromal cells was noted. In all treated males and females, very slight to moderate (32 mg/kg-day only) adrenal cortical cell vacuolation was found, but it did not appear to be dose-related. The LOEL, which was the lowest dose tested, was 8 mg/kg-day, based on adrenal organ weight and histopathological changes at all doses.

Ordram (R-4572, 99.5 percent purity) was administered in the diet (0, 450, 900, or 1,800 ppm) to beagle dogs (two dogs/sex/group) for 13 weeks (Woodard, 1964). The approximate corresponding doses were 0, 15, 30, or 60 mg/kg-day. The dogs were not provided food on Sundays, but received double rations on Saturdays. In the 900 ppm group, one male and three females instead of two males and females were started on the study, but another male dog was added at week 5 and remained in the study to week 13. Survival was not affected, nor were there any significant consistent effects on body weight, weight gain, or food consumption. The treated dogs appeared quieter than controls and receded to the rear of their cages. There were slight decreases in hemoglobin levels in one female dog from each exposure level. Serum glutamic-pyruvic transaminase levels showed a marked elevation in one male (at 4 weeks) and one female (at 13 weeks) given 1,800 ppm Ordram. Thyroid weights were increased compared to controls in 3 of 4 dogs given 1,800 ppm. There were no histopathological changes related to treatment. The NOAEL was 900 ppm, or about 30 mg/kg-day, based on thyroid weight changes and enzyme changes at 1,800 ppm.

Groups of ten Sprague-Dawley rats per sex were exposed to technical grade molinate (purity not specified) via inhalation at nominal levels of 0, 2, 10, or 50 mg/m^3 for six hours/day, five days/week for 13 weeks (Biodynamics, 1979). The actual exposure concentrations, based on gas chromatographic analysis, were 0, 2.2, 11.1, and 42 mg/m^3. Food and water were provided ad libitum only when the animals were outside of the exposure chamber. Physical observations of mucoid nasal discharge, excessive lacrimation and salivation, aggressive behavior, and rapid and labored breathing appeared to be treatment-related, with the highest incidence at the 50 mg/m^3 exposure level. Body weights and body weight gains at the 50 mg/m^3 exposure were significantly less than controls throughout the exposure period. Erythrocyte counts were significantly increased in males exposed to 10 mg/m^3 but not 50 mg/m^3 at weeks 5 and 13, and in females exposed to 50 mg/m^3 at week 13. Various changes in clinical chemistry parameters were

measured that appeared to be treatment-related: depressed serum glutamic pyruvic transaminase and lactate dehydrogenase activities, elevated blood urea nitrogen, and decreased potassium and calcium levels in males and females exposed to 50 mg/m^3; decreased potassium in females at 10 mg/m^3 (week 5 only); and decreased lactate dehydrogenase in males exposed to 10 mg/m^3 (week 13 only). Significantly inhibited brain cholinesterase activity was measured at week 13 in males and females exposed to 50 mg/m^3, and males exposed to 10 mg/m^3. Plasma cholinesterase activity also was inhibited at weeks 8 and 13 in males exposed to 50 mg/m^3. RBC cholinesterase activity was not affected by molinate exposure. Organ weight changes most likely related to treatment, rather than a result of body weight reduction, include: at 10 mg/m^3, significant increase in absolute (males) and relative adrenal weights (both sexes) and absolute and relative thyroid weights (males); at 50 mg/m^3, significant increase in relative brain weight, absolute and relative adrenal, left and right kidney, and thyroid (females) weights, and an increase in relative liver weights (females). Bilateral degeneration of the seminiferous tubules was treatment- and dose-related; the incidence was 0 mg/m^3, 0/10; 2 mg/m^3, 4/10; 10 mg/m^3, 3/10; and 50 mg/m^3, 8/10. Hypospermia was observed in 1/10 males at 2 mg/m^3 and 6/10 males at 50 mg/m^3 and abnormal spermatozoa were observed in 2/10 males at 2 mg/m^3, 1/10 at 10 mg/m^3 and 10/10 at 50 mg/m^3. The only histopathological change observed in female rats was an increase in hemosiderosis in spleens of 1/10 controls, 4/10 mid-dose, and 8/10 high-dose animals. A NOEL was not determined in this study. The LOAEL was 2 mg/m^3, based on testicular effects in male rats at all exposure levels.

Neurotoxicity

The acute neurotoxicity of molinate was assessed in rats. Single oral doses of molinate (96.8 percent purity; 0, 25, 100, or 350 mg/kg) were administered to male and female Alpk:ApfSD rats (12/sex/group), and then the animals were observed for 15 days (Horner, 1994a). Body weights of male and female rats given 350 mg/kg were significantly less than controls ($p<0.01$) on days 8 and 15, and for males given 25 or 100 mg/kg on day 8. There was a moderate reduction in food consumption during week 1 for males at all doses and females administered 100 or 350 mg/kg and during week 2 for males given 350 mg molinate/kg body weight. Clinical observations included a dose-dependent decrease in activity in males and females, upward curvature of spine, hunched posture, lacrimation, salivation, and urinary incontinence. Recovery of these clinical signs generally occurred by day 3. Results of the functional observation battery showed a dose-related increase in time-to-tail flick and a decrease in motor activity on day 1 for treated animals that was significantly different from controls. Any other differences between control and treated animals with respect to landing foot splay or forelimb or hindlimb grip strength were not dose-related or statistically significant. Brain cholinesterase activity (n=6/sex/group) was significantly decreased in males and females treated with 100 or 350 mg/kg compared to controls; the difference was 23 percent or less. RBC cholinesterase activity in males at 350 mg/kg was significantly less than controls (21 percent decrease). Plasma cholinesterase activity was significantly decreased only in female rats given 100 mg/kg. Neuropathy target esterase (NTE) activity was not

affected by molinate treatment. In males only there was a marginal dose-related increase in glial fibrillary acidic protein (GFAP) levels, but the difference was not statistically significant. The small number of animals (n=3/sex/group) examined for NTE and GFAP makes interpretation of these results difficult. Some minor but statistically significant brain changes (decrease in weight and length) were found in males given 350 mg/kg. Macroscopic findings were limited to reduced testes with white areas or spots in 6 of 12 males at 350 mg/kg. Minimal neuronal cell necrosis in the pyriform cortex of the brain and sciatic nerve degeneration were noted in females (4/6 and 3/6, respectively) at the 350 mg/kg dose level, but not 25 or 100 mg/kg and not in males. A NO(A)EL for acute effects was not determined in this study. The LO(A)EL was 25 mg/kg, based on increased time-to-tail flick and reduced motor activity and body weight changes at all doses.

Ordram technical (98.6 percent purity) was tested for its ability to induce acute delayed neurotoxicity in hens (Sprague, 1983). In the initial study, adult White Leghorn hens (10 or 26 per group) in full egg production were administered two doses of either Ordram (0, 0.02, or 2.0 g/kg) or TOCP (0.5 g/kg) [tri-ortho-cresyl-phosphate, a positive control for acute delayed neurotoxicity], by gavage three weeks apart. Animals were terminated on day 43. Adverse effects observed with administration of 2.0 g/kg Ordram and 0.5 g/kg TOCP included listlessness, non-vocalization, unsteady gait, difficulty standing, and wide stance. Wing or neck droop, ptosis, and soft-shelled eggs were observed only with 2.0 g/kg Ordram. Diarrhea was observed in hens treated with both doses of Ordram and TOCP, but not in control hens. Neurohistopathological changes in both Ordram and TOCP-treated hens included axonal degeneration and focal gliosis in the brain and spinal cord and bilateral degeneration of the sciatic nerve. For Ordram-treated hens, the severity of the neuropathologic effects was predominantly mild and the incidence was greater in the brain and upper spinal cord than in the lower spinal cord. For TOCP-treated hens, however, the severity of the effects was mild to moderate and the incidence was similar at all levels of the spinal cord (90 to 100 percent). In addition, bilateral swelling of the sciatic nerve axis cylinders was observed in the TOCP-treated hens but not the Ordram-treated hens. The second part of the study was designed to determine if the effects observed in the first part were reproducible, dose-dependent, and reversible. Hens (10 to 30 per group) were given either two doses of Ordram (0, 0.063, 0.20, 0.63, or 2.0 g/kg) or 0.5 g/kg TOCP by gavage three weeks apart. Again, the study was terminated on day 43, except that recovery animals (control, TOCP, and 0.63 and 2.0 g/kg Ordram groups) were observed an additional 120 days, and then sacrificed on day 163. Mortality in the high dose Ordram (2.0 g/kg) group was 67 percent. Observed signs included weight loss, reduced food consumption, lack of vocalization, listlessness, incoordination, ptosis, and moderate to severe diarrhea. The incidence was highest in the 0.63 and 2.0 g/kg groups. Egg production was reduced only with 2.0 g/kg Ordram or 0.5 g/kg TOCP. The hens that survived in the 0.63 or 2.0 g/kg groups recovered from the adverse clinical effects and resumed egg production during the recovery observation period. Egg production in the TOCP-treated hens did not recover to the same extent as with Ordram treatment. TOCP produced mild to moderate swelling or destruction of axon filaments, some associated fragmentation of myelin sheaths, and focal gliosis in the brain and all three levels of the spinal cord. This was accompanied by clinical signs of delayed neurotoxicity. Although

the severity of the lesions was somewhat less in the recovery animals, the number of animals affected generally remained constant (approximately 100 percent). All hens treated with either 0.63 or 2.0 g/kg Ordram had axonal degeneration in the brain, spinal cord, and peripheral nerves. The lesions found in the myelinated pathways or tracts were similar to those produced by TOCP; however, the severity of the Ordram-induced lesions generally was slight to moderate, and the lesions were most frequent and severe in the brain stem and upper spinal cord, rather than in the pathways that control muscle coordination. Additionally, these changes were somewhat reversible within the 120-day recovery period. Nevertheless, the veterinary pathologist for the study determined that the histopathologic changes in the hens receiving 2.0 g/kg Ordram were characteristic of delayed neurotoxicity. The NOAEL for acute neurotoxicity is 0.2 g/kg, based on the presence of clinical neurological signs and axonal degeneration in the brain, spinal cord, and peripheral nerves at 0.63 or 2.0 g/kg Ordram.

Horner (1994b) assessed the subchronic neurotoxicity of molinate to rats. Molinate (96.8 percent purity) was administered at concentrations of 0, 50, 150, or 450 ppm in the diets of Alpk:ApfSD rats (12/sex/group) for 13 weeks. The corresponding mean doses were 0, 4.0, 11.7, and 35.5 mg/kg-day for males and 0, 4.5, 13.9, and 41.0 mg/kg-day for females. A functional observational battery included landing foot splay, sensory perception (tail flick test), and muscle weakness (forelimb and hindlimb grip strength). Cholinesterase activity (n=6/sex/group, left half of brain) and NTE activity and GFAP measurements (n=3/sex/group, right half of brain) also were taken. Survival was not affected by molinate treatment. Body weights were significantly reduced in male rats fed 150 ppm (weeks 2 to 4 only) or 450 ppm and in female rats fed 50, 150, or 450 ppm. Food consumption was reduced in males and females given 450 ppm molinate and in females given 50 or 150 ppm during the first three weeks only. There were no adverse clinical findings related to molinate exposure. The only consistent treatment-related effect reported for the functional observational battery was a slight reduction in forelimb and hindlimb grip strength of males and females at 450 ppm. Statistically significant inhibition of brain cholinesterase activity was measured in females given 50, 150 or 450 ppm and males given 150 or 450 ppm. RBC cholinesterase activity was significantly inhibited in females given 150 or 450 ppm and in males given 450 ppm molinate. Plasma cholinesterase activity of treated animals was not different from controls. NTE activity was significantly inhibited in a dose-related manner in all treated animals, but there were no clear treatment related effects on GFAP measurements. Absolute brain weight in males and females given 450 ppm was less than controls; however, relative brain weight was decreased only in males. Microscopic findings included minimal to slight neuronal cell necrosis in the dentate gyrus of the brain in males and females fed 450 ppm, but the incidence in these animals was not different from controls. There was a slight increase in the incidence of sciatic nerve fiber degeneration (minimal grade) in males at 450 ppm (4/6) compared to controls (1/6). The NOAEL for structural/functional neurotoxicity was 150 ppm, or about 12.8 mg/kg-day, based on brain weight changes and slight muscle weakness in males and females and slight sciatic nerve degeneration in males at 450 ppm. Inhibition of enzymes associated with neurological changes (NTE and brain cholinesterase) occurred at all exposure levels in female rats; the LOAEL for this endpoint was 50 ppm, or 4.5 mg/kg-day. A NOAEL was not apparent for systemic

toxicity. The LOAEL for systemic effects was 50 ppm, or 4.5 mg/kg-day, based on reductions in body weight and marginal decreases in food consumption in females at all exposures.

Genetic Toxicity

Bacterial Cell Mutagenicity Assays

Salmonella Reversion Assay

Several studies were found where molinate (purity ranging from 97.6 to 99.8 percent) was tested for mutagenicity using the Salmonella reversion assay. Various strains of *Salmonella typhimurium*, including TA 1535, TA 1537, TA 1538, TA 98, TA 100, and TA 102, were incubated with molinate doses ranging from 1 to 5,000 μg/plate with and without metabolic activation using liver S9 mix. All reported results were negative (Callander, 1988; Moriya *et al*., 1983; Ruiz and Marzin, 1997; Shirasu *et al*., 1977). Negative mutagenic results in *S. typhimurium* also were reported by Litton Bionetics (1975) and Woodard Research Corporation (1975), but these studies were described inadequately for thorough evaluation.

Shirasu *et al*. (1977) tested the mutagenic potential of molinate (60 or 200 mg/kg) in a host-mediated assay using *S. typhimurium* G46. Six male ICR mice per group were given 0 (corn oil), 60, or 200 mg/kg molinate (purity 99.8 percent) in two equal doses via gastric intubation over a 24-hour period, and then were inoculated immediately with *S. typhimurium* G46 (his−) intraperitoneally. Positive control animals were provided 50 mg/kg of dimethylnitrosamine and treated in the same manner as above. Three hours later peritoneal fluid was plated in triplicate and incubated for two days. The plates from the molinate-treated animals were negative for mutagenicity, whereas those from dimethylnitrosamine-treated animals were positive for mutagenicity.

Genotoxicity Assays in Other Bacteria

Molinate (99.8 percent purity) with or without metabolic activation was not mutagenic to the PQ37 strain of *Escherichia coli* in a test using the B-galactosidase gene under the control of the SOS-gene *sfiA* (SOS chromotest) (Ruiz and Marzin, 1997). The dose range tested was 5 to 500 μg/plate with S9 mix or 1 to 1,000 μg/plate without S9 mix. Additionally, molinate at doses up to 5,000 μg/plate was not mutagenic in the bacterial reversion assay using the WP2 *hcr* strain of *E. coli* (Moriya *et al*., 1983). Shirasu *et al*. (1977) found similar results using molinate (99.8 percent) in the *E. coli* WP2 *hcr* strain with or without S9 mix at doses ranging from 10 to 1,000 μg/plate.

Molinate (1 mg/plate) was negative in a rec assay using *Bacillus subtilis* M45(rec^-) and H17(rec^+), but the dose was insufficient to produce a killing zone on the *Bacillus* spore plates (Kuroda *et al*., 1992). Shirasu *et al*. (1977) also reported negative results in a rec assay using molinate (99.8 percent purity) concentrations per plate of 1 to 100 percent (v/v).

Mammalian Cell Mutagenicity Assays

Mouse Lymphoma Forward Mutation Assay

Molinate (50 to 200 μg/ml, purity not specified) was positive in the mouse lymphoma L5178Y TK+/– assay in the presence of metabolic activation, but was negative in the absence of S9 fraction (Kim *et al*., 1997). Stauffer Chemical Company (1984) reported a marginally significant increase (2 to 5 times) in mutation frequency in L5178Y TK+/– mouse lymphoma cells exposed to molinate (98.8 percent purity) at doses of 0.01 to 0.10 μl/ml with metabolic activation using both rat and mouse liver S9 mix. Although relative cell survival was generally less than 20 percent at the doses with positive responses, the results were reproducible.

Cytogenetic Assays

Chromosome Aberrations / Sister Chromatid Exchange (SCE)

Molinate (98.8 percent purity) was tested for its ability to induce either chromosomal aberrations or sister chromatid exchange (SCE) in L5178Y mouse lymphoma cells in the presence or absence of metabolic activation with rat liver S9 fraction (Stauffer Chemical Company, 1983b). Without S9 mix, molinate at dose levels of 0.0125 to 0.20 μl/ml did not increase the frequency of aberrations or SCE. With S9 mix, molinate treatment (0.0025 to 0.04 μl/ml) resulted in some statistically significant increases in aberrations and SCE, but none of the effects were dose-related or reproducible.

The frequency of sister chromatid exchange increased in a dose-dependent manner when *Vicia faba* root meristems were treated with 25, 50, and 75 ppm of molinate (72 percent purity) (Calderón-Segura *et al*., 1999). Metaphase cells were not observed after treatment with 100 ppm molinate.

Molinate (0, 10, 20, 40, or 100 μg/ml) did not induce sister chromatid exchanges in Chinese hamster V79 cells (Kuroda *et al*., 1992). The purity of the test compound was unknown.

Micronucleus Test

A statistically significant ($p<0.05$) dose-related increase in the frequency of micronucleated reticulocytes was observed in 48-hour blood samples from male ICR mice injected intraperitoneally with molinate (91, 182, or 363 mg/kg; purity not specified) (Kim *et al*., 1997). Pintér *et al*. (1990) determined that molinate (350 or 525 mg/kg; 97.4 percent purity) administered by gavage to male and female CFLP mice produced a statistically higher incidence of micronucleated polychromatic erythrocytes (PCE) compared to control animals at 48 hours after treatment. The frequency of mean micronucleated PCE in a low-dose group given 175 mg molinate/kg was not different from the vehicle control. In a study by Stauffer Chemical Company (1983a), $B6C3F_1$ mice were administered molinate (98.8 percent purity) by gavage at the following dose levels: 0, 200, 400, or 600 mg/kg to males and 0, 100, 200, or 400 mg/kg to females.

Molinate compared to the solvent control did not produce a significant increase ($p>0.01$) in the average number of micronuclei per 1,000 PCE at 24, 48, or 72 hours.

Unscheduled DNA Synthesis Assays

Exposure of primary rat hepatocyte cultures to molinate (97.6 percent purity) at concentrations of 10^{-8} to 10^{-4} M for 17 to 20 hours did not induce unscheduled DNA synthesis (Trueman, 1989). At all concentrations, the net nuclear grain count was less than zero.

