**MOLINATE**
**(ORDRAM )**

# RISK CHARACTERIZATION DOCUMENT

Medical Toxicology and Worker Health and Safety Branches

Department of Pesticide Regulation

California Environmental Protection Agency

March 1, 1996

# EXECUTIVE SUMMARY

Molinate (Ordram ), a selective, pre-emergence herbicide, is registered in California for use in rice fields. There were 1.4 million pounds of molinate active ingredient used in California in 1992. Two products, Ordram 10G (10% granular formulation) and Ordram 8E (8 pounds per gallon emulsifiable concentrate), are sold in California. Virtually all molinate is applied by aerial spraying. The duration of molinate application and, therefore, potential exposure is approximately four to six weeks each spring. Molinate can be absorbed through the skin, lungs (inhalation), or gut from oral ingestion.

In 1989, results of a new study on molinate were submitted to the Department of Pesticide Regulation (DPR). These results showed adverse effects on the ovarian cells of the test animals. These findings triggered a re-evaluation of the entire data base on molinate toxicity regarding its effects on fertility.

An Interim Risk Characterization Document was completed in March, 1990 to evaluate the possible adverse effects of molinate exposure on human health. The interim assessment prompted DPR to implement extensive mitigation measures for workers for the 1990 application season. The registrant was also required to conduct additional toxicity and worker exposure studies to better define the potential exposure, the threshold level for adverse effects, and the relevance of existing studies to humans. The process was repeated for the 1991, 1992, 1993, and 1994 growing seasons. This revised risk assessment incorporates all new toxicological and worker exposure data submitted by the registrant, new monitoring studies conducted by DPR (up to 1994), 1990 environmental monitoring data for water, 1992 environmental monitoring data for air, and the updated software programs from Technical Assessment System (TAS) for the dietary analyses.

## Risk Assessment Process

Molinate was entered into the risk assessment process because of possible adverse effects identified in reproductive and chronic toxicity studies. The risk assessment process consists of four aspects: hazard identification, dose response assessment, exposure evaluation, and risk characterization.

Hazard identification entails review and evaluation of the toxicological properties of each pesticide. The dose-response assessment then considers the toxicological properties and estimates the amount which could potentially cause an adverse effect. The amount which will not result in an observable or measurable non-oncogenic effect is called the No-Observed-Effect Level, NOEL. Oncogenic effects of a chemical are generally assumed to not have biological thresholds, and therefore occur at all dosages. The relative ability of a chemical to cause tumors is indicated by its potency.

A basic premise of toxicology is that at a high enough dose, virtually all substances will cause some toxic manifestation. Chemicals are often referred to as "dangerous" or "safe", as though these concepts were absolutes. In reality, these terms describe chemicals which require low or high dosages, respectively, to cause toxic effects. Toxicological activity is determined in a battery of experimental studies which define the kinds of toxic effects which can be caused, and the exposure levels (doses) at which effects may be seen. State and federal testing requirements mandate that substances be tested at doses high enough to produce toxic effects, even if such testing involves chemical levels many times higher than those to which people might be exposed.

In addition to the intrinsic toxicological activity of the pesticide, the other parameters critical to determining risk are the level, frequency and duration of exposure. The purpose of the exposure evaluation is to determine the potential exposure pathways and the amount of pesticide likely to be delivered through those routes.

The risk characterization then integrates the observed toxic effects (generally obtained from laboratory studies conducted with high dosages of pesticide) with potential human exposures at low dosages. The likelihood of potential, non-oncogenic adverse health effects in people is generally expressed as a margin of safety. The margin of safety is a ratio of the dosage which produced no effects in laboratory studies to the dosage humans might potentially receive. For oncogenic effects, an additional lifetime risk of cancer may be calculated by multiplying the cancer potency of the pesticide times the estimated average daily exposure dosage a person could potentially receive over their lifetime (assumed to be 70 years).

Toxic Effects

Based on the currently available toxicity information, DPR concluded that molinate causes adverse effects on spermatogenesis and ovarian function in rodents, resulting in decreased fertility. In addition, molinate causes neuropathies in dogs, mice, and rats. DPR has further concluded that, in the absence of additional data to the contrary, molinate has the potential to cause similar effects in humans.

Potential Human Exposure

Members of the general public with potential exposure to molinate include people who eat rice, people served by water utilities using the Sacramento River as the source of drinking water, and people residing in communities in the proximate vicinity where molinate is applied to rice fields. In addition, farmers entering rice fields shortly after molinate application may be potentially exposed to molinate in the ambient air. Workers involved in molinate application can also be exposed via ingestion, inhalation, and skin contact.

Potential exposure of the general public to molinate through rice consumption or via drinking water was more than 1000 times lower than the dosage of molinate which was found to have no adverse effects in animals. Margins of safety for potential ambient air exposures to molinate for the general public and farmers were also more than 1000. All workers involved in the application of molinate (*i.e.*, mixers/loaders, flaggers, and pilots) have margins of safety over 100, when the required protective measures are taken.

Mitigation Measures

Based on evaluations presented each year since 1990, DPR has implemented increasingly stringent mitigation measures for the 1990, 1991, 1992, 1993, and 1994 use seasons which require mixers/loaders and flaggers to wear appropriate, chemically resistant protective clothing, chemical-resistant gloves, and respirators. A new formulation of molinate has been required, and the numbers and types of bags of molinate which may be loaded during the season have been limited. Total amount loaded must not exceed 228,000 pounds per loader during the seasn. Upon implementation of the mitigation measures, the resulting margins of safety were considered more than the values conventionally recommended to protect people from the toxic effects of molinate.

Conclusion

Based on current toxicity and exposure data, margins of safety for current potential short-term, seasonal, annual, or lifetime exposures of workers, the general public, and farmers to molinate are greater than the values conventionally recommended to protect people from the toxic effects of a chemical.

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Although molinate caused kidney tumors in male rats, the weight of evidence suggested that the oncogenic potential of molinate was equivocal. 1) The incidence of hepatocellular adenomas and carcinomas in treated male rats exhibited a statistically significant trend, but the occurrence of tumors at any dose was not significantly different from that in concurrent controls. 2) Only male rats developed a significant incidence of kidney tumors (combined adenomas and carcinomas) associated with molinate exposure, and only at the high dose. 3) The kidney tumors appeared at a dose which may have exceeded the maximum tolerated dose, as indicated by a 14% decrement in body weight gain and clear evidence of systemic toxicity (peripheral neuropathies). 4) There was no indication of substance-related oncogenicity in the mouse. 5) The evidence of molinate's genotoxic potential was equivocal.

The slope of the dose-response curve for the combined incidence of kidney adenomas and carcinomas was zero between the control animals and the animals at the next two doses. When the slope in the low-dose portion of the dose response curve is zero, mathematical models which assume that there is no threshold (e.g. the linearized multistage model) are not applicable for estimating the slope of the curve. In this circumstance, where a toxicological threshold appears to exist, a margin of safety approach for lifetime exposure may be applied. By convention, when the procedure is applied to a potentially carcinogenic endpoint, an additional uncertainty factor of 10 may be applied. The LOEL for kidney tumors in male rats was 13 mg/kg-day with a NOEL of 1.8 mg/kg-day.

**Dietary Exposure-** Field studies have never detected molinate in rice. For the purposes of conducting a dietary risk assessment, it was assumed that the anticipated residue level of molinate in or on rice grain for potential acute exposure was at the minimum detection limit of 0.05 ppm and that for potential annual exposure was 0.025 ppm (50% of the minimum detection limit). The population subgroup of non-Hispanics other than black and white had the highest potential acute dietary exposure of 0.397 ug/kg-day. The potential annual dietary exposure for non-Hispanics other than black or white was 0.028 ug/kg-day.

**Residential Exposure-** Potential environmental exposure sources of molinate were the air and drinking water. Infants living in the town of Maxwell had the greatest potential exposure from the air-0.22 ug/kg-day. Children, ages 1 to 6 years, living in Sacramento had the greatest potential exposure from drinking water- 0.43 ug/kg-day.