Dominant Lethal Assay

Twenty male Sprague-Dawley rats per group were exposed to 0, 5, 10, or 15 ppm molinate (96.8 percent purity) in the diet for 12 weeks as part of a multigenerational reproduction study (Moxon, 1995). They were transferred to the dominant lethal study and fed the same diet for another two weeks, but only during the day. Methylmethane sulfonate (MMS), administered to 20 male rats in a single intraperitoneal dose of 40 mg/kg, served as a positive control. During the first week, the males were each mated with two untreated female rats for seven consecutive overnight periods. During the second week, the males were mated as above, but with different untreated female rats. Twenty days after the first overnight mating period, the females were sacrificed and evaluated for corpora lutea, live implantations, and early and late intrauterine deaths. Molinate treatment had no effect on body weight or clinical condition, but males fed 15 ppm had reduced fertility, as indicated by a significant ($p<0.01$) decrease in the number of implantations. There was no increase in the number of intrauterine deaths as a result of molinate exposure; therefore, molinate did not induce dominant lethal mutations. MMS treatment, however, did produce dominant lethality, as indicated by statistically significant ($p<0.01$) post-implantation loss (early intrauterine death) after both mating periods.

Developmental Toxicity

Minor (1990) assessed the effects of R-4572 technical (molinate, 97.6 percent purity) on pregnant rats and unborn conceptuses when administered orally to dams. Twenty-six female Crl:CD® rats per group were gavaged with 0, 2.2, 35, or 140 mg/kg-day molinate in corn oil on gestation days 6 to 15 and sacrificed on gestation day 21. One gravid dam in the 140 mg/kg-day group was sacrificed on day 15 with chromodacryorrhea, dehydration, and weight loss. Necropsy findings included moderately enlarged adrenals and focal discoloration in the stomach mucosa. Ovaries had 14 corpora lutea and the uterus had three implants, which were being resorbed. All pregnant females in the 0, 2.2, and 35 mg/kg-day groups had live fetuses on gestation day 21. In the 140 mg/kg-day group, 2 of 23 surviving pregnant dams had only resorbing conceptuses, and two started delivery before the scheduled sacrifice. Clinical findings in the 140 mg/kg-day group included a significant increase in the incidence of salivation, which was first observed on day 14, and a significant reduction in mean RBC cholinesterase activity (56 percent of

control) on sacrifice day 21. Mean corrected body weights and mean absolute body weights were significantly reduced at gravid day 12 and thereafter in the 140 mg/kg-day group animals compared to controls. Food consumption for the 35 mg/kg-day animals was significantly less on gestation day 16 to day 21 and for the 140 mg/kg-day animals on day 6 to day 21 compared to controls. There were no necropsy findings in the dams that appeared to be treatment-related. No differences were observed between control and treated groups in the mean number of corpora lutea per dam, implants per dam, or in preimplantation losses. In the 140 mg/kg-day group, there was a significant increase in postimplantation losses with a concomitant decrease in live fetuses and an increase in early and mid resorptions and affected implants. The mean fetal weights and mean weights of the reproductive tract were significantly reduced at this dose, too. None of these effects occurred at molinate doses of 2.2 or 35 mg/kg-day. Malformations were not increased in any group. Fetal observations at 140 mg/kg-day included a statistically significant increase in the incidence of fetuses considered to be runts externally, fetuses with moderately dilated cerebral ventricles, and fetal skeletons with incompletely ossified fifth sternebrae. The NOAEL for maternal toxicity was 35 mg/kg-day, based on cholinesterase depression, salivation, body weight decrements, and reduced food consumption in dams at the 140 mg/kg-day dose. The NOAEL for developmental toxicity also was 35 mg/kg-day, based on postimplantation losses, increased resorptions, reduced fetal weights, dilated fetal brain ventricles, and incomplete ossification of fetal vertebrae at the 140 mg/kg-day dose.

Ordram (96.5 percent purity) was fed in the diet at doses of 0, 8, or 24 mg/kg-day to pregnant mice on gestation days 6 to 15 or 18 (Scott and Beliles, 1967). Ten animals per group were allowed to deliver naturally and ten were delivered by Cesarean section. All female mice survived and the duration of pregnancy in treated mice allowed to come to term was comparable to controls. There were no changes in internal organs. Some ovarian capsules had a balloon-like appearance, but this effect occurred in control and treated animals. There were no teratological changes, changes in ossification rate, or changes in sex ratio induced in fetal or newborn mice by Ordram administration. A NOAEL for maternal toxicity or developmental toxicity could not be identified from this study.

Wilczynski *et al*. (1985) studied the effects of Ordram technical on the pregnant rabbit and conceptuses. Mated New Zealand White female rabbits (age 26 to 29 weeks) were administered Ordram technical (98.8 percent purity) via gavage in 13 consecutive daily doses of 0, 2, 20, or 200 mg/kg-day from gestation day 7 to day 19. Sixteen females were assigned to each group, except the 200 mg/kg-day group had 17 animals. Pregnant females were sacrificed on day 29 of gestation. Overall fertility rate was 94 percent (61 of 65 females pregnant), and the rates for each group were comparable. One female in the 20 mg/kg-day group died, and the cause of death was not determined. The incidence of abortions for each group was: control, 1/16 (6 percent); 2 mg/kg-day, 1/15 (7 percent); 20 mg/kg-day, 1/13 (8 percent); and 200 mg/kg-day, 4/17 (24 percent).

Although there were no statistically significant differences between the control and treated animals, the incidence of abortions in the high dose group (24 percent) is much higher than the incidence in historical controls from three separate studies (0 percent),

and is considered treatment-related. With respect to body weight, there were no significant differences between control and treated animals for does with live fetuses or for all does. However, reductions in body weight occurred in the 200 mg/kg-day group compared to controls during days 14 to 21, but the changes were statistically significant only for all does, and not for does with live fetuses.

Food consumption also was somewhat reduced in the 200 mg/kg-day group on days 14 through 21. The only organ weight changes were significant increases in absolute and relative liver weights in 200 mg/kg-day females compared to controls. This group also had a treatment-related increase in the incidence of dark brown livers (38 percent versus 6.7 percent for controls). There were no significant differences between treated and control animals in the number of corpora lutea per doe, number of implants per doe, ratio of implants to corpora lutea, or the ratio of the number of live fetuses to implants. A statistically significant increase in the number of live fetuses in the 20 mg/kg-day group compared to the control was not considered biologically relevant. Mean fetal body weight or mean number of ossified caudal vertebrae were not significantly different between treated and control animals. The only increase in a treatment-related malformation was for unossified 5th sternebrae in the 200 mg/kg-day group (21.48 percent versus 7.23 percent for control), but the incidence was not statistically significant, nor was it greater than historical controls.

The NOAEL for this study was 20 mg/kg-day for both maternal and developmental toxicity, based on body weight and liver changes in the does, and an increase in the incidence of abortions at the 200 mg/kg-day dose.

Horner (1996) exposed rat dams to molinate (96.8 percent purity) to determine the developmental neurotoxicity to offspring. Alpk:Ap$_f$SD dams (30 per group) were exposed to dietary levels of 0, 20, 75, or 300 ppm molinate on gestation day 7 through lactation day 11. Developmental neurotoxicity evaluations were performed on offspring shortly after cessation of treatment of dams and again on postnatal day 63. These evaluations included motor activity, learning and memory testing, auditory startle responses, histopathology, and morphometric analyses of brain transverse sections at seven levels. Pup weights, clinical signs, and preputial separation and vaginal opening were also monitored.

Reductions in food consumption and body weights (9 percent) were observed during gestation and lactation in dams fed 300 ppm molinate. In the 300 ppm group offspring, slight delays were observed in learning and memory skills in tests administered within two weeks of maternal exposure; most of the effects seen at day 21 to day 23 resolved by day 59 to day 62. An exception to this was a decrease in the amplitude of response to auditory stimuli and/or an increase in delay to maximum amplitude of response, which persisted to the end of the study (day 61 or 63). Pup mortality was increased during the maternal exposure period, and pup body and brain weights were decreased 14 to 16 percent at 300 ppm for both male and female offspring. The brain weight decrements persisted through day 63. A statistically significant reduction occurred in the thickness of the molecular layer of the prepyramidal fissure of the cerebellum at day 12, but not at day 63 at 75 ppm and above. A slight delay occurred in time to preputial separation or

vaginal opening in pups from dams fed 300 ppm molinate. There were no histopathological changes at day 12 or day 63.

The NOAEL for maternal toxicity in this study conducted by Horner (1996) was 75 ppm, based on decrements in body weight and food consumption at 300 ppm. The NOAEL for developmental neurotoxicity was 20 ppm, based on brain changes at 75 ppm.

Williams (1997) confirmed the delayed vaginal opening in rat pups after exposure of F_0 dams and F_1 offspring to 300 ppm molinate in the diet that was reported by Horner (1996) and Moxon (1997). Williams (1997) determined that the delayed vaginal opening could be inhibited by treatment of 0.5 μg estradiol per rat on day 28 post partum. The author stated that the developmental toxicity was due to a lack of estrogen at this sensitive time in development.

When molinate (dose not provided) was administered with malathion, an organophosphorus pesticide, to chick embryos, teratogenic signs were observed, including severe micromelia, beak deformities, feathering abnormalities, and abdominal edema (Jin and Kitos, 1996). Molinate alone was found to inhibit carboxylesterase in chick embryos. Furthermore, molinate sulfoxide inhibited the serine esterase in vitro in extracts of chick embryo tissues, suggesting that the sulfoxide was the active inhibitor in vivo. Since malathion is metabolized to a less toxic metabolite by carboxylesterase, inhibition of this enzyme by molinate apparently increased the toxicity of malathion and resulted in a teratogenic synergy (Jin and Kitos, 1996).

Reproductive Toxicity

Numerous studies investigating the reproductive toxicity of molinate were available for review. These included the reproductive toxicity studies required under FIFRA (Federal Insecticide, Fungicide, and Rodenticide Act) guidelines for pesticide registration, as well as many studies to elucidate the mechanism of molinate reproductive toxicity. Rats appear to be the species most sensitive to molinate. Consistent findings in the rat studies include abnormal sperm, decreased sperm motility and sperm numbers, decreased litter size, decreased percentage of pups born live, organ weight changes and increased incidence of microscopic lesions in the ovary, testes, and adrenal gland, delayed vaginal opening, and decreased brain weight. The individual studies are described below and have been grouped according to whether they were performed using animals of one or both sexes.

Studies Exposing Both Sexes

Moxon (1997) performed a two-generation reproductive toxicity study in rats using molinate (96.8 percent purity) administered in the diet. The dietary exposure levels were 0, 5, 10, or 15 ppm for males and 0, 20, 50, or 300 ppm for females. Different dietary levels were chosen for males because previous studies showed males were more sensitive than females. Matings were as follows: 0 ppm males with 0 ppm females, 5 ppm males with 20 ppm females, 10 ppm males with 50 ppm females, and 15 ppm males with 300 ppm females. Respective doses (mg/kg-day) during the pre-mating period were: 0, 0.4,

0.8, and 1.3 (F_0 males); 0, 1.9, 4.7, and 28.8 (F_0 females); 0, 0.5, 1.1, and 1.6 (F_1 males); 0, 2.2, 5.6, 34.5 (F_1 females). During matings, the female rats were fed the males' diets. Forty male and female rats per dietary exposure group were mated to produce the F_{1A} generation. From these animals, 40 males and females per group were selected and put through two subsequent matings to produce the F_{2A} and F_{2B} generations.

None of the intercurrent deaths or changes in clinical condition of adult rats was related to administration of molinate in the diet. Significant reductions in adjusted mean body weights generally were limited to females fed 300 ppm molinate, including F_0 and F_1 adults during the premating period, gestational period, and lactation. In addition, there were occasional statistically significant reductions in adjusted mean body weights for lactating F_0 and F_1 females (A and B litters) fed 20 or 50 ppm molinate, but these were not consistently dose-related. Food consumption was significantly reduced in F_0 and F_1 females by exposure to 300 ppm molinate compared to controls during the premating and gestational periods and lactation. Some reductions also were apparent at 20 and 50 ppm, but these were not consistently dose-related.

Comparison of premating vaginal smears between control and treated groups indicated that molinate had no effect on the number of cycles in 22 days or on pre-coital interval of the F_0 and F_1 adult females. Gestation length for F_0 adult females given 300 ppm molinate and for F_1 adult females (litter A) given 50 ppm (but not 300 ppm) molinate was significantly greater than for controls. In the absence of a consistent dose-response effect and of a consistent response between generations, the differences in gestation length were not considered to be related to molinate treatment. There were fewer successful matings of adult males and females of the F_0 and F_1 generations in the high dose group (15 and 300 ppm for males and females, respectively) compared to controls, but the differences were statistically significant only for the F_1 adults (litter A). With respect to litters, the incidence of whole litter losses was not different between control and molinate-treated animals. In the F_{1A} and F_{2B} litters but not the F_{2A} litter, the proportion of pups born live was significantly reduced in the animals treated with 300 ppm molinate in comparison to control animals. Litter size from day 1 to day 29 postpartum was statistically significantly reduced in the 300 ppm group compared to the controls for the F_{1A}, F_{2A} and F_{2B} litters. Pup survival to day 22 was significantly reduced at 300 ppm compared to controls only for the F_{2B} litter.

There were no clinical observations in the pups that could be attributable to dietary molinate exposure. By day 29, adjusted mean body weights of pups from the F_{1A}, F_{2A}, and F_{2B} litters given 300 ppm molinate were significantly reduced compared to controls. Significant reductions were observed in total litter weights as well, but this was probably a result of the reduction in litter size and individual pup body weights. With respect to developmental landmarks, molinate treatment did not affect the time when preputial separation occurred in F_1 males, but dietary exposure to 300 ppm compared to controls did cause a statistically significant delay in the age at which vaginal opening occurred in the F_1 females. Ano-genital distance of the F_{2A} pups on day 1 post partum was not affected by molinate exposure.

Post mortem investigations of adult rats revealed that molinate had no effect on sperm velocity of F_0 males, but exposure to 15 ppm compared to controls caused a significant

reduction in percent motile sperm and total sperm in the right cauda. These effects also were observed in F_1 males, in addition to a significant reduction in curvilinear and average path velocity, but not straight line velocity, at 15 ppm. Effects on sperm morphology of the F_0 and F_1 males include a statistically significant reduction in percent normal sperm, an increase in percent abnormal sperm, and an increase in the percent of abnormal and detached heads in animals administered 10 or 15 ppm molinate in the diet, and a significant increase in abnormally shaped heads, abnormal acrosomes (F_0 only), and coiled/kinked tails (F_1 only) with 15 ppm molinate. There were no statistically significant differences in small, growing, or large oocytes of the left ovary from F_0 and F_1 adult females treated with 300 ppm molinate compared to controls.

Organ weight changes included: statistically significant reductions in absolute brain weight for F_0 and F_1 males given 5, 10, or 15 ppm molinate and F_0 and F_1 females given 20, 50, or 300 ppm molinate; significant reductions in absolute spleen weight for F_0 and F_1 females given 20 (F_1 only), 50, or 300 ppm molinate; significant reductions in absolute kidney and liver weights, and significant increases in relative adrenal weight in F_0 and F_1 females given 300 ppm molinate; and significant reductions in absolute and relative right cauda weights and absolute right and left epididymides weights in F_0 and F_1 males given 15 ppm molinate. Other statistically significant changes in absolute or relative organ weights were not considered to be related to molinate administration.

Macroscopic findings for adult rats included an increase in the number of F_0 females without uterine implantation sites (incidence was 1/40, 2/40, 5/40, and 6/40 in the 0, 2, 50 and 300 ppm groups, respectively) and a decrease in the weight of the epididymides (8/40) and testes (7/40) of F_1 males given 15 ppm molinate compared to control animals (2/40 and 0/40, respectively). Microscopic findings for adult males included increased sperm precursor cells in the epididymis of F_1 males given 15 ppm and bilateral focal tubular degeneration of testes from F_1 males given 10 or 15 ppm. Nothing remarkable was noted for F_0 males. For adult females, microscopic findings included an increase in diffuse fine cortical fat vacuolation of the adrenal glands and interstitial cell vacuolation/hypertrophy of ovaries (F_0 and F_1 females, 50 and 300 ppm), increased ovarian interstitial tissue (F_0 females, 300 ppm), increased incidence of cystic follicles (F_0 and F_1 females, 300 ppm), and increased incidence of hemosiderin deposition in the spleen (F_0 and F_1 females, 300 ppm).

Post mortem examination of the pups revealed the following statistically significant organ weight changes: reduction in absolute brain weights of F_{1A} males (50, 300 ppm), F_{1A} females (300 ppm), F_{2A} males and females (300 ppm), F_{2B} males (300 ppm), and F_{2B} females (50, 300 ppm); reduction in absolute spleen weights of F_{1A} males (50, 300 ppm), F_{1A} females (300 ppm), and F_{2A} and F_{2B} males and females (300 ppm); reduction in relative spleen weights of F_{1A} males (50, 300 ppm), F_{1A} females (300 ppm), F_{2A} males (300 ppm), F_{2A} females (50, 300 ppm),and F_{2B} males and females (300 ppm); reduction in absolute ovaries weights in F_{1A}, F_{2A} and F_{2B} females (300 ppm); reduction in absolute and relative testes weights of F_{1A} males (50, 300 ppm), and F_{2A} and F_{2B} males (300 ppm); reduction in absolute and relative thymus weights of F_{1A} males (50, 300 ppm), F_{1A} females (300 ppm), and F_{2A} and F_{2B} males and females (300 ppm). All other significant differences in organ weights were generally inconsistent across the litters, or were

attributable to reductions in body weights. There were no remarkable macroscopic or microscopic findings in pups up to and including day 18 post partum or over day 18 post partum that could be attributable to molinate exposure.

In this study of Moxon (1997), the NOAEL for male rats for reproductive effects was 5 ppm (approximately 0.4 mg/kg-day), based on changes in sperm parameters and testicular effects at 10 and 15 ppm molinate. The NOAEL for female rats for reproductive effects was 20 ppm (approximately 2 mg/kg-day), based on ovarian interstitial cell vacuolation/hypertrophy that was dose-related in incidence and severity at 50 and 300 ppm. A NOAEL was not identified for systemic effects; however, the LOAEL was 5 ppm for males and 20 ppm for females, based on reductions in absolute brain weight at all exposure levels. The NOAEL for developmental effects was 50 ppm, based on delayed vaginal opening in female pups exposed to 300 ppm.