**Occupational Exposure-** Monitoring data obtained in 1994 indicated mean work-related average daily dosages (ADDs) ranged from 0.56 ug/kg-day for drivers (wearing carbon suits) to 10.5 ug/kg-day for direct loaders (wearing Tyvek). The 95th percentile of the ADDs ranged from 5.0 ug/kg-day for drivers (wearing carbon suits) to 15.9 ug/kg-day for direct loaders (wearing Tyvek). Seasonal average daily dosages (SADDs) ranged from 0.43 ug/kg-day for drivers (wearing carbon suits) to 4.12 ug/kg-day for direct loaders (wearing Tyvek). Annual average daily dosages (AADDs) ranged from 0.04 ug/kg-day for drivers (wearing carbon suits) to 0.39 ug/kg-day for direct loaders (wearing Tyvek). The greatest Lifetime Average Daily Dose (LADD) for occupational or non-occupational exposure to molinate was 0.22 ug/kg-day for direct loaders wearing Tyvek suits.

**Risk Characterization-** Residential exposures- Margins of safety (MOSs) for potential seasonal dietary risks from consuming rice contaminated with molinate ranged from 17,000 to 96,000, with the lowest MOS in the population subgroup- non-nursing infants less than 1 year old. MOSs for drinking seasonally contaminated water ranged from 1,000 to 4,000, and the range of MOSs for combined seasonal exposure to contaminated drinking water and rice ranged from 1,000 to 3,000. The lowest MOS was associated with the potential exposure of non-nursing infants, less than the age of one year. The MOSs associated with potential seasonal air exposures ranged from 2,000 to 12,000 in the towns of Maxwell and Williams. Infants, potentially, had the lowest MOS.

two years, the Lowest Observed Effect Level (LOEL) for neurotoxicity (skeletal muscle atrophy, peripheral nerve degeneration, and distal spinal cord changes) was 0.3 mg/kg-day. Indications of reproductive toxicity in rats at two-years were oligospermia (NOEL = 1.8 mg/kg-day), and ovarian thecal/interstitial cell vacuolation/hypertrophy (NOEL = 2 mg/kg-day). In mice, the NOEL for neurotoxicity (increased incidence and severity of sciatic nerve degeneration/demyelination and Schwann cell hyperplasia; increased frequency of eosinophilic bodies in the spinal cord and the medulla of the brain) was 10.4 mg/kg-day and 13.9 mg/kg-day for males and females, respectively; the NOEL for ovarian effects was 13.9 mg/kg-day; and the NOEL for testicular degeneration was 1.0 mg/kg-day. No effects of molinate on testicular function were reported in dogs. The NOEL for neurotoxic effects (clinical signs) in dogs was 1 mg/kg-day. The 1-year NOEL for reduced hematocrit in dogs was 1 mg/kg-day.

**Genotoxicity-** The genotoxicity of molinate was examined in several assay systems. One study with mouse lymphoma cells *in vitro* demonstrated mutagenicity with metabolic activation. Molinate was positive in one bone marrow micronucleus test, and negative in another. Elevated frequencies of both chromosome aberrations and sister chromatid exchanges with activation were noted. However, these cytogenetic effects were not consistent in repeat assays, and were not dose-related (none occurring at the highest dose). Molinate did not cause unscheduled DNA synthesis. No mutagenic activity was indicated in microbial systems, with or without metabolic activation.

**Reproductive Toxicity-** In addition to causing decreased male fertility and increased sperm abnormalities, molinate induced reduced fertility in female rats. The NOEL for reduced fertility in the female was 50 ppm (approximately 3.7 mg/kg-day). The NOEL for histopathological changes in the rat ovary was 0.44 mg/kg-day. Human epidemiological data from workers engaged in the production of molinate were not utilizable for risk assessment purposes because of inadequate quality assurance/quality control and problems in the study design.

**Developmental Toxicity-** Molinate was not teratogenic in rats or rabbits. In rats, the NOEL for maternal toxicity (cholinergic signs, decrement in food consumption and weight gain) and developmental toxicity (increased resorptions, intrauterine growth retardation) was 35 mg/kg-day. In rabbits, the NOEL for maternal toxicity (decrement in weight gain) and developmental toxicity (increased resorptions, intrauterine growth retardation) was 20 mg/kg-day.

**Neurotoxicity-** The NOEL for acute neurotoxicity in chickens was 200 mg/kg based on observations of microscopic lesions, walking behavior deficits, and other neuromuscular weakness. The 1-day LOEL in rats for clinical signs and performance decline in the functional observational battery tests was 25 mg/kg. The 1-day NOEL for neuronal cell necrosis of the pyramidal neurons in the pyriform cortex in rats was 100 mg/kg.