Woodard (1977c) exposed three generations of CD rats to Ordram (purity not specified) in the diet to determine the reproductive toxicity of the pesticide. The dose levels were 0, 0.063, 0.2, or 0.63 mg/kg-day. Twenty-five F_0 males and females per dose group were mated at 100 days of age to produce F_{1a} and F_{1b} offspring. One-half of the F_{1a} offspring were examined for visceral abnormalities, and the other half were examined for skeletal abnormalities. The F_{1b} animals were weaned, placed on their respective diets until 100 days of age, and then mated to produce the F_{2a} and F_{2b} offspring. The F_{2a} offspring were examined after weaning and the F_{2b} continued on their respective diets for between 50 and 85 days, and then they were mated to produce the F_{3a} and F_{3b} offspring. Because of a heating system failure, which resulted in poor pup survival, the F_{3a} pups were discarded at birth. The F_{3b} pups were weaned, and then examined for gross necropsy and organ histopathology. In the 0.63 mg/kg-day group, the numbers of litters were reduced, but not the average litter size, for all generations. There appeared to be some effect on pup survivability, as reflected by the number of females available for mating in subsequent generations.

Food consumption was not affected by Ordram treatment. Mean weekly body weights were slightly reduced for F_{1b} males fed 0.63 mg/kg-day of Ordram, but not for F_0 or F_{2b} rats. The mean body weight at weaning for the F_{3b} pups administered 0.63 mg/kg-day was 10 to 20 percent lower than controls. Consequently, the mean absolute heart, liver, and kidney weights also were lower, but the relative organ weights were not different from controls. Teratogenic examination did not reveal any abnormalities of the skeleton or viscera that could be attributed to Ordram treatment. Likewise, tissues of the F_{3b} pups examined for histopathological lesions showed no differences between treated and control animals. Microscopic appearances of the testes indicated that male F_{3b} pups had not reached sexual maturity, but the testicular effects were noted among the treated and control animals. The NOAEL was 0.2 mg/kg-day for both systemic and reproductive toxicity.

Woodard (1975b) performed a "crossover" mating study to determine whether the male or female rat was responsible for reduced fertility in Ordram treated rats. CD rats (8 animals per sex per group) were placed into one of six groups where either the males were fed control diet and the females were fed diets containing 8, 16, or 32 mg/kg-day of Ordram (purity not specified), or the females were fed control diet and the males were fed

diets containing 8, 16, or 32 mg/kg-day Ordram. Animals were on the diets for 12 weeks, and then were paired for mating (8 pairs per dose group). When males were fed the control diet and females the treated diets, 8 litters were produced per dose group. However, when females were fed the control diet and males the treated diets, the number of litters produced were: 2 at 8 mg/kg-day and 0 at 16 or 32 mg/kg-day. From these data, it appeared that Ordram primarily affected the male rat to reduce expected numbers of litters.

CD rats (25/sex/group) were fed Ordram (purity not specified) in the diet at levels of 0, 8, 16, or 32 mg/kg-day for 18 weeks, and then the diets were reduced and the same animals were fed 0, 0.63, 2.0, or 6.3 mg/kg-day, respectively, for another 11 weeks (Woodard, 1975a). Males and females within each group were paired for a 10-day mating period at weeks 7 and 21. At week 7, the number of litters produced was 22, 4, 3, and 0 for the 0, 8, 16, and 32 mg/kg-day groups, respectively. At week 21, the number of litters was 19, 12, 9 (24 pairs only), and 3 for the 0, 0.63, 2.0, and 6.3 mg/kg-day groups, respectively. These results indicated that 0.63 mg/kg-day was nearing a NOEL for reproductive effects in CD rats and that the effects of Ordram on fertility appeared to be reversible.

Wickramaratne (1997b) administered molinate (96.8 percent) via gavage to male and female Crl:CD(SD)BR rats to examine the morphological effects of the herbicide on the ovary, adrenal, and testis. Five females per group were given 0, 10, 40, 100, or 150 mg/kg-day for 7 days. Males (12 to 14 per group) were given 0, 10, 30, or 60 mg/kg-day for 35 days. Although weights were not taken, adrenal glands of female rats given 100 or 150 mg/kg-day were visibly enlarged compared to controls. Morphological examination revealed adrenal cortex hypertrophy, normal adrenal medulla, enlarged cells of the zona fasciculata with centrally-located nuclei and grossly vacuolated cytoplasm, and accumulation of lipids in the adrenal cortex cells.

In the females receiving 100 or 150 mg/kg-day molinate, marked hypertrophy was observed in the interstitial compartment of the ovary; the hypertrophied cells exhibited a vacuolated appearance, and had marked lipid accumulation. There were no changes in the adrenal glands or ovaries at 10 or 40 mg/kg-day. Body weights of male rats were significantly lower at all doses compared to controls. Epididymis weights were significantly reduced at 30 and 60 mg/kg-day and testis weights were significantly reduced at 60 mg/kg-day. (The adrenal glands were not weighed.) Morphological examination of testes revealed significant atrophy of the seminiferous tubules (Stages VII to IX) at 60 mg/kg-day. The lesion was similar but less severe at 30 mg/kg-day. The testicular lesion was characterized by retention of spermatids at the base of the tubules, nuclear degeneration of early spermatids, exfoliation of degenerated spermatids from the germinal epithelium, formation of multinucleated spermatid bodies in the lumen of affected tubules, some vacuolation of Sertoli cell cytoplasm, and some focal regions of mild hyperplasia in Leydig cells of the atrophied testes. There were no testicular effects at 10 mg/kg-day. At 60 mg/kg-day, few mature sperm were present in the lumen of the epididymis, but there were many round and multinucleated spermatids present. Similar but less severe effects were observed at 30 mg/kg-day, but the epididymis was normal at 10 mg/kg-day. At 30 and 60 mg/kg-day, sperm exhibited detached heads and tails, backwardly flexed heads, and fractured midpiece membranes with fine filaments

protruding from the membranes. The NOAEL for female rats was 40 mg/kg-day and for male rats was 10 mg/kg-day.

Studies Exposing Males Only

Minor (1981) conducted a fertility study in male rats to determine the mechanism and site of action of Ordram's antifertility effect in rats. The study was conducted in four parts:

I. Twelve male rats/group were administered 0, 12, or 60 mg/kg-day Ordram (98.2 percent purity) orally for five days, and then were mated with a new female rat each week for ten weeks. Females were sacrificed nine to ten days after cohabitation and the number of corpora lutea, implants, viable fetuses, and resorptions were counted.

II. Twenty male rats/group were administered 0 or 12 mg/kg-day Ordram orally for ten weeks, and then were mated with two females each week for two weeks. Females were sacrificed nine to ten days after cohabitation and the same fertility parameters were measured as in Part I. In addition, serum triiodothyronine (T_3), thyroxine (T_4), thyroid-stimulating hormone (TSH), luteinizing hormone (LH), follicle-stimulating hormone (FSH), and testosterone concentrations, sperm viability, motility, morphology, and concentration, and adrenal and testes plus epididymides weight and histology were monitored.

III. Twelve male rats/group were administered 0, 12, or 30 mg/kg-day Ordram orally for five weeks, and then were mated with two females per week for one week. Females were sacrificed 15 days after cohabitation and the same fertility parameters were measured as in Part I. The additional parameters described in Part II also were monitored.

IV. Twelve male rats/group were administered 0, 0.2 or 4 mg/kg-day Ordram orally for five weeks, and then were mated with two females per week for one week. The experimental design was the same as in Part III.

Body weights of treated animals from all four groups were not different from control animals. Clinical observations were unremarkable. In Part I, female rats mated to males given 60 mg/kg-day of Ordram showed a significant reduction in the number of pregnancies, implants, viable fetuses, and the implantation index (pre-implantation loss, or total number of implants/total number of corpora lutea) during the third mating, as well as the number of implants during the fourth mating. These results suggested that the major effect of Ordram was on the later spermatid stage.

In Part II, the number of pregnancies were significantly reduced during the second mating, the numbers of implants, viable fetuses, and pre-implantation losses were significantly reduced during the first and second matings, and the total resorptions and post-implantation losses were significantly reduced during the second mating for treated animals compared to controls. Serum hormone concentrations of treated males were not different from controls. Percent viable sperm, percent motile sperm, sperm cells $\times$ 10^6/ml, and number of implants/female were significantly reduced, and percent abnormal sperm was significantly increased in treated animals compared to controls.

In Parts III and IV, the numbers of implants (12, 30 mg/kg), viable fetuses (4, 12, 30 mg/kg) resorptions (30 mg/kg), pre-implantation losses (12, 30 mg/kg), percent viable (4, 12, 30 mg/kg) and percent motile sperm (4, 12, 30 mg/kg), cells × 10^6/ml (4, 12, 30 mg/kg), and number of implants/female (4, 12, 30 mg/kg) were significantly reduced and percent abnormal sperm (4, 12, 30 mg/kg) was significantly increased in treated compared to control rats. Testosterone (12, 30 mg/kg), FSH (30 mg/kg), and T_4 (30 mg/kg) concentrations were significantly increased in treated versus control animals. There were no differences between treated and control animals with respect to absolute and relative adrenal and testes weights. The incidence of testicular and epididymal lesions was not different between control and treated animals, but a dose-related trend in spermatid/spermatocyte degeneration was apparent in males given Ordram doses >4 mg/kg-day. Treatment at 5 or 10 weeks at doses >4 mg/kg-day produced detached sperm heads and tails, heads and tails bent at abnormal angles, and rupture of sperm membranes at the head-midpiece junction and the midpiece-tail junction. The NOAEL was 0.2 mg/kg-day, based on reduced sperm parameters, and decreased number of viable fetuses at ≥4 mg/kg-day.

Hodge (1993) performed a gavage study exposing male rats to molinate (96.8 percent) to define the NOEL for changes in sperm morphology. Groups of 12 Crl:CD(SD)BR rats were administered molinate in corn oil at dose levels of 0, 0.5, 1.0, 2, 3, 4, or 8 mg/kg-day for 35 days. One hundred sperm per rat were examined for abnormalities of the head, midpiece, and tail. There were no treatment-related changes in clinical observations. Body weight was reduced <4 percent from day 10 onward in the 8 mg/kg-day group compared to controls, but the difference was not statistically significant. The percentage of midpiece abnormalities was significantly increased at dose levels of 2 mg/kg-day and above, whereas the percentage of head abnormalities only were significantly increased at 1 mg/kg-day (i.e., there was no dose-response). The NOAEL was identified as 1 mg/kg-day, based on midpiece abnormalities at ≥2 mg/kg-day.

Killinger (1982) compared the effects of three thiocarbamate compounds, benthiocarb, benthiocarb sulfoxide, and Ordram®, on male rat fertility. Twelve CR®Crl:CD® (SD) BR rats/group were gavaged with 0 (corn oil), 20, 70, or 140 mg/kg-day of benthiocarb or benthiocarb sulfoxide, or 20 mg/kg-day of Ordram for 12 weeks. Each male was mated with two untreated females after 10 weeks of treatment; the males were housed with females for one week. For benthiocarb and benthiocarb sulfoxide-treated males, sperm samples were analyzed from the cauda epididymides, and for control and Ordram-treated males, sperm samples were analyzed from the cauda and caput epididymides. Females were necropsied mid-gestation and the number of corpora lutea, implant sites and viable fetuses were counted.

Body weights of treated animals (except 20 mg/kg-day benthiocarb sulfoxide) were significantly reduced throughout much of the treatment period compared to control rats. Clinical observations included wheezing and "dull" behavior in the 140 mg/kg-day benthiocarb sulfoxide group and excessive salivation in all treated animals compared to controls. Mating and fertility indices were slightly but not significantly reduced in Ordram treated males compared to controls. However, pregnancy index was significantly reduced. Significant reductions also were observed in the number of implants and viable

fetuses, with a corresponding decrease in the implantation index (number of implants/corpora lutea) from females mated with Ordram-treated males, but not with the other groups. There was a low but statistically significant increase in the number of resorptions and a decrease in the implant viability index (number of viable fetuses/number of implants) in the benthiocarb (70 and 140 mg/kg-day) and benthiocarb sulfoxide (20 and 70 mg/kg-day) groups. The only changes in sperm parameters were a significant reduction in cauda epididymal sperm motility, cell concentration, and percent abnormal (damage or detachment at the head-midpiece or midpiece-tail junctions) in the Ordram-treated males compared to controls. Transmission electron micrographs of sperm samples showed areas of absent plasma membrane in the midpiece in both the caput and cauda epididymal samples. Since only one Ordram dose was used, a NOEL for Ordram-induced male fertility effects could not be identified.

Groups of ten male Sprague-Dawley rats were exposed via inhalation to technical grade molinate (purity not specified) at exposure levels of 0, 2.2, 11.1, or 42 mg/m^3 (nominal levels of 0, 2, 10, or 50 mg/m^3) for six hours/day, five days/week during two separate studies (Biodynamics, 1979). The first study was a typical subchronic inhalation study where the males were exposed for three months. This is described in more detail under the Subchronic Toxicity section. The second study was a reproductive fertility study in which animals were exposed under four different scenarios. Under the first scenario, males were exposed for one month, and then each male was mated with two untreated females. Under the second scenario, males were exposed for three months, and then each male was mated with two untreated females during the last ten days of the three-month period. Under the third scenario, each male was exposed for three months and then allowed to recover for one month before being mated with two untreated females. Under the final scenario, each male was exposed for three months and allowed to recover for three months before being mated with two untreated females. Mating under all four scenarios continued for ten consecutive days or until the presence of sperm or vaginal plug was observed. In both studies, animals were given food and water ad libitum outside of the exposure chamber only.

Adverse reproductive or endocrine effects observed in male rats during the subchronic inhalation study included a statistically significant increase in absolute and relative adrenal weights (11.1 and 42 mg/m^3), a significant reduction in absolute testes weight (42 mg/m^3), an increase in the incidence of bilateral degeneration of the seminiferous tubules (2.2, 11.1, and 42 mg/m^3), and hypospermia and abnormal spermatozoa (42 mg/m^3). A NOEL was not determined in this study. The LOAEL was 2.2 mg/m^3, based on testicular effects at all exposure levels.

During the second study (Biodynamics, 1979), mating indices (number of females mated/number of females exposed to males and number of males mating with females/number of males exposed to females) of the treated groups were not different from controls for the one- and three-month treatment and the one- and three-month recovery periods. The pregnancy index (number of females producing offspring/number of females mated) was significantly reduced after the one-month treatment (11.1, 42 mg/m^3), the three-month treatment (42 mg/m^3), and the three-month treatment with one-month recovery (42 mg/m^3). The fertility index (number of males producing

offspring/number of males mated) was significantly reduced after the one-month (11.1, 42 mg/m^3) and three-month treatment periods (42 mg/m^3), and was reduced but not significantly after the three-month treatment with one-month recovery period (42 mg/m^3). There were no significant differences in either the pregnancy or fertility indices between the control and treated animals after the three-month treatment and three-month recovery period. After the one-month treatment period, females mated to treated males had significantly fewer implantations and fetuses (2.2, 11.2, and 42 mg/m^3) and significantly more early resorptions (42 mg/m^3). After the three-month treatment period, females mated to treated males had significantly fewer implantations and fetuses (2.2, 11.1 mg/m^3).

Males exposed to 42 mg/m^3 did not impregnate any females. There were no differences in reproductive parameters in the females that were mated to males allowed to recover for one or three months after molinate treatment. Mean testes weight was significantly lower in males treated with 42 mg/m^3 compared to controls. Male reproductive performance was reversibly impaired at all exposure levels; a LOAEL was identified at 2.2 mg/m^3.

Knapp (1982b) exposed male Sprague-Dawley CD rats (48/group) to Ordram vapors (98.2 percent purity) for 6 hours/day, 5 days/week for 13 weeks to evaluate the effects on male fertility. The cumulative mean exposure levels after 28 days were 0, 0.1, 0.83, 2.1, or 4.0 mg/m^3, and after 65 days were 0, 0.1, 0.59, 1.8, or 4.0 mg/m^3 (nominal exposure levels were 0, 0.1, 0.4, 1.2, or 4.8 mg/m^3). Male rats received food and water during inhalation exposure to Ordram. Only 24 males per group were used to measure mating/fertility parameters, and each was mated with two untreated females for up to 10 consecutive nights. The first mating was after 4 weeks of exposure (interim), the second was after 12 weeks of exposure (terminal), and the last was 7 weeks after the last exposure day (recovery; only 12 males/group). Males were removed from the exposure chambers during mating (~15 hours per night). Fertility parameters included fertility and mating indices (males), pregnancy index, number of corpora lutea, total implants, and viable implants, and the ratio of implants to corpora lutea (females).

There were no treatment-related effects of Ordram on mortality, food consumption, body weight, clinical observations, hematology, clinical chemistry, serum enzymes, serum hormones, or brain cholinesterase levels. Slight to moderately severe necrotizing rhinitis was found in all of the exposed males, but the animals showed some signs of recovery after Ordram exposure was discontinued. The only other microscopic finding was necrosis of spermatids and/or spermatocytes in all males (terminal sacrifice), but the degree of severity in the treated groups was significantly greater than in the controls. The incidence of spermatid/spermatocyte necrosis also was significantly increased in the recovery males in the 1.8 and 4.0 mg/m^3 groups.

After 4 weeks of inhalation exposure, fertility index and pregnancy index were significantly reduced in the 0.83, 2.1, and 5.0 mg/m^3 groups. Mean counts of total and viable implants in the cohabited females and ratios of implants to corpora lutea in the cohabited or gravid females were significantly reduced in females mated with males exposed to 2.1 or 5.0 mg/m^3. After 12 weeks of exposure, there were significant reductions in the number of corpora lutea and viable implants in cohabited females, and in total implants and ratio of implants to corpora lutea in the cohabited or gravid females.

Fertility indices of treated males were not different from controls. After the seven-week recovery period, there were some significant reductions in total implants and ratio of implants to corpora lutea in the 0.1, 1.8, and 4.0 mg/m^3 groups, but not in the 0.59 mg/m^3 groups, but there was no consistent dose-response relationship. The investigator attributed these effects to a skewed distribution of corpora lutea counts in the females, rather than to Ordram exposure. However, the recovery group only contained one-half of the number of rats that the other two groups contained, which makes it difficult to draw any conclusions in light of the significant changes in some of the treated groups compared to controls.

The NOEL for male fertility effects after 4 weeks of exposure was 0.10 mg/m^3 and after 12 weeks exposure was 1.8 mg/m^3, based on impaired fertility characterized chiefly as pre-implantation losses. The greater than 10-fold difference in NOELs between the two exposure periods was possibly due to the variation in exposure chamber concentrations that occurred during the first four weeks. Moreover, the actual exposure received by the treated rats may be inaccurate due to possible intake via food and water, or grooming, since the exposure was whole body instead of nose only.