**Hazard Identification-** The weight of evidence suggests molinate should be considered a potential human reproductive toxicant. A NOEL of 11.5 mg/kg for reduced fertility of male rats after 5 days exposure was used as the toxicological basis for estimating margins of safety (MOSs) for potential acute human exposure to molinate. The NOEL of 0.48 mg/kg-day for sperm abnormalities in rats was used as the basis to calculate margins of safety for potential seasonal occupational exposures to molinate.

Peripheral nerve degeneration was observed in dogs, mice and rats as a result of long-term exposure to molinate. Also, non-classical delayed neurotoxicity (microscopic lesions, walking behavior deficits, and other neuromuscular weakness) was observed in hens. As the neuropathies were not ameliorated with time, a NOEL of 1mg/kg-day for intermittent clinical signs in dogs was used to establish margins of safety for potential annual occupational and non-occupational exposure to molinate.

Although molinate caused kidney tumors in male rats, the weight of evidence suggested that the oncogenic potential of molinate was equivocal. 1) The incidence of hepatocellular adenomas and carcinomas in treated male rats exhibited a statistically significant trend, but the occurrence of tumors at any dose was not significantly different from that in concurrent controls. 2) Only male rats developed a significant incidence of kidney tumors (combined adenomas and carcinomas) associated with molinate exposure, and only at the high dose. 3) The kidney tumors appeared at a dose which may have exceeded the maximum tolerated dose, as indicated by a 14% decrement in body weight gain and clear evidence of systemic toxicity (peripheral neuropathies). 4) There was no indication of substance-related oncogenicity in the mouse. 5) The evidence of molinate's genotoxic potential was equivocal.

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Occupational exposures- Margins of safety estimated for potential mean short-term exposures ranged from 1,095 for direct loaders (wearing Tyvek) to 32,000 for ground applicators using the 8E formulation. When the 95th percentile of short-term exposure was considered for each of the job categories, the MOSs ranged from 214 for direct loaders (wearing Tyvek) to 6,765 for flaggers. The MOS for farmers entering the fields for 1 hour a day immediately after molinate application was 96,000. MOSs for potential seasonal exposures were lower, ranging from 117 for direct loaders (wearing Tyvek) to 1,700 for ground applicators using the 8E formulation. MOSs for potential annual exposures ranged from 3,000 for direct loaders (wearing Tyvek) to 33,000 for ground applicators using the 8E formulation.

The MOS for potential lifetime exposure to molinate for the most exposed group (direct loaders wearing Tyvek suits), based on the oncogenic potential of molinate, was 8,182.

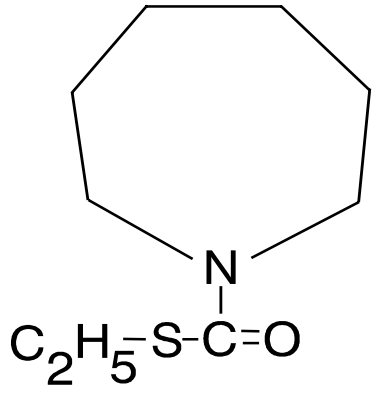
Conclusions- Margins of safety for potential short-term, seasonal, annual, and lifetime exposures to workers associated with handling and application of molinate, the general public, and farmers were greater than the values conventionally recommended to protect people from the toxic effects of a chemical.

Implementation of specific mitigation measures in the 1991, 1992, 1993, 1994 application seasons resulted in reductions of exposure. The mitigation measures included: the requirement that most handlers wear two layers of protective clothing (one of which may be chemical resistant), chemical resistant hand and foot coverings; a full or half face respirator (depending on the activity); and head covering (if not chemical resistant, then or tightly woven fabric). As contact with molinate increases, so does the personal protective equipment requirement. In addition, the total number of pounds of molinate handled by mixer/loaders during a season was limited.

# II INTRODUCTION

## A. CHEMICAL IDENTIFICATION

Molinate (S-ethyl hexahydro-1H-azepine-1-carbothioate) is a selective, pre-emergence, thiocarbamate herbicide registered in California for use on rice. Molinate entered the risk assessment process in the Department of Pesticide Regulation (DPR) because of its reproductive toxicity and neurotoxicity. Its chemical structure is shown in the figure below. The mechanism of herbicidal action for molinate is unknown. The trade names for molinate are Ordram , Hydram and Yalan . Hydram and Yalan are not sold in the State of California.