Because of the discrepancy in the two NOELs between the two exposure periods, Knapp repeated the 4-week study exposing rats to Ordram (Knapp, 1982a). The changes made from the first study were use of 24 male rats/group and exposure levels of 0, 0.07, 0.16, 0.30, 0.64, or 1.6 mg/m^3 (nominal exposures of 0, 0.1, 0.2, 0.4, 0.8, or 1.6 mg/m^3) for 6 hours/day, 5 days/week for 20 days. In addition, treated males were mated with two untreated females for a maximum of 7 days, or until females showed positive signs of mating. The first 10 males from each group that had mated successfully were sacrificed for sperm analyses, including concentration, motility, morphology, and viability of sperm from the cauda epididymides.

There were no treatment-related effects of Ordram on mortality, clinical observations, body weight, or testes weights. Chromorhinorrhea was the most frequent observation, but it occurred in both control and treated animals. Statistically significant changes in sperm analyses included an increase in percent abnormal sperm (detached heads, broken midpiece regions) in the 0.64 and 1.6 mg/m^3 groups, a reduction in sperm motility in the 1.6 mg/m^3 group, and a reduction in the number of implants per female mated with males exposed to 1.6 mg/m^3 compared to controls. In the gravid female rats, the mean number of implants and implants per corpora lutea were significantly reduced in the 0.64 and 1.6 mg/m^3 groups, and the number of viable implants was significantly reduced in the 1.6 mg/m^3 group. The NOAEL for male fertility effects was 0.30 mg/m^3.

Jewell *et al*. (1998) showed that a single intraperitoneal injection of molinate (≥200 mg/kg) produced testicular toxicity in rats. The testicular lesion was characterized by Sertoli cell vacuolation, failed spermiation, and phagocytosis of spermatids, particularly evident at Stages X and XI. With increasing dose, the time was shortened when the lesion became evident (i.e., 1 week after 200 mg/kg and 48 hours after 400 mg/kg). The severity of the lesion also increased with time, and appeared irreversible at 400 mg/kg. Administration of molinate sulfoxide (200 mg/kg intraperitoneally) caused testicular damage similar in severity to that seen with 400 mg/kg molinate.

Molinate (40, 140 mg/kg-day), molinate sulfoxide (10, 20 mg/kg-day), 4-hydroxymolinate (10 mg/kg-day), or HMI (10 mg/kg-day) was administered continuously for seven days to male Sprague-Dawley rats via a subcutaneously implanted osmotic mini-pump (Ellis and Farnworth, 1999a; Ellis *et al*., 1998). Rats were sacrificed 28 days after implantation. Testes were weighed and examined for morphological changes and epididymal sperm samples were examined for abnormalities using light microscopy and scanning electron microscopy.

None of the treatments produced any changes in testicular weights. Testes from rats receiving molinate at 140 mg/kg-day showed tubular atrophy, but testes from all other treatment groups failed to show any morphological changes. Light microscopy revealed a significantly increased incidence of detached heads in sperm samples from animals administered molinate (26 percent and 36 percent for 40 and 140 mg/kg-day, respectively) or molinate sulfoxide (~25 percent for 10 and 20 mg/kg-day). Midpiece lesions and tail abnormalities also were increased in these animals. Scanning electron microscopy also revealed an increase in midpiece abnormalities, but the incidence was much lower than with light microscopy (0.5 percent for 140 mg/kg-day molinate and 0.63 percent for 20 mg/kg-day molinate sulfoxide). Neither 4-hydroxymolinate nor HMI induced any abnormal changes in sperm morphology.

Both plasma and testicular testosterone concentrations were markedly decreased in a dose- and time-dependent manner after oral administration of 50, 100, or 200 mg/kg molinate to adult male rats (Ellis *et al*., 1998; Ellis and Farnworth, 1999b). The greatest reduction occurred between 0 and 6 hours after dosing; after that testosterone concentrations gradually increased between 6 and 24 hours. A similar dose- and time-dependent reduction in plasma testosterone levels was observed after intraperitoneal injection of 40 mg/kg molinate or 20 mg/kg molinate sulfoxide and in testicular interstitial fluid testosterone levels after intraperitoneal injection of 20 mg/kg molinate sulfoxide.

In another study, male rats were administered molinate (40 mg/kg), molinate sulfoxide (1, 10, or 20 mg/kg), 4-hydroxymolinate (10 mg/kg), or HMI (5 mg/kg) via intraperitoneal injection to determine the effect on the concentration of plasma cholesterol and testicular interstitial fluid progesterone, 17α-hydroxyprogesterone, androstenedione, and testosterone (Ellis *et al*., 1998; Ellis and Farnworth, 1999a). Neither molinate nor any of the metabolites had any effect on plasma cholesterol levels. Testicular interstitial fluid levels of progesterone and 17α-hydroxyprogesterone were significantly lower in animals given molinate (40 mg/kg) or molinate sulfoxide (10 or 20 mg/kg) compared to controls. Androstenedione and testosterone levels were significantly reduced in animals given molinate (40 mg/kg) or molinate sulfoxide (1, 10, or 20 mg/kg) compared to controls. Neither 4-hydroxymolinate or HMI had any effect on hormone levels. These effects suggest that molinate or its sulfoxide may be causing a block in the biosynthesis of testosterone prior to progesterone.

The effect of molinate on rat testicular esterase activity and testosterone levels was examined by Lovatt (2000b). Molinate (0, 6, 12, or 25 mg/kg) was administered to male rats (six/group) by gavage six hours before sacrifice. Testosterone levels were measured

in blood and testicular interstitial fluid, and esterase activity was measured in testicular homogenates via the hydrolysis of *para*-nitrophenyl acetate. All three measurements were decreased in a dose-dependent manner. The values expressed as a percent of control activity were: 34, 10, and 9 percent for plasma testosterone; 69, 34, and 21 percent for testicular interstitial testosterone; and 10, 9, and 4 percent for testicular esterase activity, for animals given 6, 12, and 25 mg/kg, respectively. The investigators proposed that the inhibition of esterase activity prevented testicular tissues from acquiring cholesterol from high-density lipoprotein for testosterone synthesis. This proposed mechanism is discussed in more detail under the section *Proposed Mechanism of Reproductive Toxicity*.

Ordram (98.2 percent purity) was administered to male mice to determine if it had an effect on fertility (Killinger, 1980a). Twenty proven-breeder CD-1 males per group were gavaged with 0, 2, 20, 100, or 200 mg/kg-day Ordram in corn oil daily for seven weeks. Each male was mated to two females after the males were dosed for two, four, and six weeks. At the end of the treatment period, five males/group were sacrificed and necropsied. The remaining males were allowed to recover for four weeks, and then were again mated each with two females. All pregnant female mice were examined for number of implant sites, resorbing fetuses, viable fetuses, and corpora lutea.

There were no clinical observations in the treated males that could be related to Ordram exposure, nor did treatment have any effect on body weight on mating index. Statistically significant reductions in male and female fertility indices were observed with the males treated with 100 or 200 mg/kg-day, but these differences were not seen after the four-week recovery period. There was a statistically significant dose-related reduction in the number of uterine implants and viable fetuses in pregnant females mated to males treated with 100 or 200 mg/kg-day after four and six weeks. Some reductions also were seen after two weeks of treatment, but these differences were not dose-related. Again, there were no differences between control and treated animals after the four-week recovery period. Neither absolute or relative testes plus epididymides weights of the treated animals were different from the controls at the interim sacrifice (study day 71, after dosing on days 22-70), or the final sacrifice (study days 120-121).

The only histological finding in the male mice (interim and final sacrifices) was a mild testicular cell degeneration affecting single or small clusters of seminiferous tubules. This was observed in both the controls and treated mice, with no apparent dose-related trends. The NOAEL in male mice was 20 mg/kg-day, based on a decrease in the number of pregnancies (fertility index) and the number of implants and viable fetuses at 100 and 200 mg/kg-day.

Killinger (1980b) treated male rabbits with Ordram to determine if the pesticide had any affect on fertility. Dutch Belted rabbits (six to nine months of age) of proven fertility were dosed with 0 (corn oil), 2, 20, or 200 mg/kg-day of Ordram (98.2 percent purity) via oral capsule daily for six weeks. Each group contained nine rabbits, except for the control, which had ten rabbits. During the sixth week of dosing, each male was mated with the two females used in a pretreatment mating. At the end of the dosing period, five control and four males from each treated group were sacrificed for necropsy. The remaining males were allowed to recover for five weeks, and then were mated again to

examine fertility. Male and female rabbits were sacrificed after the post-recovery littering.

Clinical observations that were dose-related were limited to eye irritation, which increased in severity and duration with increasing dose. There were no differences between control and treated males with respect to mating index, male and female fertility indices, numbers of litters and pups/litter, pup weight, length of gestation, and organ weights that could be related to Ordram treatment, either during the treatment or post-treatment recovery periods. The only histopathological lesion observed was a dose-related increase in germinal giant cell formation of the testes. With the absence of any effect of Ordram on fertility, this lesion is not considered toxicologically significant. The NOAEL for reproductive effects in male rabbits was >200 mg/kg-day. A LOAEL for general toxicity was 2 mg/kg-day, based on eye irritation at all doses.

Three additional studies were performed to assess the effects of molinate on fertility of male rabbits. In the first two studies, male New Zealand White rabbits (10 per group), six to eight months of age, were dosed with either 0 (corn oil), 10, 100, or 200 mg/kg-day of molinate (99 percent purity) daily via gavage for up to 84 days (Tinston, 1991a,b).

In the first study (Tinston, 1991a), the rabbits given 200 mg/kg-day were dosed only for 16 days and the other groups only for 49 days, due to unexpected mortality at 100 and 200 mg/kg-day. Males were mated each with one untreated female prior to dosing and then at 4, 8, and 12 weeks of dosing (8 and 12 week matings for second study only). In the first study, 10 of 10 males in the 200 mg/kg-day group died or were killed for humane reasons by day 16, and 4 of 10 males in the 100 mg/kg-day group died or were killed by day 26. The number of pregnancies from the 100 and 200 mg/kg-day males prior to dosing with molinate (week –1) were low, suggesting that the males were immature. At week 4, there was no statistically significant difference in pregnancy rate between the control and treated groups. However, the number of implantations and live fetuses from the 100 mg/kg-day rabbits was lower than controls, due to a significant increase in pre-implantation loss. Fertility was not affected by treatment with 10 mg/kg-day molinate. Molinate treatment did not affect sperm motility or sperm count, but did cause a significant increase in sperm abnormalities, in particular mid-piece abnormalities, in the 100 mg/kg-day group, as observed with light microscopy. Interestingly, the same differences in sperm abnormalities were not observed with scanning election microscopy. The reason for this was unknown.

Because the results of this first study were considered inconclusive; it was repeated (Tinston, 1991b). Again, mortality was high at 100 and 200 mg/kg-day and the number of pregnancies in weeks –1, 4 and 8 were low (generally ≤60 percent), which precluded any meaningful interpretation of the data. By week 12, pregnancy rates had improved to ≥70 percent, which implied that the male rabbits again were immature at the beginning of the study. At week 12, the mean number of live fetuses per litter was significantly less at 200 mg/kg-day, compared to controls, and this was associated with higher pre- and post-implantation losses. As in the first study, there was a slightly higher incidence of total sperm abnormalities in the 100 and 200 mg/kg-day groups as seen by light microscopy,

but this effect was not dose-related. Again, the abnormalities were not confirmed with scanning electron microscopy.

The study was repeated a third time, with some changes in the study design (Tinston, 1992). Molinate (98.1 percent) was administered via gavage to male New Zealand White rabbits of proven fertility (15 per group) at doses of 0 (corn oil), 40, 80, or 160 mg/kg-day (reduced to 120 mg/kg-day after 5 weeks of treatment) daily for 13 weeks. Untreated females were inseminated with sperm from the treated males at weeks –7, 5, 9, and 13 of treatment. Fertility parameters included total sperm number and motility, sperm morphology by light and scanning electron microscopy, and in inseminated females, number of corpora lutea per ovary, live fetuses, and early and late intrauterine deaths.

Evidence from the previous studies (Tinston, 1991a,b) suggested that the mortality in rabbits followed periods of inappetance, which was probably due to gavage dosing. Therefore, food consumption was monitored in this study. Animals showing substantial reduction in food consumption were not dosed with molinate until consumption increased to ≥50 percent of control (or after week 5, ≥40 g). The maximum number of days on which any one rabbit was not dosed was 5 for controls, 16 for 40 mg/kg-day, 13 for 80 mg/kg-day, and 12 for 160/120 mg/kg-day. The investigators did not discuss what effect, if any, the inconsistent dosing may have had on the study results.

There were seven rabbit mortalities that were probably related to molinate treatment (n=1, 2, and 4 for the 40, 80, and 160 mg/kg-day groups, respectively). Clinical observations prior to death included severe diarrhea, subdued appearance, and reduced food consumption and body weights 1 to 2 days beforehand. There were no treatment related effects of molinate on body weight or food consumption. Red blood cell cholinesterase activity was significantly reduced in all treated animals at weeks 4, 8, and 12, but brain cholinesterase activity at week 14 was not affected. No consistent dose-response was observed, but the reduced activity indicates that molinate was absorbed.

Pregnancy rate and mean number of live fetuses were somewhat reduced and pre-implantation loss was increased in females mated with treated males compared to controls at weeks 5 and 9, but not week 13. Molinate had no effect on total sperm number or semen volume, but did produce a statistically significant reduction in motility of epididymal sperm from rabbits given 80 mg/kg-day. The only morphological change observed in sperm with scanning electron microscopy was a significant increase in the percentage of total abnormal sperm in 160/120 mg/kg-day group compared to controls at week 9 only. This was due to an increase in tail abnormalities. With electron microscopy, the incidence of total abnormal sperm, in particular atypically stained sperm heads, was significantly increased in the 160/120 mg/kg-day group at all time points. This effect was noted in the 80 mg/kg-day group, too, but the increase was statistically significant only at week 13. The inconsistency between findings from light microscopy and SEM could not be explained. There were no macroscopic findings or organ weight changes of the testes or epididymides. A NOAEL of 40 mg/kg-day was identified for sperm morphology, based on a dose- and time-related atypical staining of sperm heads under light microscopy. A NOAEL for transient pre-implantation loss could not be established.

Minor reproductive effects were observed in male dogs exposed to molinate during a chronic (one-year) gavage study (Pettersen and Wadsworth, 1990). Beagle dogs (four per group) were administered molinate (R-4572; 97.6 percent purity) in gelatin capsules at doses of 0, 1.0, 10, or 50 mg/kg-day for one year. A high dose group received 100 mg/kg-day, but administration of molinate was discontinued after 14 weeks due to severe toxicity. These animals continued in the study as a recovery group (0 mg/kg-day for the remaining 38 weeks). Sperm analyses were completed at 6 and 12 months, but not at 3 months when any effects of the highest molinate dose would have been more apparent. Measured parameters included sample volume, percent motile, cell count (millions) per ml and per sample, percent abnormal and percent live.

In the 50 mg/kg-day group compared to controls, sperm analysis at 12 months, but not at 6 months, showed a statistically significant reduction in ejaculate sample volume and percent motile sperm, and an increase in percent abnormal sperm that was not statistically significant. The mean values of all of the sperm parameters in the 100 mg-kg-day recovery group compared to the control group indicated that molinate may have had some adverse effect at 6 months, since these values had returned to control levels at 12 months. However, considerable inter- and intra- animal variability was observed between the 6- and 12-month time points, and without measurements at 3 months, it is impossible to determine specific trends in recovery. Histopathological evaluation revealed minimal to slight epithelial cystic degeneration of the epididymides (control, 1/4; 1 mg/kg, 1/4; 10 mg/kg, 2/4; 50 mg/kg, 3/4; 100 mg/kg-recovery, 1/4), but this was deemed incidental in nature and unrelated to molinate exposure by the study's pathologist. The NOAEL for reproductive toxicity was identified as 10 mg/mg-day, based on changes in sperm parameters at 50 mg/kg-day. [For more details on systemic effects, see the section on Chronic Toxicity.]

Killinger (1981) administered Ordram (98.6 percent purity) in corn oil via nasal gastric tube at dose levels of 0, 0.2, 10, or 50 mg-kg-day for five days per week for 12 weeks to twenty-eight adult male cynomolgus monkeys (seven per group). The study objective was to determine if Ordram had any effect on sperm production and reproductive hormone levels.

Ordram treatment did not have an effect on body weight, the incidence of clinical observations, temperature, pulse, respiration rate, or pupillary measurements. The only statistically significant change in clinical chemistry measurements was a reduction in mean plasma cholinesterase activity (34 to 43 percent of control) in the 50 mg/kg-day group at treatment weeks 4, 8 and 12. This reduction occurred with an increase in the treatment time. RBC cholinesterase activity in treated animals was not different from controls. Additionally, there were no statistically significant changes in hematological parameters, including clotting time, or in reproductive hormone levels (serum FSH, LH, or testosterone) in monkeys treated with Ordram compared to controls. Statistically significant changes in sperm parameters only were observed in the 0.2 mg/kg-day group; they included a reduction in percent motile sperm at treatment week 9, an increase in percent abnormal sperm at treatment week 2, and a reduction in sperm cell concentration (10^6 cells/ml) at treatment weeks 1, 2, and 9. The changes in sperm parameters were not dose-related, and they were not different from pretreatment values. However, the great

variability in the measured parameters limits the usefulness of the data. Nevertheless, these effects were considered to be a characteristic of the monkeys assigned to this treatment group. A NOEL for reproductive toxicity was >50 mg/kg-day. For systemic toxicity, the NOAEL was 10 mg/kg-day, based on the reduction in plasma cholinesterase activity at 50 mg/kg-day.

Zuhlke and Bee (1991) exposed male cynomolgus monkeys (ten per group) via gavage to 0 (corn oil), 0.2, 10, or 50 mg/kg-day molinate (purity not specified) for 12 weeks. No changes in sperm morphology were identified with either light or electron microscopy, nor were any adverse effects indicated. The study was of limited value for evaluating the possible effects of molinate on sperm morphology due to great variability in the measured parameters. In addition, the study did not attempt to measure the potential adverse effects on fertility.

Studies Exposing Females Only

Gilles and Richter (1989) performed a two-generation study to determine the reproductive toxicity of molinate to female rats. R-4572 (97.6 percent purity) was administered in the diet to 52-day old CD female rats (25 per group) at dietary concentrations of 0, 6, 50, or 450 ppm. After 60 days of treatment, the females (P_0 generation) were mated with untreated proven males to produce the F_1 offspring. The F_1 pups were weaned at 21 days and 25 females/dietary group were selected to continue as the P_1 generation. The P_1 females were on dietary treatment until approximately 108 days of age, at which time they were mated with fresh, untreated proven males to produce the F_2 offspring. Both the P_0 and P_1 dams were necropsied between 201-207 days of age, after their pups had been weaned. The preweaning pups (F_1, F_2) that were culled on day 4, the F_1 weanling pups not selected for the P_1 generation, and the F_2 weanling pups were sacrificed and subjected to head and soft tissue internal examinations.

There were no deaths of the parental females or any increases in clinical signs that could be attributed to molinate treatment. Mean body weights and food consumption were significantly reduced in P_0 and P_1 females fed 450 ppm molinate compared to controls prior to mating, during gestation, and during lactation. Mean body weights also were significantly reduced during gestation and lactation in the P_1 females only fed 50 ppm. Body weight gains were reduced at 450 ppm, but statistically significant changes were sporadic. Organ weight changes included a significant reduction in mean absolute liver, spleen, brain, and heart weights in P_0 and P_1 females, kidney weights in P_1 females fed 450 ppm, and brain weights in P_1 females fed 50 ppm. Mean relative weights for these same organs were significantly greater in the P_0 and P_1 females treated with 450 ppm, compared to controls; therefore, the changes in absolute organ weights probably were due to the reduction in body weights, rather than an effect of molinate treatment. Absolute and relative adrenal weights were significantly increased with 450 ppm molinate in both the P_0 and P_1 females.

The only histopathological lesion observed was a statistically significant increase in the incidence of vacuolation/hypertrophy of ovarian thecal/interstitial cells in both P_0 and P_1

females fed 450 ppm molinate. This lesion was slightly increased in 50 ppm females as well, but the incidence was not statistically significant.

With respect to reproductive indices, the mean number of uterine implants was significantly reduced in P_0 and P_1 females fed 450 ppm molinate only. In addition, a slight but not statistically significant reduction in the fecundity, fertility, and gestation indices occurred at 450 ppm in the P_1 but not P_0 females. Mean litter size (number of live pups and total born) was significantly reduced on days 0 and 4 post partum at 450 ppm for both F_1 and F_2 litters. Litters were culled on day 4, so litter size was the same in control animals compared to the 450 ppm group after this point. Mean pup weights by litter (F_1 and F_2) also were significantly reduced at 450 ppm compared to controls on post partum days 7, 14, and 21. Molinate treatment had no effect on pup survival indices, nor were there any remarkable necropsy or macroscopic findings in either F_1 or F_2 pups that could be attributed to molinate exposure.

The NOAEL for general or systemic toxicity was 6 ppm (at least 0.44 mg/kg-day of molinate), based on body weight reductions in P_0 and P_1 females during gestation and lactation. The NOAEL for reproductive toxicity was 50 ppm (at least 3.7 mg/kg-day) based on histopathological findings on the ovaries, and a reduction in uterine implants and mean litter size of both P_0 and P_1 females. The NOAEL for developmental toxicity was 50 ppm, based on significant reductions in pup weights at 450 ppm.

Horner (1992a) examined the potential ovarian changes in pregnant female rats exposed to a short-term high-dose molinate regime. Molinate (98.1 percent purity) was administered in corn oil by gavage at doses of 0, 75, 135, or 200 mg/kg-day to mated Crl:CD (SD) BR females rats (ten per group) on gestation days 7 to 9. Fourteen rats died as a result of molinate exposure: five rats in the 135 mg/kg-day group were killed on day 10 and five rats in the 200 mg/kg-day group were killed between days 8 to 10 for humane reasons, and four rats in the 200 mg/kg-day group were found dead on day 10.

Clinical signs observed prior to death included piloerection, signs of urinary incontinence, pinched-in sides, subdued behavior, hunched posture, salivation, abnormal gait, and perinasal and perioral staining. There were no macroscopic findings in these animals that could be attributed to molinate treatment. Clinical signs in surviving animals included head held twisted to one side (135, 200 mg/kg-day), rolling gait (135 mg-kg-day), and eye discharge (75, 135, 200 mg/kg-day). Body weights and body weight gains were significantly less in the 135 and 200 mg/kg-day groups when compared to controls on days 8 to 10. Although body weights and weight gains were less in the 75 mg/kg-day group compared to controls, the differences were only sporadically significant.

There were no consistent treatment-related trends in clinical chemistry parameters, i.e., serum progesterone levels. Adrenal weights were significantly greater in all treatment groups compared to controls, and ovarian weights were slightly greater in the 200 mg/kg-day group, but the difference was not statistically significant. No consistent treatment-related effects of molinate were observed on the number of corpora lutea, uterine implantation sites, or percentage pre-implantation loss. Histopathological changes included a dose-related increase in cellular swelling and vacuolation in the zona fasciculata and zona reticularis and degeneration of the zona fasciculata of the adrenal

cortex (135, 200 mg/kg-day), fatty accumulation of ovarian corpora luteal cells (75, 135, 200 mg/kg-day), and slight liver congestion (200 mg/kg-day). Lipid analysis indicated that no dose-related changes were apparent in cholesterol or cholesterol esters. However, a dose-related increase in neutral lipids (free fatty acids and glycerides) was noted in adrenal extracts but not ovary extracts. The morphological changes indicate a functional disturbance in adrenocortical and corpora luteal cells, which would be consistent with a perturbation of steroid hormone synthesis by both tissues (Horner, 1992a). A LOAEL of 75 mg/kg-day is indicated, based on dose-related changes in body weight, adrenal weights, and ovarian histopathology.

The effect of molinate on rat ovarian esterase activity was examined by Lovatt (2000a). Molinate (0, 10, 40, 100, or 150 mg/kg) was administered to 9- to 12-week old female SD rats (three/group) by gavage daily for seven days, and the animals were sacrificed 24 hours after the last dose. In addition, single-dose groups received either 0 or 40 mg/kg molinate by gavage six hours before sacrifice. Esterase activity, indicated by hydrolysis of *para*-nitrophenyl acetate, was measured in ovarian homogenates.

All doses of molinate caused a decrease in esterase activity compared to the control group. Activity expressed as a percent of control was: 51, 38, 45, and 25 percent for the 10, 40, 100, and 150 mg/kg groups, respectively. In the 40 mg/kg single-dose group, esterase activity was 50 percent of control activity. The investigators proposed that the inhibition of esterase activity prevented ovarian tissues from acquiring cholesterol from high-density lipoprotein for reproductive hormone synthesis. However, they did not measure either estrogen or progesterone levels to show that the decrease in esterase activity is causally linked to a decrease in female sex hormone production.

Proposed Mechanism of Reproductive Toxicity

Several investigators (Foster and Ellis, 1998; Ellis and Farnworth, 1999c; Foster, 1999; Wickramaratne, 1997a; Wickramaratne *et al*., 1998) have proposed that rats are the species most sensitive to the reproductive toxicity observed after molinate exposure, because the proposed mechanism by which the primary lesion, disruption of the plasma membrane in the midpiece region of maturing sperm, occurs is unlikely to happen in non-rodent mammalian species. Briefly, it is hypothesized that the sulfoxide metabolite of molinate binds to an esterase in testicular Leydig cells that is responsible for the hydrolysis of cholesteryl esters to cholesterol. Cholesterol is the precursor lipid from which steroid hormones, such as testosterone and progesterone, are produced. In male rats, inhibition of cholesteryl ester hydrolysis causes a reduction in cholesterol levels, and thus, a reduction in testosterone biosynthesis. The reduction in testosterone levels occurs at a critical period in the maturation of sperm, resulting in the characteristic sperm lesion that is observed in rats.

Although the reproductive toxicity observed in female rats after molinate exposure is not as specific as male rats, the ovarian effects (see Reproductive Toxicity section) are hypothesized to be produced by a similar mechanism (decreased steroidogenesis). These investigators contend that rats are more sensitive to gonadal damage from molinate exposure because the source of cholesterol for steroidogenesis is different than in other

mammals. In rodents, high density lipoproteins (HDLs) are the principal source of cholesterol, whereas in other mammals (e.g., humans, monkeys, dogs, rabbits), low density lipoproteins (LDLs) are the primary source. Cholesterol is released from the LDLs via lysosomal acidic hydrolases, an enzyme system different from the neutral cholesteryl ester hydrolase (nCEH) that releases cholesterol from HDLs. The LDL route of cholesterol derivation is via acetyl CoA, which is not inhibited by molinate or its metabolites. The specificity of molinate for blocking the HDL pathway suggests that the gonads of humans and other non-rodent mammals will not be affected by molinate exposure. Evidence to support or refute this proposed mechanism is provided below.

Molinate sulfoxidation produces a reactive electrophilic intermediate that is capable of binding to proteins, such as chick embryo proteins (Faiman *et al*., 1991), and inhibiting various enzymes, such as aldehyde dehydrogenase (Hart and Faiman, 1995; Quistad *et al*., 1994), carboxylesterase (Jin and Kitos, 1996), neuropathy target esterase (Horner, 1994b), and acetylcholinesterase (Horner, 1994a, b; Biodynamics, 1979; Pettersen and Richter, 1990). A highly reactive intermediate such as molinate sulfoxide would have to be produced in the testes, because the same metabolite produced in the liver would likely bind to circulating proteins or tissue enzymes prior to reaching the testes and be unavailable to react with testicular sites. Evidence suggests that the metabolic oxidation of molinate to form molinate sulfoxide does occur in the rat testes. Jewell *et al*. (1998) found molinate sulfoxide to be the primary metabolite of molinate when incubated with rat liver or testes microsomes, with testes microsomes producing only slightly less molinate sulfoxide than liver microsomes. A minor metabolite was hydroxymolinate, with testes microsomes producing substantially less than liver microsomes.

The enzyme which molinate sulfoxide is thought to inhibit is an esterase. Nonspecific esterase activity (NSE), using *para*-nitrophenyl acetate as the substrate, was found to be decreased in rat testicular and ovarian homogenates (Lovatt, 2000a, b) and rat Leydig cell suspensions (Ellis *et al*., 1998) after the animals were exposed to various doses of molinate. Ellis *et al*. (1998) showed that a substantial reduction in esterase activity in the Leydig cells only occurred at those dose levels that caused morphological damage within the seminiferous tubule (100 and 150 mg/kg for 10 days). Using alpha naphthyl acetate as the substrate, Winder *et al*. (1999) found that liver and testes NSE activity remained inhibited as long as 7 days after male rats were exposed to a single intraperitoneal injection of 100 or 200 mg/kg molinate (Winder *et al*., 1999). NSE activity also was inhibited at doses of 5 or 50 mg/kg, but recovery was complete by 2 days. Concentrations of molinate, molinate sulfoxide, and molinate sulfone that gave 50 percent inhibition (IC_{50}) of microsomal NSE activity in vitro were 150 μM, 2 μM and 40 μM for liver and 200 μM, 2 μM, and 10 μM for testes, respectively (Jewell and Miller, 1998). IC_{50} values for in vitro inhibition of esterase activity in rat Leydig cell suspensions were >4 μM for molinate, 2.5×10^{-3} μM for molinate sulfoxide, and 2.5×10^{-5} μM for molinate sulfone (Ellis *et al*., 1998). These studies show that molinate and its sulfoxide or sulfone metabolites are capable of inhibiting NSE activity that is found in the rat ovary and testis.

The evidence demonstrating that molinate sulfoxide inhibits the specific enzyme that converts cholesteryl esters to cholesterol is more limited. Yan *et al*. (1995) (cited in Jewell and Miller, 1998) showed that testicular NSE activity was largely due to a single

carboxylesterase identified as Hydrolase A and that this protein was localized in the Leydig cell. Jewell and Miller (1998) tentatively identified the protein present in both rat liver and testis to which molinate, molinate sulfoxide, and molinate sulfone were bound as Hydrolase A, a carboxylesterase. This enzyme has also been called neutral cholesterol ester hydrolase (nCEH) (Foster, 1999).

Winder *et al*. (1999) found that the hydrolysis of cholesteryl oleate, a cholesterol ester, was decreased in rat liver and testes microsomal preparations after intraperitoneal molinate administration. These studies suggest that molinate may inhibit the release of cholesterol from cholesteryl esters. Even so, the studies above have not demonstrated that Hydrolase A is the same enzyme that converts cholesteryl esters to cholesterol, that nCEH is bound by and inhibited by molinate, or that Hydrolase A and nCEH are the same enzyme. Furthermore, data are not available to show the effect (or lack thereof) of molinate on lysosomal acidic hydrolases.

While some investigators have reported a reduction in blood or testicular testosterone levels in rats after molinate exposure (Ellis *et al*., 1998; Lovatt, 2000b), others have reported increased levels or no changes after molinate exposure, even though sperm abnormalities were present (Minor, 1981; Knapp, 1982b). In female rats exposed to molinate (75 to 200 mg/kg-day for 3 days), there were no dose-related changes in cholesterol or cholesterol esters, or no consistent treatment-related trends in serum progesterone levels, even though there were dose-related changes in ovarian corpora luteal cell histopathology (Horner, 1992a). Furthermore, Lovatt (2000a) reported that female rats exposed to molinate (10 to 150 mg/kg-day for 7 days) demonstrated a dose-related decrease in NSE activity.

If inhibition of esterase activity prevents reproductive tissues from acquiring cholesterol from HDLs to synthesize sex steroids, then Horner (1992a) should have seen a decrease in serum progesterone levels in the female rats, since they were exposed to similar molinate doses as the rats in the Lovatt (2000a) study. This was not the case. Furthermore, Winder *et al*. (2001) reported that the IC_{50} for NSE activity (using *para*-nitrophenyl acetate as the substrate) by molinate sulfoxide in cultured Leydig cells was 1.9 nM, whereas the IC_{50} for inhibition of testosterone production was 60 µM. These results suggest that the inhibition of esterase activity and the inhibition of steroidogenesis may not be directly related. In addition, a direct link between reductions in testosterone or progesterone levels and the reproductive toxicity observed in male or female rats, respectively, has not been demonstrated.

With respect to the argument that LDLs are the primary source of cholesterol in non-rodent species, some in vitro studies indicate that human granulosa cells from ovarian follicles actively use HDL cholesterol for steroidogenesis (Azhar *et al*., 1998; Parinaud *et al*., 1987). Therefore, the claim that humans and other non-rodent mammals rely on LDL as the cholesterol source for steroidogenesis may not hold true.

Another proposed mechanism for a rat-specific effect is that molinate is metabolized via different pathways in rats and humans. This does not appear to be a relevant mechanism, since data show that both rats and humans produce molinate sulfoxide, and that the production is dependent on the dose of molinate received (see Metabolism and

Pharmacokinetics section). In any case, any metabolic differences do not eliminate the possibility of an effect in humans and there is not enough information to quantitatively estimate the difference in effective dose between rodents and humans.

A recent study from Winder *et al*. (2001) suggests a novel mechanism by which molinate exposure could impair testicular function in rats. The investigators found that molinate sulfoxide, but not molinate, inhibited the production of retinoic acid in rat liver and testis S9 fractions via the inhibition of retinal dehydrogenation. Retinoic acid, which is required for spermatogenesis, is produced in the liver by the action of aldehyde dehydrogenase on retinal, which in turn can be derived from the hydrolysis of retinyl esters by an esterase. Others have reported that molinate inhibits aldehyde dehydrogenase activity (Quistad *et al*., 1994; Hart and Faiman, 1995). In fact, Hoover *et al*. (1991) reported that disulfiram, an inhibitor of aldehyde dehydrogenase used to treat alcohol abuse, caused a decrease in testes weight and moderate to severe reduction in the number of spermatids and spermatocytes in young male rats exposed to 10, 30, or 100 mg/kg on postpartum days 6 through 36.

The Winder *et al*. (2001) results suggest that molinate may have more than one mechanism by which it induces reproductive toxicity. This offers an explanation why some effects on sperm parameters were evident in rabbits and dogs, albeit not to the same extent as in rats; whereas rats appear to be the only species demonstrating the characteristic sperm lesion. Until the mechanism of reproductive toxicity is elucidated, we will assume that molinate has the potential to cause reproductive toxicity in humans. This endpoint will be taken into consideration when calculating the proposed PHG.

Other Reproductive Effects

Molinate induced a dose-related, but not statistically significant increase in interstitial cell tumors in male rats exposed to 7, 40, or 300 ppm in the diet for two years (Pettersen and Richter, 1990). In addition, a significant positive trend was observed in the incidence of testicular mesotheliomas. This study is described in more detail in the Carcinogenicity section.

Chronic Toxicity

Three studies were available for evaluating the chronic oral toxicity of molinate -- a two-year rat study, an 18-month mouse study, and a one-year dog study. Several adverse effects frequently were observed in the three animal species, including neuropathological changes, organ and body weight changes, and testicular and ovarian effects. Details of the studies are provided below. Two other chronic studies using rats and mice, respectively (Woodard, 1977a, b), provided few useful data to evaluate the chronic toxicity of molinate. Nevertheless, a summary of each also is provided below.

In a combined chronic toxicity/oncogenicity study, molinate (R-4572; 97.6 percent purity) was administered in the diet to Crl:CD(SD)BR rats (60 or 70 animals/sex/group) at concentrations of 0, 7, 40, or 300 ppm for 104 weeks (Pettersen and Richter, 1990). A satellite group of 20 rats/sex (for examining non-neoplastic pathology) was administered

600 ppm dietary molinate for 52 weeks. Corresponding doses of molinate were 0, 0.3, 1.8, 13, and 29 mg/kg-day for male rats and 0, 0.4, 2.0, 15, and 35 mg/kg-day for female rats. Twenty rats/sex from the 0 and 600 ppm groups, and ten rats/sex from the 7, 40, and 300 ppm groups were sacrificed at 12 months for interim evaluation.

Body weights and body weight gains of male and female rats receiving 300 and 600 ppm molinate were significantly less than controls throughout most of the study. Food consumption was significantly depressed in male and female rats fed 300 and 600 ppm during much of the first year, but only sporadically at 300 ppm during the second year.

Clinical signs indicative of neurological impairment (hindlimb ataxia, adduction and atrophy, atrophied thigh and sacral region) were observed in the 300 ppm males and females starting at 19 months. Small but statistically significant treatment-related decreases in mean corpuscular volume and mean corpuscular hemoglobin were noted in male and female rats mostly during the first year, but the differences were not considered to be biologically relevant. RBC, but not serum or brain, cholinesterase activity was significantly depressed in male rats fed 40 ppm (at 12 months only), 300 ppm (at 6, 12, and 24 months), or 600 ppm molinate (at 6 and 12 months). RBC cholinesterase activity was significantly decreased in female rats fed 300 ppm (6 and 18 months) or 600 ppm (6 and 12 months), and brain cholinesterase activity was significantly less than controls in female rats fed 300 or 600 ppm molinate (at 12 months).