Molinate

## B. REGULATORY HISTORY

In 1984 the California Department of Food and Agriculture set an 8 day holding period for water in rice paddies treated with molinate. The holding period allowed the molinate concentration in rice field water to decline before the water was returned to nearby rivers. A holding period was instituted because of the large number of fish kills which occurred in the rivers with high concentrations of molinate. The holding period for the 1993 rice growing season was 24 days. In April of 1989, based on concerns for human health, the California Department of Health Services set a Maximum Contaminant Level (MCL) of 20 *u*g/L for molinate in drinking water (CDHS, 1989). USEPA lists molinate as a C(q*) carcinogen, based on a significant incidence of kidney tumors in male rats (USEPA, 1995). The $q_1$* derived by USEPA is 0.11 $[mg/kg\text{-}day]^{-1}$, which is an estimate of the upper bound on potency. The USEPA RfD is 0.002 mg/kg-day, based on a NOEL of 0.2 mg/kg-day from a subchronic fertility study in the rat showing increased preimplantation loss and increased sperm abnormalities (USEPA, 1994).

In 1990, the Department of Pesticide Regulation (DPR) required the registrant to conduct additional toxicity and exposure studies to better define the potential occupational exposure, the inhalation NOEL, and the relevance of existing studies to humans (Fong, 1991).

Interim Risk Characterization Documents (RCDs) were issued for the 1990 (Tsai, 1990), 1991 (Tsai, 1991), 1992 (Cochran, 1992), 1993 (Nelson, 1992), and 1994 (Cochran, 1994) rice growing seasons recommending successive restrictions on the amount of molinate that could be handled by mixer/loaders, increasing the protective clothing to be worn, and requesting additional monitoring studies. Permits, issued by DPR, have made these recommended restrictions into requirements for continued use of molinate (Okumura, 1993).

## C. TECHNICAL AND PRODUCT FORMULATIONS

Ordram is commercially available in a 10% granular formulation containing 10 pounds (10G) of active ingredient per 100 pounds of product; and as an emulsifiable concentrate containing 8 pounds (8E) active ingredient per gallon. Ordram 10G and 8E are the only formulations registered in California. They are recommended for pre-plant, pre-flood use on water seeded rice only. Approximately 1.4 million pounds of molinate active ingredient were used in California in 1992 (CDFA, 1994). The majority of the products were applied by aerial spraying (Formoli et al., 1994).

## D. ILLNESS REPORTS

Between 1982 and 1992, there was one reported systemic illness, two eye injuries, and two skin injuries associated with molinate use in California (Appendix B). In 1982, 1983, 1984, and 1985, no illnesses were reported to be associated with molinate use.

## E. PHYSICAL/CHEMICAL PROPERTIES[a]

| | |
|---|---|
| Chemical Name: | S-Ethyl hexahydro-1H-azepine-1-carbothioate |
| Common Name: | molinate |
| Trade Name: | Ordram |
| CAS # | 2212-67-1 |
| Empirical Formula: | $C_9H_{17}ONS$ |
| Molecular Weight: | 187.32 g/Mol |
| Physical State | amber liquid at room temperature |
| Specific Gravity: | 1.0626 g/ml at 20$^{o}$C |
| Vapor Pressure: | 5.0 x $10^{-3}$ mm Hg (25$^{o}$C) |
| Henry's Law Constant: | 1.3 x $10^{-6}$ $m^3$-atm/g-mol |
| Solubility: | 800 mg/L in water at 20$^{o}$C<br>miscible with most common organic solvents such as acetone, ethanol, kerosene, methyl-isobutyl-ketone (MIBK), and xylene. |
| Partition Coefficient Octanol:Water | 756 |

a/ Myers, 1987; 1988; Stauffer, 1971.