Statistically significant reductions in absolute brain, kidney, and liver weights of male and female rats fed 300 and 600 ppm molinate were considered to be related to body weight changes. Relative adrenal weights were significantly increased in males and females at 600 ppm (12 months) and males only at 300 ppm (24 months). Absolute testes weights were significantly decreased at 600 ppm (12 months) and relative ovarian weights were significantly increased at 300 (24 months) and 600 ppm (12 months).

Neoplastic findings are described under the section *Carcinogenicity*. Statistically significant non-neoplastic histopathological findings included atrophy and reserve cell hyperplasia of the gluteus muscle and thigh skeletal muscle, degeneration/demyelination of the sciatic nerve, and active chronic inflammation of the sacral region of the spinal cord in male rats given 7, 40, and 300 ppm molinate. Degeneration of and presence of eosinophilic bodies (which represent swollen, degenerated axons) in the sacral region of the spinal cord and the incidence of oligospermia were significantly increased in males at 300 ppm only, compared to controls. For female rats, sciatic nerve degeneration/demyelination and atrophy and reserve cell hyperplasia of the thigh skeletal muscle were significantly elevated at all exposure levels, the presence of ovarian cysts was statistically greater with 300 ppm molinate, and ovarian thecal interstitial cell vacuolation/hypertrophy was greater at 40 and 300 ppm. A NO(A)EL was not determined. A LOAEL of 7 ppm (males, 0.3 mg/kg-day; females; 0.4 mg/kg-day) was identified, based on the presence of adverse neurological effects in male and female rats at all exposure levels.

In another chronic study, Ordram (98.8 percent purity) was administered in diets of Fischer rats (60 animals/sex/group) initially at doses of 0, 8, 16, or 32 mg/kg-day (Woodard, 1977b). At week 18, the doses were reduced to 0, 0.63, 2.0, or 6.32 mg/kg-

day and remained at this level to the end of the study at 104 weeks. According to the author, the doses were reduced because a decrease in weight gain was evident. However, after 16 to 18 weeks on the original diet, the body weights in the high dose (32 mg/kg-day) were depressed less than 10 percent. At week 78, body weights of males and females administered 6.32 mg/kg-day averaged 93 percent of controls, and at week 104, they averaged 97 percent of controls for males and 101 percent of controls for females. These results indicate that the maximum tolerated dose was not reached; thus, the dose levels may have been too low to identify any adverse effects.

The author reported that there were no differences between control and treated groups with respect to general appearance and behavior, food efficiency, survival, clinical chemistry, hematological parameters, interim organ weights (at 56 weeks and 78 weeks), or frequency of neoplastic or non-neoplastic lesions. Testicular interstitial cell tumors were observed in control and treated animals during the two interim sacrifices (56 and 78 weeks) and the terminal sacrifice; their occurrence was not treatment- or dose-related. A NOEL could not be determined due to poor study quality.

Potrepka and Morrissey (1991) administered molinate (R-4572; 97.6 percent purity) in the diet to Crl:CD-1(ICR)BR mice (50 animals/sex/group) at concentrations of 0, 10, 100, 1,000, or 2,000 ppm for 18 months. Corresponding doses of molinate were 0, 1.0, 10.4, 105, or 200 mg/kg-day for male mice and 0, 1.3, 13.9, 133, or 249 mg/kg-day for female mice.

Clinical observations included hindlimb muscle weakness, adducted hindlimbs, and ataxia in males and females fed 2,000 ppm molinate, and atrophied hindlimb muscles and splayed hindlimbs only in females fed 2,000 ppm molinate. In general, these signs occurred more frequently in females than in males and mostly on or after the 70^{th} week of treatment. Red blood cell count, hemoglobin and hematocrit were significantly decreased in a dose-dependent manner in both males and females fed 1,000 or 2,000 ppm molinate.

Male and female body weights, body weight gains, and food consumption were significantly reduced at 2,000 ppm compared to controls. Females fed 1,000 ppm also had significant reductions in body weights and body weight gains, and males fed 1,000 ppm had sporadic but statistically significant reductions in body weight gains during the first three months. Brain and liver weights, relative to body weight, were significantly increased in male and female mice at 2,000 ppm. Absolute testicular weights were significantly reduced in males and absolute and relative adrenal weights were significantly increased in females at 2,000 ppm compared to controls. Statistically significant histopathological findings of treated animals compared to controls at 18 months included: ceroid/lipofuscin degeneration of the adrenal gland (male and female mice, 1,000 and 2,000 ppm); mineralization of the adrenal gland (females, 1,000 and 2,000 ppm); eosinophilic bodies (red-staining spherical structures interpreted to represent degenerated axons) in the medulla of the brain (males and females, 1,000 and 2,000 ppm); centrilobular hypertrophy of the liver (males, 2,000 ppm); clara cell hyperplasia of the lungs (males and females, 2,000 ppm); sciatic nerve demyelination and Schwann cell hyperplasia (males and females, 1,000 and 2,000 ppm); eosinophilic bodies in the sacral and thoracic regions of the spinal cord (males, 2,000 ppm; females, 1,000 and 2,000 ppm); eosinophilic bodies in the lumbar region of the spinal cord (females,

1,000 and 2,000 ppm); testicular degeneration (males, 100, 1,000, and 2,000 ppm); mammary gland atrophy (females, 2,000 ppm); hyperplasia of ovarian thecal/interstitial cells (1,000 and 2,000 ppm); and uterine atrophy (females, 2,000 ppm). A NOAEL of 10 ppm, or 1.0 mg/kg-day, was identified, based on testicular degeneration in male mice administered 100, 1,000, or 2,000 ppm molinate in the diet.

Ordram (purity not specified) was administered in diets of CAF_1 hybrid mice (20 mice/sex/group) at dose levels of 0, 3.6, 7.2, or 14.4 mg/kg-day (Woodard, 1977a). There were no interim sacrifices because an insufficient number of animals would have been exposed for a lifetime had the interim sacrifice taken place. Food consumption, clinical chemistry, hematological, and urine sample data were not collected. Survival at week 99 was only 50 percent of males (10/20) and 40 percent of females (8/20, including 3 mice that escaped but could not be found) in the 3.6 mg/kg-day group, so the study was terminated at this point.

Control animals did not differ from treated animals with respect to appearance and behavior and body weights. There was considerable variability in organ weights, but treatment-related changes were not apparent. Insufficient numbers of tissue samples were available for necropsy/histopathology due to loss of animals from early deaths or escape, or from advanced autolysis of tissues. According to the author, there were no neoplastic or non-neoplastic changes that could be related to Ordram treatment. Because of the poor study quality, a NOEL could not be determined.

Beagle dogs (four animals/sex/group) were administered molinate (R-4572; 97.6 percent purity) in gelatin capsules at doses of 0, 1.0, 10, or 50 mg/kg-day for one year (Pettersen and Wadsworth, 1990). A high dose group received 100 mg/kg-day, but administration of molinate was discontinued after 14 weeks due to severe toxicity. These animals (four dogs/sex) continued in the study as a recovery group (0 mg/kg-day for the remaining 38 weeks).

There were no unscheduled deaths in any of the dose groups. Clinical observations included ataxia, cyanosis, dyspnea, general pallor, reduced locomotor activity, splayed hindlimbs, and tremors in the 50 and 100 mg/kg-day dose groups. Many of these clinical signs were first observed within the first two weeks of the study. Several dogs had attenuated bark or inability to bark.

Mean body weights were lower than controls at all doses, but the differences were statistically significant only at 50 and 100 mg/kg-day. Body weight gains were significantly lower at 50 and 100 mg/kg-day for males and 100 mg/kg-day for females. In the 100 mg/kg-day group, body weights and weight gains of males were significantly less than controls even during the recovery period (weeks 14-53). There was no significant difference in food consumption between control and treated animals.

Sperm analysis at 12 months, but not at 6 months, showed statistically significant reductions in ejaculate sample volume and percent motile sperm in the 50 mg/kg-day group compared to controls. Abnormal neurological findings, such as depressed postural reactions and reduced muscle tone, increased in severity with dose (50 and 100 mg/kg-day), and males responded more severely than females. At 12 months, these findings were not completely reversible in the 100 mg/kg-day recovery group, but females were

more likely to have a normal reaction than males. Mild anemia, as indicated by decreases in erythrocyte count, hemoglobin concentration, and hematocrit, was observed at 3 months in males given 10, 50, or 100 mg/kg-day and in females given 50 or 100 mg/kg-day; at 6 months in males given 10 or 50 mg/kg-day and in females given 50 mg/kg-day. At 12 months, these parameters were still slightly depressed in males and females given 50 mg/kg-day, but had returned to control values in the 100 mg/kg-day recovery group. Increases in platelet levels were statistically significant at 6 and 12 months for males and females given 50 mg/kg-day.

There were no treatment-related effects on RBC or brain cholinesterase activities. However, serum cholinesterase activity was depressed in dogs of both sexes in the 50 mg/kg-day (20 to 27 percent decrease), but the difference between control and treated animals was statistically significant only for females. In the 100 mg/kg-day group dogs, serum cholinesterase activity was decreased only at the 3-month time point (statistically significant decrease for females); activity had recovered to control levels by 6 months. Other changes in clinical chemistry include a statistically significant increase in serum cholesterol (males and females 50 mg/kg-day; males only, 100 mg/kg-day-recovery), a significant increase in alkaline phosphatase activity (males only, 50 mg/kg-day), and a significant decrease in serum creatinine levels (females, 10 mg/kg-day at 12 months, 50 mg/kg-day and 100 mg/kg-day-recovery at 3 and 12 months).

Significant changes in organ weight relative to body weight for female dogs were observed for liver (10 and 50 mg/kg-day, and 100 mg/kg-day-recovery), adrenals (50 mg/kg-day), and kidneys (1, 10, and 50 mg/kg-day). For male dogs, relative weights for liver and adrenals were significantly increased at the 50 mg/kg-day dose level.

Histopathological findings included degenerative changes in the brain, spinal cord, and peripheral nerve, increased hemosiderin (iron) deposition in the liver and spleen, extramedullary hematopoiesis in the spleen, and increased lipofuscin pigment in the kidney. A statistically significant positive trend was found for many effects, especially spinal cord and peripheral nerve demyelination, in dogs of both sexes. The lowest dose tested, 1 mg/kg-day, is considered to be the LOAEL, based on a dose-related incidence of minimal to slight demyelination of the cervical, lumbar, and thoracic regions of the spinal cord and the sciatic nerve. However, only the combined male/female incidence of demyelination in the thoracic region of the spinal cord, 0/8, 5/8, 4/8, and 8/8 for 0, 1, 10, and 50 mg/kg-day, respectively, was statistically significant at the low dose level ($p<0.05$ using Fisher's exact test).

The small number of dogs used in the study (four per sex, eight total per dose) limits the power to detect statistical significance. Nevertheless, similar neuropathologic injury was found in rats and mice; therefore, the minimal to slight grade of injury is considered to be biologically relevant, even though most of the responses at the 1 mg/kg-day dose level did not reach statistical significance.

Carcinogenicity

In a two-year combined chronic toxicity/oncogenicity study, R-4572 (Ordram®; 97.6 percent purity) was administered in the diet to Crl:CD(SD)BR rats (60 or 70 animals/sex/group) at concentrations of 0, 7, 40, or 300 ppm for 104 weeks (Pettersen and Richter, 1990). A satellite toxicity group of 20 animals/sex was administered 600 ppm dietary R-4572 for 52 weeks. Corresponding doses of R-4572 were 0, 0.3, 1.8, 13, and 29 mg/kg-day for male rats and 0, 0.4, 2.0, 15, and 35 mg/kg-day for female rats. Twenty rats/sex from the 0 and 600 ppm groups, and ten rats/sex from the 7, 40, and 300 ppm groups were sacrificed at 12 months for interim evaluation. Survival was not affected by treatment with molinate, except for some improvement in the 300 ppm group. Body weight gains were decreased about 15 percent in the 300 ppm males.

Tumor incidences are presented in Table 4. Statistical analyses of the data were performed using Tox_Risk (Version 3.5). Among male rats, a significant positive trend ($p<0.05$) was observed for kidney cortical adenomas, kidney carcinomas, combined kidney tumors, and testicular mesotheliomas. Only the incidence of combined kidney cortical adenomas and carcinomas (counting animals with either adenomas or carcinomas) was significantly increased ($p<0.05$ using Fisher's exact test) and only at the high dose (300 ppm, or 13 mg/kg-day). The incidence of testicular interstitial cell tumors in male rats, while increasing with dose, showed neither a statistically positive trend (Mantel-Haenszel test) nor increased incidence at any dose relative to controls (by Fisher's exact test). There were no significant increases in tumor incidences in female rats dosed with molinate compared to control animals.

A ten-day oral dosing study using rats showed that molinate at doses of 0, 15, 75, or 150 mg/kg-day did not promote accumulation of α-2u-globulin in the male rat kidney at any dose level (Horner, 1992b). However, a positive control, 2,2,4-trimethylpentane (200 mg/kg-day), a known promoter of α-2u-globulin, did cause a marked increase in the male rat specific protein in the kidney. Accumulation of this protein in the kidney has been associated with male rat specific kidney tumor induction (Swenberg *et al*., 1989). The results of the Horner (1992b) study suggest that the mechanism of kidney tumor induction in the male rat with molinate is not related to α-2u-globulin.

Table 4. Tumor Rates in Male Crl:CD® (SD) BR Rats Receiving Molinate in the Diet for 104 Weeks (Pettersen and Richter, 1990)

Tumor Site and Type	Molinate in Diet (ppm)			
	0	7	40	300
Kidney tumors[a]				
Cortical adenoma	0/48[d]	0/46	0/49	2/48
Carcinoma	0/48[d]	0/46	0/49	3/48
Combined cortical adenoma + carcinoma	0/48[d]	0/46	0/49	5/48[e]
Liver tumors[b]				

Hepatocellular adenoma	1/40	1/31	1/36	3/45
Carcinoma	0/40	0/33	2/37	2/46
Combined hepatocellular adenoma + carcinoma	1/40	1/33	3/37	5/46
Testis tumors[c]				
Interstitial cell tumor	3/45	5/40	5/42	7/48
Mesothelioma	0/41[d]	0/35	0/40	2/47

[a] Number of tumor bearing animals/number of animals examined, excluding those that died before week 55. First adenoma observed on day 738; first carcinoma observed on day 644.

[b] Number of tumor bearing animals/number of animals examined, excluding those that died before observation of the first tumor. First adenoma observed on day 603 at the 40 ppm level; first carcinoma observed on day 601 at the 40 ppm level

[c] Number of tumor bearing animals/number of animals examined, excluding those that died before observation of the first tumor. First interstitial cell tumor observed on day 535 at the 40 ppm level; first mesothelioma observed on day 579 at the 300 ppm level.

[d] Positive Mantel-Haenszel trend test denoted at control.

[e] Statistically increased incidence over control animals using Fisher's exact test ($p<0.05$).

Woodard (1977b) administered Ordram (98.8 percent purity) to Fischer rats (60 animals/sex/group) in the diet at dose levels of 0, 8, 16, or 32 mg/kg-day to determine chronic toxicity/oncogenicity. At week 18, the doses were reduced to 0, 0.63, 2.0, or 6.32 mg/kg-day and remained at this level to the end of the study at 104 weeks. According to the author, the dose levels were reduced because a decrease in weight gain was evident. However, after 16 to 18 weeks on the original diet, the body weights in the high dose (32 mg/kg-day) were depressed less than 10 percent. At week 78, body weights of males and females administered 6.32 mg/kg-day averaged 93 percent of controls, and at week 104, they averaged 97 percent of controls for males and 101 percent of controls for females. These results indicate that the maximum tolerated dose was not reached; thus, the dose levels may have been too low to identify any oncogenic effects.

The author reported that there were no differences between control and treated groups with respect to survival or frequency of neoplastic or non-neoplastic lesions. Testicular interstitial cell tumors were observed in control and treated animals during the two interim sacrifices (56 and 78 weeks) and the terminal sacrifice; their occurrence was not treatment- or dose-related. The results of this study are insufficient to evaluate the oncogenic potential of molinate.

In an 18-month oncogenicity study, molinate (R-4572; 97.6 percent purity) was administered in the diet to Crl:CD-1(ICR)BR mice (50 animals/sex/group) at concentrations of 0, 10, 100, 1,000, or 2,000 ppm (Potrepka and Morrissey, 1991). Corresponding doses of molinate were 0, 1.0, 10.4, 105, or 200 mg/kg-day for male mice and 0, 1.3, 13.9, 133, or 249 mg/kg-day for female mice.

Survival of female mice was affected at the 2,000 ppm exposure level (58 percent compared to >70 percent for controls and the other exposure levels at 80 weeks). Male mice survival rate was not affected by molinate treatment. The only neoplastic lesions observed were benign testicular interstitial cell tumors in male mice. The incidences at

corresponding exposure levels were: 0 ppm, 0/50; 10 ppm, 1/48; 100 ppm, 1/48; 1,000 ppm, 5/49; and 2,000 ppm, 0/50. Only the tumor incidence at 1,000 ppm was statistically increased compared to controls using Fisher's exact test ($p<0.05$). The lack of a dose-response at the highest exposure level indicates that the incidence of testicular tumors at 1,000 ppm was not related to molinate exposure.

Other Toxicological Endpoints

Molinate is an inhibitor of low K_m aldehyde dehydrogenase ($ALDH_2$) activity. Molinate (≥95 percent purity) was injected intraperitoneally into adult mice at a dose of 8 mg/kg body weight. Two hours after administration, liver mitochondrial $ALDH_2$ activity was inhibited 49 percent compared to control animals (Quistad *et al*., 1994). In rats, the dose of molinate that inhibited liver $ALDH_2$ activity by 50 percent (IC_{50}) eight hours after intraperitoneal administration was calculated to be 3.1 mg/kg (Hart and Faiman, 1995). Molinate sulfoxide appears to be the active metabolite, since equimolar doses of both the parent compound and the sulfoxide inhibited $ALDH_2$ activity to the same degree. Furthermore, molinate inhibited liver mitochondrial $ALDH_2$ activity *in vitro* only after metabolic activation with isolated rat liver microsomes (Hart and Faiman, 1995). Levels of acetaldehyde, the potentially toxic metabolite of alcohol, were increased about six-fold in blood and about three-fold in brain after intraperitoneal administration of 40 mg molinate/kg body weight, followed two hours later with 1,000 mg/kg of ethanol (Quistad *et al*., 1994). Hart and Faiman (1995) also found an increase in blood acetaldehyde in molinate-treated rats given ethanol. In addition, they showed that molinate–treated rats challenged with ethanol exhibited a hypotensive response indicative of a disulfiram-like ethanol reaction. Disulfiram, also known as Antabuse, is used in the treatment of alcohol abuse (Parkinson, 1996).

To evaluate the potential effects of Ordram on the immune system of mice, technical grade Ordram (purity not specified) was given to female mice via gavage at dosages ranging from 20 to 320 mg/kg-day for 12 days (Smialowicz *et al*., 1985). The herbicide had no significant consistent effects on the following parameters: body, spleen, and thymus weights, natural killer cell activity, B and T cell lymphoproliferative responses, and delayed-type hypersensitivity and antibody responses to sheep red blood cells. However, 4/10 animals died in the 160 and 320 mg/kg-day groups, and these animals had lost approximately 25 percent of their initial body weight. The only statistically significant response was an increase in absolute and relative spleen weights of the surviving mice gavaged with 320 mg/kg-day of Ordram.

Toxicological Effects in Humans

Acute, Subchronic, or Chronic Toxicity

Hayes and Laws (1991, citing Minakawa *et al*., 1978) reported an incident of molinate exposure and toxicity in four families in Japan. After molinate was applied to rice paddy fields (60 kg per two hectares), four families, including a total of 17 people, began to

notice an odor in a nearby well, which was the source of their water. Soon after, eight people, including five children, developed nausea, diarrhea, abdominal pain, fever, weakness, and conjunctivitis, and four others had abdominal pain only. The families recovered after they stopped using water from the well. Chemical analysis of the well water was not done until 15 days later, at which time the average molinate concentration in five samples was 0.006 ppm. The well water still had an odor one month after the contamination occurred.

Molinate was evaluated for its allergenic and irritant potential using patch tests (Lisi *et al*., 1987). A one percent solution in "pet" (possibly petroleum ether, but not defined) was applied to the backs of 294 patients, who had been admitted for contact dermatitis or non-allergic skin disorders; allergic and irritant reactions were evaluated after 48 and 72 hours. Molinate was negative for both irritation and allergic reactions in all tested patients.

Between 1980 and 1982, an epidemiological assessment of fertility was performed of male workers exposed to molinate at the Stauffer molinate production plant in Cold Creek, Alabama, and at two molinate formulation plants in Richmond, California, and North Little Rock, Arkansas (Taves *et al*., 1984; Paddle, 1992). The study design had two components. The first was an assessment of the effect of molinate exposure on sperm parameters and hormone levels in male volunteers both before the study period and at four sampling points after alternating periods of exposure (production or formulation) and non-exposure (no production or formulation). The second was an assessment of whether molinate exposure had any effect on the fertility of employees' wives through the use of reproductive history questionnaires. The Taves *et al*. (1984) study has been criticized for its lack of clarity and incomplete reporting. Because of this, the exposure and effects data from this study were subsequently reanalyzed using more recently developed statistical methodology (Tomenson *et al*., 1995, 1999; Zeneca Agrochemicals, 1995).

Respiratory exposure for each period ($\mu g/m^3 \times hr$) was based on industrial hygiene measurements collected before and during the study period and estimated hours worked in a molinate work area (Tomenson *et al*., 1995, 1999). The average number of hours worked and the concentration × hours measures were highly variable. Mean exposures to molinate of workers at the three plants during different monitoring periods ranged from 13 to 211 $\mu g/m^3$. The corresponding doses, assuming a respiratory rate of 20 m^3/day and body weight of 70 kg, range from 3.7 to 60 μg/kg-day, respectively. The highest mean exposure occurred at the Richmond plant during monitoring period 2, a formulation period. Mean exposure hours ranged from 14.4 hours (Richmond, period 1, formulation) to 119.1 hours (Cold Creek, period 3, production). There are a number of uncertainties with respect to the exposure assessment in the presented data. Exposure estimates provided by Tomenson *et al*. (1995, 1999) were based on vapor inhalation only and did not include the potential for dermal absorption, oral ingestion, or inhalation of particulates with adsorbed or absorbed molinate liquid. Therefore, the exposure may have been underestimated.

Taves *et al*. (1984) estimated the combined respirable/dermal "absorbed dose" in the workers (historical dose: ~0.26 mg/kg-day; current dose: <0.07 mg/kg-day), but did not provide a clear basis for these estimates. The appropriateness of using participants as their own controls is questionable based on findings of similar exposures in the absence of production or handling: mean exposures at the three plants during non-production or non-formulation periods ranged from 13 to 58 $\mu g/m^3$, whereas mean exposures during the production or formulation periods ranged from 27 to 211 $\mu g/m^3$. In addition, personal protective equipment (e.g., respirators and protective clothing, including gloves) was used with varying degrees of compliance throughout the plants, but this was not taken into account in the exposure estimates.

A total of 268 of 404 workers provided semen samples in at least one time period; this excludes those workers who had undergone a vasectomy. In addition, 222 married workers provided reproductive-history information. There was no evidence that sperm parameters or serum hormone levels at the start of the study were related to the total number of hours of molinate exposure before the study. Mean sperm concentration (10^6/mL) ranged from a low of 60.6 (North Little Rock, period 4, non-formulation) to a high of 114.6 (Richmond, period 4, formulation). A value less than 16.4 million/mL was considered low. Mean percentage sperm with normal morphology ranged from 77 percent (Cold Creek, period 2, non-production) to 83 percent (North Little Rock, periods 1, 3, and 4, formulation and non-formulation). A value of 68.7 percent was considered low. A motility score of 65.0 was considered low; the score for workers ranged from 98 (North Little Rock, period 4, no formulation) to 144 (Cold Creek, period 1, production).

There was no evidence that serum hormone levels were related to molinate exposure before the study or during the four monitoring periods. There appeared to be no effect of molinate exposure on the ratio of observed to expected births. With respect to the sperm parameters, semen samples were usually collected at home and brought into the plant, so that the sample temperature and time to analysis were not well controlled. These variables may have had a substantial effect on sperm parameter measurements. In addition, sperm parameter measurements varied considerably both intra-individually and between monitoring periods.

In spite of the limitations noted in both the exposure assessment and semen analyses, this study was considered adequate to detect any effect of molinate on male fertility. The data from this study do not indicate a relationship between molinate exposure of male workers and adverse effects on sperm or serum hormone measurements or fertility. It should be noted, however, that the estimated human worker doses, 0.004 to 0.060 mg/kg-day, are lower than the lowest NO(A)EL identified for male rat reproductive effects in a subchronic study (0.2 mg/kg-day, based on sperm abnormalities and reduction in number of viable fetuses at 4 mg/kg-day given orally for five weeks; Minor, 1981). If the inhalation exposure is indeed an underestimate of total potential exposure, the estimated human doses could be comparable to those producing adverse reproductive effects in rats.

Genetic Toxicology

Human lymphocytes from two donors were exposed to molinate (97.6 percent purity) at dose levels of 24, 95, or 190 μl/mL to determine the clastogenic potential of molinate (Howard and Richardson, 1988). There were no statistically or biologically significant increases in chromosomal damage seen at any dose level, with or without rat liver S9 fraction.

Sister chromatid exchanges were not increased in human lymphocyte cultures treated with 25, 50, 75, 100, or 200 ppm molinate (72 percent purity). However, 300 ppm molinate was cytotoxic to the lymphocyte cultures (Calderón-Segura *et al*., 1999). Exposure of human lymphocyte cultures to extracts of *Vicia faba* roots treated with 100 to 2,000 ppm molinate resulted in a significant increase in sister chromatid exchanges compared to a control group for the 100, 200, and 300 ppm treatment groups only. For exposure levels of 400 to 2,000 ppm, the frequency of sister chromatid exchanges was not different from the control. The authors (Calderón-Segura *et al*., 1999) suggested that the lack of response at the higher concentrations was due to a phytotoxic effect of molinate on the plant roots, in part because the pH of the solutions decreased with increasing molinate concentration.

Carcinogenicity

No studies were found that evaluated the carcinogenicity of molinate in humans.

Summary of Evidence for Carcinogenicity

Tumors were found at three sites in male rats exposed to molinate: kidney, liver, and testes. Only the incidence of combined kidney adenomas and carcinomas was statistically significant and only for the highest dose (300 ppm). However, there was a significant positive trend ($p<0.01$ by the Mantel-Haenszel test) for the incidence of benign, malignant, and combined kidney tumors. The mean historical control incidence of combined kidney tumors in the same strain of rat in the same laboratory was 1.5 percent (range 0 to 3.3 percent) (Table 5). The incidence of kidney tumors in the present study (10.4 percent) was above the mean and range for historical controls; therefore, these tumors are considered to be rare. In addition, kidney tumor induction in the male rat with molinate did not appear to be related to accumulation of α 2u-globulin (Horner, 1992b).

The incidence of combined hepatocellular adenoma and carcinoma in male rats increased with dose, but the incidence was not statistically significant at any dose relative to control (by Fisher's exact test) nor was the trend test positive (Mantel-Haenszel test). Furthermore, the incidence of hepatocellular adenomas, carcinomas, or combined adenomas and carcinomas is within the range of historical control data for these tumors provided by Pettersen and Richter (Table 5).

With respect to testicular tumors, both benign interstitial cell tumors and mesotheliomas were observed. The incidence of interstitial cell tumors, while increasing with dose,

showed neither a statistically significant positive trend (Mantel-Haenszel test) nor increased incidence at any dose relative to controls (by Fisher's exact test). The incidence in the high dose group, however, was more than double the incidence in the concurrent control (14.6 vs. 6.7 percent). The range of historical control incidence of this tumor type is 0 percent to 6.7 percent (Table 5). The hepatocellular and testicular tumor data are presented as for reference only, and are not included in the risk calculation.

There was a significant positive trend (p=0.01) for mesothelioma in male rats, but the incidence of tumors (4 percent) did not reach statistical significance at any dose. The authors did not provide historical control data for this tumor type. However, the historical incidence of benign mesotheliomas in the same Sprague-Dawley strain of rats that were maintained on a restricted diet (3.3 to 3.4 kcal/gram of feed) was three lesions per 1685 testes (0.18 percent). This incidence was from a total of 26 studies initiated between December 1989 and April 1995 in four different laboratories (Charles River Laboratories, 1998). While the Charles River dataset may not be completely relevant to the Pettersen and Richter study because of the dietary restrictions, the incidence data it provides indicate that the benign mesotheliomas are a rare tumor in control Sprague-Dawley rats. Therefore, the incidence of mesotheliomas is included in the weight of evidence for carcinogenic potential.

Molinate treatment did not affect tumor formation in female rats. Additionally, there was no treatment-related effect on the incidence of any tumors in male or female mice exposed to molinate. There were no studies available to evaluate carcinogenicity in humans.

Table 5. Historical Control Incidence of Selected Neoplastic Findings for Male Rats[a]

		Number of Control Animals with Tumors[b]				
		Liver		*Kidney*		*Testes*
		Hepatocellular		Tubular Cell		Interstitial Cell
Study No.	Start Date	Adenoma	Carcinoma	Adenoma	Carcinoma	
A	1/29/85	3 (5%)	0 (0%)	1 (1.7%)	1 (1.7%)	1 (1.7%)
B	6/03/80	3 (5%)	0 (0%)	2 (3.3%)	0 (0%)	1 (1.7%)
C	8/01/80	5 (8.3%)	0 (0%)	0 (0%)	0 (0%)	3 (5%)
D	5/19/86	0 (0%)	3 (5%)	0 (0%)	0 (0%)	0 (0%)
E	2/22/83	0 (0%)	0 (0%)	0 (0%)	0 (0%)	4 (6.7%)
F	2/12/85	8 (13.3%)	1 (1.7%)	0 (0%)	2 (3.3%)	3 (5%)
G	4/10/82	1 (1.7%)	0 (0%)	0 (0%)	0 (0%)	3 (5%)
H	6/24/82	6 (10%)	0 (0%)	1 (1.7%)	0 (0%)	0 (0%)
I	6/24/82	8 (13.3%)	0 (0%)	1 (1.7%)	0 (0%)	1 (1.7%)
	Mean	***3.8 (6.3%)***	***0.4 (0.7%)***	***0.6 (0.9%)***	***0.3 (0.6%)***	***1.8 (3%)***

[a] Data are from Pettersen and Richter (1990), and represent the historical control data from two-year oncogenicity studies using the Crl:CD(SD)BR strain of male rats (Sprague–Dawley). The conducting laboratory was ICI Americas, Inc.

[b] Each study consisted of 60 male rats per group, including 10 animals sacrificed at 12 months. The start date of the Pettersen and Richter study was August 28, 1987, whereas start dates of the studies included here ranged from June 1980 to May 1986. In addition, the historical data are non-censored.

The evidence for a genotoxic mechanism for molinate carcinogenicity is equivocal. There were positive results for mammalian cell mutagenicity (with metabolic activation), but negative results for bacterial mutagenicity. There were both positive and negative chromosomal aberration, sister chromatid exchange, and micronucleus assays, but the DNA repair and the dominant lethal assays were negative.

DOSE-RESPONSE ASSESSMENT

Noncarcinogenic Effects

Several animal studies have described adverse noncarcinogenic effects resulting from exposure to molinate. The most sensitive endpoint was a dose-related neurotoxic effect observed in rats, mice, and dogs. Rats were the most sensitive species, with males being slightly more sensitive than females. The neurotoxicity was characterized by degeneration and demyelination of the sciatic nerve, the severity of which increased with increasing dietary molinate exposure (7, 40, 300 ppm). Clinical signs indicative of neurological changes included ataxia, atrophy, and some paralysis of the hind limbs. The LOAEL was 7 ppm, or about 0.3 mg/kg-day. The other major endpoint observed was reproductive toxicity, characterized by abnormal sperm morphology and reductions in fertility. The lowest NOAEL for a reproductive toxicity endpoint was 0.2 mg/kg-day, based on reduced sperm parameters (percent viable sperm, percent motile sperm, and sperm cells $\times 10^6$/ml), a reduction in the number of implants and viable fetuses, and an increase in percent abnormal sperm in rats receiving molinate doses of 4, 12, and 30 mg/kg-day. A single case report of human exposure from contaminated well water was available, but the report was limited by inadequate estimation of exposure levels and by insufficient exposure duration for establishing effects that may result from long-term exposure. An epidemiological study of workers in molinate production and formulation plants did not find any link between molinate exposure and adverse effects on male fertility.

The U.S. EPA reference dose (RfD) for chronic dietary exposure to molinate is 0.001 mg/kg-day (Rowland and Taylor, 1998). This value is based on the toxicity endpoint of sciatic nerve degeneration/demyelination and muscle atrophy/reserve cell hyperplasia observed in the two-year rat chronic toxicity/carcinogenicity study (Pettersen and Richter, 1990). A cumulative uncertainty factor (UF) of 300 was applied to the LOAEL of 0.3 mg/kg-day to derive the chronic RfD; it included factors of 10 for interspecies extrapolation, 10 for intraspecies variation, and 3 for the use of a LOAEL (i.e., lack of a NOAEL in the critical study). The U.S. EPA cumulative UF differs from that used by

OEHHA (see Calculation of proposed PHG below), in that OEHHA incorporated a 10-fold factor, rather than 3-fold, for the use of a LOAEL in the critical study.

In addition to the chronic RfD, U.S. EPA has derived an FQPA (Food Quality Protection Act) adjusted chronic dose of 0.0001 mg/kg-day (cPAD, or chronic Population Adjusted Dose) (Taylor, 1999; Dobozy, 2001). The FQPA Safety Factor Committee determined that the 10x FQPA safety factor should be retained for chronic dietary risk assessment for "All Populations," which include infants and children (Tarplee, 1998). The Committee's recommendation was based on the increased susceptibility demonstrated in both the prenatal developmental toxicity and developmental neurotoxicity studies in rats.

Carcinogenic Effects

For the purpose of establishing the most sensitive site of tumor formation in the most sensitive sex and strain of experimental animals, cancer potencies (q_1*) and slope factors (CSF) were calculated for the exposure-related tumor incidences reported as statistically significant ($p<0.05$) in the oral studies by Pettersen and Richter (1990) (Table 6). OEHHA used the incidence of combined kidney cortical adenomas and carcinomas in male rats given the highest dose level of 300 ppm (13 mg/kg-day). Kidney tumor formation did not appear to be related to an accumulation of α-2u-globulin. Historical control data suggest that the observed kidney cortical adenomas and carcinomas are rare. Increases in liver or testicular tumor incidences in male rats were not statistically significant. There were no significant increases in tumor incidences in female rats, or male or female mice, dosed with molinate compared to controls.

Table 6. Tumor Incidence Used for Estimation of Cancer Potency

Data Set / Study	Concentration (Dose)	Tumor Incidence
Kidney adenoma + carcinoma – male rats[a]	0, 7, 40, 300 ppm (0, 0.3, 1.8, 13 mg/kg-day), 7 days/week in diet	0/48, 0/46, 0/49, 5/48[b]

[a] Data from Pettersen and Richter (1990).

[b] Number of tumor-bearing animals/number of animals examined, excluding those that died before week 55. First adenoma observed on day 738; first carcinoma observed on day 644.

To generate the potency estimates, the Tox_Risk (Version 3.5) program was used, which fit the linearized multistage (LMS) model to the data on tumor incidence (Tox_Risk, 1993). The results of these analyses are presented in Table 7 below. In place of the default rat food intake and body weight values of 17.5 g and 0.35 kg, respectively, the means for male rats from Pettersen and Richter (1990) were used, i.e., food intake of 23 g/day (based on feed disappearance) and mean body weight of 0.575 kg (average weight from weeks 0 to 78, prior to weight loss). The carcinogenic potency for humans, q_1*, is the upper 95 percent confidence limit on the cancer potency slope calculated by the LMS model, and the cancer slope factor, CSF, is defined as $0.1/LED_{10}$, where LED_{10} is the

lower 95 percent confidence limit on the dose giving a 10 percent tumor incidence. Interspecies scaling of cancer potencies from experimental animals (CSF_{animal} or q_{animal}) to human potencies (CSF_{human} or q_1*) were based on the following relationship:

$$CSF_{human} = CSF_{animal} \times \left(\frac{bw_h}{bw_a} \right)^{\frac{1}{4}}$$

where bw_h and bw_a are human and animal body weights, respectively. The default human body weight is 70 kg.