## F. ENVIRONMENTAL FATE

**Summary.** Aerobic metabolism of molinate under normal use conditions resulted in a half life of 28 days. The estimated half-life of molinate under anaerobic conditions was 129 days. Molinate did not penetrate soil, and thus, is unlikely to become a groundwater contaminant. Dissipation of molinate from flooded rice paddies occurs as the result of vaporization from the water. Molinate did not hydrolyze at pH 5, 7, or 9 within a 30 day test period, nor did it break down under the equivalent of sunlight for 34 days. The lack of breakdown of molinate by hydrolysis or photolysis has probably contributed to the documented presence of molinate in streams and rivers receiving run-off from rice fields.

### Hydrolysis

The hydrolysis of molinate was examined at 25 and 40°C in aqueous solutions buffered at pH 5, 7 or 9 (Lee, 1988). The studies were carried out in the dark under sterile conditions, with concentrations of unlabeled molinate in the 100 mg/L range. Under the experimental conditions employed, no significant hydrolytic breakdown of molinate occurred within a 30-day test period.

### Photolysis

The photolysis of molinate was examined under natural sunlight in an aqueous solution buffered at pH7 at 25°C (Eya, 1989). The test was conducted under sterile conditions with concentrations of unlabeled molinate in the 100 mg/L range. Under the experimental conditions used, no significant photolytic breakdown of molinate occurred within the duration of the study (33.9 days of sunlight).

$^{14}$C-labeled molinate on 0.4 mm films of Biggs clay was exposed continuously under a filtered xenon sun lamp in closed photoreactors for up to 3 days (equivalent to 30 days of outdoor exposure)(Haag and Mill, 1989). No significant photolysis of molinate occurred on the soil studied. The average recovery of radioactivity after the equivalent of 30 days outdoor exposure was 97±2%. More than 92% of the recovered radioactivity was extractable from the soil. More than 96% of the extractable radioactivity consisted of molinate.

### Aerobic Soil Metabolism

$^{14}$C-Molinate (98% purity; specific activity 27.3 mCi/mmol) was applied at a rate of 4.2 ppm (dry soil basis) to the water layer covering clay loam soil (Lay, 1990). The test system was incubated under aerobic conditions at 30°C for 0 to 30 days after treatment. The two major soil metabolites were hexamethyleneimine (HMI; maximum 0.66%) and molinate sulfoxide (1.91%). Metabolites in the flood water were mainly molinate sulfoxide (6.58%), HMI (8.96%), carbamyl chloride (an artifact, 3.26%), 3-ketomolinate (0.82%), and 4-ketomolinate (0.76%). The half life of molinate under these experimental conditions was 28 days.

### Anaerobic Soil Metabolism

Biometer flasks containing a clay soil were flooded with water and preincubated in a nitrogen atmosphere (Tarr, 1990). [*ring*-2-$^{14}$C]molinate was applied in each flask at a level of 5.1 ppm (dry soil weight basis) and incubated under nitrogen at 30°C for up to 365 days. The estimated half-life of molinate under these conditions was 129 days. Metabolism to carbon dioxide was the principal fate of the herbicide.

Soil Mobility

The adsorption and desorption of molinate was studied on four soils and one aquatic sediment by the batch equilibrium method (Dohn, 1988). The soils used were Keeton sandy loam (less than 1% organic mater), Columbia loamy sand, Manteca sandy loam, and Biggs clay. The aquatic sediment used was obtained from the Colusa canal, and was classified as a clay loam. The values determined for the Freundlich adsorption coefficient ($K_d$) of molinate ranged from 0.74 to 2.04. The values for the Freundlich desorption coefficient ranged from 1.14 to 2.94. The $K_{oc}$ values ($K_d$ values corrected for soil organic carbon content) ranged from 121 to 252 for adsorption, and from 155 to 388 for desorption. These values suggest that molinate will have high to medium mobility in soil.

Field Dissipation

Ordram 8-E was applied twice, broadcast post-flood at a rate of 5 pounds active ingredient per acre per application to study field dissipation (Curry *et al.*, 1989). The second application was made 7 days after the first. The soil type at the site was clay. The half-life for molinate residues in the top 3.5 inches of soil was 25 days. No molinate was detected in any sample taken from below 7 inches, indicating that molinate does not migrate downwards through soil under typical use conditions. The half-life for dissipation of molinate in flood water was 3 days. No S-methyl molinate or molinate sulfoxide was detected in any soil sample. This indicates that soil metabolites either do not form to any significant extent, or they are rapidly degraded under field conditions.