Table 7. Cancer Potencies[a] for Combined Kidney Adenomas/Carcinomas in Male Rats Given Molinate by the Oral Route (generated using Tox_Risk Version 3.5)

Data Set	q_1* $(mg/kg\text{-}d)^{-1}$	χ^2	p	k	LED_{10} (mg/kg-d)	CSF_{human}[b] $(mg/kg\text{-}d)^{-1}$
Combined kidney adenoma + carcinoma – male rats (Pettersen and Richter, 1990).	3.7×10^{-2}	0.01	0.99	3	2.78	3.6×10^{-2}

[a] Cancer potencies (q_1* and CSF) are given in human equivalents.
[b] CSF is calculated as $0.1/LED_{10}$.

The most sensitive site, sex, and species for tumor development is combined kidney adenomas and carcinomas observed in male rats in the two-year cancer bioassay, with a CSF_{human} from this data set of 0.036 $(mg/kg\text{-}day)^{-1}$. This value based on a significant increase in tumors is the most appropriate for calculation of the health-protective concentration of molinate in drinking water for the carcinogenic endpoint. Other tumor increases in liver and testicles of male rats were not statistically significant.

In an earlier evaluation of the carcinogenic potential of molinate, the U.S. EPA Health Effects Division Carcinogenicity Peer Review Committee (CPRC) 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 male rat combined kidney tumor data of Pettersen and Richter (1990) should be used for quantitation of human risk (q_1*) (Taylor and Rinde, 1992). The molinate q_1* (in human equivalents) was 1.1×10^{-1} $(mg/kg\text{-}day)^{-1}$ (Fisher, 1992). This estimate was obtained by the application of the Multi-Stage model (Tox_Risk, version 3.1) to the experimental animal data and use of body $weight^{2/3}$ cross-species scaling factor. A reanalysis of the kidney tumor data in 1999, using the $body\ weight^{3/4}$ cross-species scaling factor, produced a different unit risk estimate of 4.2×10^{-2} $(mg/kg\text{-}day)^{-1}$ in human equivalents (Brunsmann, 1999, as cited in CARC, 2000 and Fort, 2000). This is similar to the value calculated by OEHHA (Table 7). The major difference between U.S. EPA and OEHHA in the approach used to calculate the q_1* appears to be the definition of animals at risk for developing tumors. Brunsmann's definition was the "number of animals examined, excluding those that died or were sacrificed before observation of the first tumor." The combined kidney tumor

incidence used by Brunsmann (1999) was 0/32, 0/30, 0/29, and 5/44 for the 0, 7, 40, and 300 ppm groups, respectively. OEHHA's definition and corresponding tumor incidence data are provided in Table 6.

The U.S. EPA Health Effects Division Cancer Assessment Review Committee (CARC), in accordance with the 1999 draft Guidelines for Carcinogen Risk Assessment (U.S. EPA, 1999), placed molinate into the classification *Suggestive as to Evidence of Carcinogenicity, but Not Sufficient to Assess Human Carcinogenic Potential* (CARC, 2000; Dobozy, 2001). This classification also was based on the occurrence of kidney tumors and the equivocal evidence for testicular tumors in male rats, as well as supportive evidence that the structurally related compound, thiobencarb, also produced testicular tumors in male rats. While the CARC reaffirmed the CPRC's previous assessment, the CARC did not calculate a potency factor for molinate because dose-response assessments were not required by the 1999 draft guidelines for chemicals in this classification.

The California Department of Pesticide Regulation (DPR, 2000c) presented both a linear and non-linear approach for estimating the carcinogenic potential of molinate. For the linear approach, DPR calculated a 95^{th} upper-bound estimate of potency, based on the combined kidney tumor data in male rats and body weight$^{3/4}$; the value was 0.077 (mg/kg-day)$^{-1}$. However, DPR did not consider this approach valid because the slope of the line between zero and the next two data points (0.3 and 1.8 mg/kg) is effectively zero, and using a mathematical model that assumes there is no threshold (i.e., linearized multistage) is not appropriate for estimating the slope of the curve. The non-linear approach was considered by DPR to be more appropriate in this circumstance. DPR identified the LOEL for kidney tumors in male rats as 13 mg/kg-day with a NOEL of 1.8 mg/kg-day, but they did not suggest a mode of action for the presumed threshold carcinogenic effect. OEHHA rejects this argument because the expected number if tumors at the two lower doses is zero or one, assuming a linear dose-response. Therefore the sensitivity of the bioassay is not adequate to distinguish between the two models. The cancer guidelines suggest a linear extrapolation when there is evidence of genotoxicity and no evidence to support a threshold mechanism (U.S. EPA, 1999, 2005a).

CALCULATION OF PHG

Calculations of concentrations of chemical contaminants in drinking water associated with negligible risks for carcinogens or noncarcinogens must take into account the toxicity of the chemical itself, as well as the potential exposure of individuals using the water. Tap water is used directly as drinking water, for preparing foods and beverages. It is also used for bathing or showering, and in washing, flushing toilets and other household uses resulting in potential dermal and inhalation exposures.

Noncarcinogenic Effects

Calculation of a public health-protective concentration (C, in mg/L) for molinate in drinking water for noncarcinogenic endpoints follows the general equation:

$$C = \frac{NOAEL/LOAEL \times BW \times RSC}{UF \times L/day}$$

where,

NOAEL/LOAEL = no-observed-adverse-effect-level or lowest-observed-adverse-effect level;

BW = adult body weight (a default of 70 kg for male or 60 kg for female);

RSC = relative source contribution (a default of 20 to 80 percent);

UF = uncertainty factors (typical defaults of a 10 to account for inter-species extrapolation, a 10 for uncertainty from the subchronic nature of the principal study and a 10 for potentially sensitive human subpopulations);

L/day = daily water consumption rate: 2 L/day for 60 to 70 kg adult, 1 L/day for 10 kg child, higher values of L equivalents (Leq/day) are used for volatile organic compounds to account for inhalation and dermal exposure through showering, flushing of toilets, and other household uses of tap water.

In the case of molinate, OEHHA identified an experimental LOAEL of 0.3 mg/kg-day from the oral chronic study using rats, based on an increased incidence of neurological effects at all doses. A NOAEL of 1.0 mg/kg-day was identified in the chronic mouse study, based on testicular degeneration, and a LOAEL of 1.0 mg/kg-day was identified in the one-year dog study, based on neurological effects. The lowest NOAEL from a rodent reproductive study was 0.2 mg/kg-day, based on effects on sperm parameters in male rats. Although the LOAEL for neurotoxicity from the chronic rat study is similar, the 10-fold uncertainty factor that would be applied to this value to obtain an estimated NOAEL makes the neurotoxicity endpoint the most sensitive indicator of noncarcinogenic molinate toxicity. In addition, there were no data available to suggest that molinate-induced neurotoxicity would be unique to rodents; in fact, it was observed in rats, mice, and dogs, and therefore, could be expected to occur in humans. This endpoint is chosen for calculation of the PHG based on a noncarcinogenic endpoint. The adult human body weight (BW) default value is 70 kg. An RSC of 50 percent was used in the calculation because inhalation of molinate vapors near rice-growing regions and ingestion of molinate-contaminated water are both considered likely routes of exposure. Data were inadequate for a specific calculation of source contributions. The OEHHA default value for adult human water consumption of 2 L/day was used; volatilization of molinate from drinking water into household air was judged to be insignificant.

The cumulative uncertainty factor of 1,000 used in the calculation incorporates uncertainty contributions for interspecies extrapolation (10), intraspecies variation (10), and a LOAEL to NOAEL extrapolation (10). The selection and application of these uncertainty values is consistent with practices of OEHHA and the U.S. EPA.

Thus the health-protective concentration (C) can be calculated for an adult as:

$$C = \frac{0.3 \text{ mg/kg - day} \times 0 \text{ kg} \times .5}{1000 \times \text{ L/day}} = 0.0053 \text{ mg/L} = 5 \text{ µg/L (rounded)}$$

The comparable calculation for an infant is:

$$C = \frac{0.3 \text{ mg/kg - day} \times 0 \text{ kg} \times .5}{1000 \times \text{ L/day}} = 0.0015 \text{ mg/L} = 2 \text{ µg/L (rounded)}$$

It is not obvious which of these values would be most relevant for protection against non-cancer effects. Because the critical endpoint is based on chronic toxicity, it may be argued that this endpoint is not appropriate to estimate a value for protection of infants. On the other hand, the developing nervous system of infants is likely to be more sensitive to the nerve degeneration/demyelinating type of effect in this case. We conclude that specifically considering children's exposure and sensitivity is appropriate in this case. Therefore the health-protective value for non-cancer effects is proposed to be 2 µg/L (ppb).

Additionally, we evaluated the Pettersen and Richter (1990) data for both the sciatic and gluteus nerve damage using several of the U.S. EPA BMD models; the data did not lend themselves to an optimal dose-response fit for the BMD models applied. Thus, we chose to base the proposed PHG value recommendation on the standard LOAEL/NOAEL approach.

Carcinogenic Effects

For carcinogens, the following general equation can be used to calculate the public health-protective concentration (C) for molinate in drinking water (in mg/L):

$$C = \frac{BW \times R}{q_1^* \text{ or CSF} \times \text{L/day}} = \text{mg/L}$$

where,

BW = adult body weight (a default of 70 kg);

R = *de minimis* level for lifetime excess individual cancer risk (a default of 10^{-6});

q_1^* or CSF = cancer potency factor (q_1^* is the upper 95 percent confidence limit on the cancer potency slope calculated by the LMS model, and CSF is a potency derived from the lower 95 percent confidence limit on the 10 percent tumor dose [LED_{10}]; CSF = 10 percent /

LED_{10}; the potency estimates are converted to human equivalent [in $(mg/kg\text{-}day)^{-1}$] using $BW^{3/4}$ scaling);

L/day = daily water consumption rate: 2 L/day for 60 to 70 kg adult, 1 L/day for 10 kg child; higher values of L equivalents (Leq/day) may be used for volatile organic compounds to account for inhalation and dermal exposure through other household uses of tap water.

The potency estimates for a carcinogen are calculated by two different methods because most of our experience is with the LMS model, and we wish to note any differences using the method first recommended by U.S. EPA (1999) in its draft guidelines for carcinogen risk assessment, which is based on the LED_{10}. The LMS model focuses on the linear low dose extrapolation. The new method, however, places a higher premium on fitting the observed data to estimate the ED_{10} and the 95 percent lower bound (LED_{10}), the point from which the low dose extrapolation is made (U.S. EPA, 1999, 2005a). As shown in Table 6, the potency estimates for molinate calculated using the two methods were consistent with each other [0.037 $(mg/kg\text{-}day)^{-1}$ and 0.036 $(mg/kg\text{-}day)^{-1}$) for q_1*and CSF_{human}, respectively].

The cancer potency selected for calculating the PHG was derived using the LED_{10} method from the U.S. EPA guidelines (1999, 2005a). For molinate, the cancer slope factor (CSF_{human}) derived from the principal study is 0.036 $(mg/kg\text{-}day)^{-1}$ and the adult human body weight (BW) default value is 70 kg. The OEHHA default value for adult human water consumption is 2 L/day. A *de minimis* risk level of 10^{-6} is used for calculating the health-protective level.

Therefore:

$$C = \frac{10^{-6} \times 70\ kg}{0.036\ (mg/kg\text{-}day)^{-1} \times 2\ L/day} = 0.000972\ mg/L = 1\ ppb\ (rounded)$$

OEHHA proposes a PHG of 1 μg/L (1 ppb) for molinate in drinking water based on its carcinogenicity in rats. This value is judged to provide adequate health protection for infants, pregnant women, the elderly, and other potentially sensitive subgroups.

RISK CHARACTERIZATION

The seasonal use of molinate poses a unique situation for consideration in the development of a PHG. The PHG is based on a daily exposure over a lifetime. Since molinate is only used during a few months in the year, it is unlikely that an individual will ever receive a daily annual exposure. However, the detection of molinate in shallow ground water wells in the Sacramento Valley indicates that there is a potential for leaching into the water supply for some residents in the north Valley who may get their drinking water from wells. The annual exposure could potentially be longer than the seasonal use period. Furthermore, molinate has been used in the Sacramento Valley for

about 40 years, and it was not until the early 1980's that the release of rice paddy tailwater was controlled to reduce the amount of molinate in the Sacramento River. Therefore, molinate exposure through drinking water and/or air is potentially an annual event, which we view as chronic exposure.

The other primary sources of uncertainty in development of the molinate PHG for drinking water are the general issues of uncertainty in any cancer risk assessment. These include the assumption of dose-response linearity to very low risk levels and the applicability of rodent tumor data to estimate cancer risk in humans. For the non-cancer extrapolation, the magnitude of the factors required for inter- and intra-species extrapolation, extrapolation from a LOAEL to a NOAEL, and the relative source contribution (RSC) are the major uncertainties.

This assessment used a linear low dose extrapolation based on male rat kidney tumors in a study in which molinate only produced tumors at the highest dose tested. A non-linear threshold approach may be used in such cases; however, there is no evidence for a threshold mechanism of action for the kidney tumor (i.e., no accumulation of α-2u-globulin in male rat kidneys). In addition, the presence of rare testicular mesotheliomas, albeit not a significant incidence, with the supportive genotoxicity data (positive forward mutation mouse lymphoma assay), is suggestive evidence that molinate is a carcinogen. The OEHHA and U.S. EPA default procedure is to assume there is no threshold and use the linear approach for estimating carcinogenic risk.

In calculating the proposed PHG level of 1 ppb a *de minimis* theoretical excess individual cancer risk level of 10^{-6} was used. The corresponding levels of molinate in drinking water for cancer risks of 10^{-5} and 10^{-4} are 10 and 100 ppb, respectively.

A somewhat unusual feature of the results obtained from this risk assessment on cancer and non-cancer effects is the narrow margin between the health-protective values for the two types of endpoints. This can be explained by the relatively low incidence of kidney tumors in the critical study (i.e., low cancer potency) versus the high potency of molinate for neurotoxicological effects. The potent effects of molinate on sperm motility should also be noted. A large amount of uncertainty is involved in both the cancer and noncancer extrapolations.

Our methods for cancer extrapolation, use of uncertainty factors, and the RSC follow the general practices of the U.S. EPA for drinking water risk assessment. For the RSC, U.S. EPA has treated carcinogens differently from noncarcinogens. For noncarcinogens, RfDs (in mg/kg-day), drinking water equivalent levels (DWELs, in mg/L) and MCLGs (in mg/L) are calculated using uncertainty factors (UFs), body weights and water consumption rates (L/day) and the RSC, respectively. The RSC range is 20 percent to 80 percent (0.2 to 0.8) depending on the scientific evidence.

U.S. EPA follows a general procedure in promulgating MCLGs:

1. if Group A and B carcinogens (i.e., strong evidence of carcinogenicity) MCLGs are set to zero,
2. if Group C (i.e., limited evidence of carcinogenicity), either an RfD approach is used (as with a noncarcinogen) but an additional UF of 1 to 10 (usually 10) is applied to

account for the limited evidence of carcinogenicity, or a quantitative method (potency and low-dose extrapolation) is used and the MCLG is set in the 10^{-5} to 10^{-6} cancer risk range,

3. if Group D (i.e., inadequate or no animal evidence) a RfD approach is used to promulgate the MCLG.

For approaches that use low-dose extrapolation based on quantitative risk assessment, U.S. EPA does not factor in an RSC. The use of low-dose extrapolation is considered by U.S. EPA to be adequately health-protective without the additional source contributions. In developing PHGs, we have adopted the assumption that RSCs should not be factored in for carcinogens grouped in U.S. EPA categories A and B, and for C carcinogens for which we have calculated a cancer potency based on low-dose extrapolation. This is an area of uncertainty and scientific debate and it is not clear how this assumption impacts the overall health risk assessment.

OEHHA believes that the proposed PHG level of 1 ppb is adequate to protect the whole population, including potentially sensitive subpopulations such as infants, children, and the elderly, against both cancer and non-cancer effects.

OTHER REGULATORY STANDARDS

The California MCL for molinate is 20 μg/L and is based on the chemical's reproductive toxicity in rats (DHS, 1988).

The World Health Organization (WHO, 1993, 1996) recommended a drinking water quality guideline of 6 μg/L. This was based on the endpoint of impaired reproductive performance in male rats, from which a total daily intake (TDI) of 2 μg/kg was derived. An allocation of 10 percent of the TDI to drinking water resulted in the guideline value of 6 μg/L.

Molinate was listed on the first U.S. EPA drinking water contaminant candidate list (U.S. EPA, 1998), and is also on the second list (CCL2) (U.S. EPA, 2005b). Molinate was under consideration in California as a potential toxic air contaminant (DPR, 2000a) until being withdrawn from this process in 2000. Molinate has had a long-term decreasing use trend in California, as shown in Figure 2. As noted earlier, 2009 is slated as the last year of molinate use in the United States. Molinate was being tracked for possible future listing under Proposition 65 as known to the State to cause reproductive toxicity (OEHHA, 1998), but has not been selected for prioritization for listing.

The U.S. EPA chronic RfD for molinate is 0.001 mg/kg-day (Rowland and Taylor, 1998), although an earlier RfD of 0.002 mg/kg-day (last revised 02/01/1991) is still listed on the U.S. EPA IRIS database (IRIS, 2007). The current U.S. EPA value is based on a LOAEL of 0.3 mg/kg-day for sciatic nerve degeneration and demyelination and muscle atrophy/reserve cell hyperplasia observed in the rat chronic toxicity/carcinogenicity study. An uncertainty factor of 300 (3-fold for LOAEL to NOAEL extrapolation, 10-fold for interspecies differences, and 10-fold for intraspecies differences) was applied to the LOAEL to calculate the RfD. Calculation of a drinking water equivalent level from this

RfD using a body weight of 70 kg, an RSC of 0.2, and a water consumption rate of 2 L/day (the usual U.S. EPA defaults) would yield an estimated safe level of 7 μg/L (ppb). The major reasons for the differences between this estimated safe level and the present OEHHA estimates are the judgment by OEHHA that the cancer data are adequate to assume a carcinogenicity hazard to humans. For the non-cancer endpoint, OEHHA has used an uncertainty factor of 10 for LOAEL to NOAEL extrapolation, compared to the 3 used by U.S. EPA, infant exposure parameters, and a higher RSC. OEHHA believes the severe nature of the effects at the LOAEL (nerve damage), as well as the reproductive toxicity at similar low exposure levels, justify a full factor of 10 for the LOAEL to NOAEL extrapolation. In addition, OEHHA agrees with the opinion of the U.S. EPA FQPA Safety Factor Committee that the effects are relevant to children (Tarplee, 1998), although we conclude that an additional children's safety factor is not necessary. The higher RSC (0.5 instead of 0.2) is justified because of other identified molinate exposure routes in California.

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