The 1986 - 1992 survey of the State's ground water found molinate in 4 private wells (Maes *et al.*, 1992). However, the contamination was ascribed to surface contamination as the wells were unsealed.

# III TOXICOLOGICAL PROFILE

## A. PHARMACOKINETICS

**Summary.** Molinate can be absorbed via ingestion, inhalation or dermal exposure. It was readily metabolized to more polar products and excreted primarily in the urine. Comparison of excretory patterns following intravenous and oral dosing suggested that oral absorption was nearly 100% in rats. Oral absorption of molinate by monkeys was estimated to be 80%. Seventy-five percent of the ring-labeled $^{14}C$-molinate was excreted within 24 hours after administration of molinate via oral gavage of the rat. Molinate did not bioconcentrate in the body tissues. The major metabolic pathway in laboratory animals involved sulfoxidation, conjugation with glutathione, and excretion in the urine as a mercapturic acid derivative. Hydrolysis of the sulfoxidized and hydroxylated derivatives represented another major metabolic process. The metabolism of [ring-$^{14}C$] Ordram in female and male rats appeared to be qualitatively similar. The major urinary metabolite in monkeys and humans appeared to be 4-hydroxy molinate. The absorption rate via the dermal route in the rat was estimated to be 55% for the occluded skin when applied in methanol, and 40% when applied in water.

### Oral- rat

Sprague-Dawley [CR\ Crl:CD\ (SD)BR] rats (5/sex/group) were given a single gavage dose with $^{14}C$-labeled molinate (94.2% - 96.7% purity; 17.5 mCi/mmol) at 10 mg/kg or 100 mg/kg (Ritter *et al.*, 1991). Molinate was rapidly absorbed and rapidly excreted (principally in the urine) at both dose levels. By 36 hours, 64-69% had been excreted in the urine. At both the high and low doses, the highest residue levels in body organs were in the liver, kidneys, lungs, and spleen. The mean concentrations of radioactivity in tissues after 96 hours ranged from 0.07 to 2.28 *u*g equivalents of molinate per gram tissue for animals at the low dose level. The concentration of radioactivity remaining in the tissues after 96 hours represented 2-3% of the dose. At the high dose level, the equivalents of molinate per gram tissue ranged from 0.7 to 23.4 *u*g. The highest residue levels were in the whole blood, and the lowest levels were in the plasma. Approximately 84% of the radioactive dose was recovered.

Sprague-Dawley [CR\ Crl:CD\ (SD)BR] rats (5/sex) were dosed by gavage with $^{14}C$-labeled molinate (94.2% purity; 17.5 mCi/mmol) at 10 mg/kg daily for a total of 14 days (Ritter, 1991a). Most of the radiolabel (73.4 - 85.4% of the dose) was excreted in the urine by both male and female rats. Radiolabel in the feces ranged from 2.5 to 10.3% of the dose, and expired $CO_2$ accounted for approximately 2% of the dose. There were no significant differences in the excretion profiles of the male and female rats, except that the male rat eliminated a slightly higher percent of the dose as $^{14}CO_2$. At termination, male rats retained 3.6% of the dose in the tissues, and females retained 3.4%. Residual radioactivity was confined mostly to the blood (2.33 *u*g equivalent/g tissue for males; 2.53 *u*g equivalent/g tissue for females), and highly vascularized organs. The mean concentrations in the liver, kidneys, and lungs ranged from 0.8 to 1.3 *u*g equivalent/g tissue.

Sprague-Dawley [CR\ Crl:CD\ (SD)BR] rats (8 males) were given single oral doses of $^{14}C$-labeled molinate (94.2% purity; 17.5 mCi/mmol) at 10 mg/kg (Peffer, 1991). The recovery of radioactivity after a 10 mg dose was 97%. Urinary excretion was 79%; fecal excretion was 14%; and $CO_2$ excretion was 1%. Approximately 75% of the dose was excreted (64% in urine; 11% in feces) by 24 hours.

I.V.- rat

Sprague-Dawley [CR\ Crl:CD\ (SD)BR] rats (5/sex) were given a single intravenous dose of 1 mg $^{14}$C-labeled molinate (94.2% purity; 17.5 mCi/mmol) and monitored over a 7 day period (Ritter, 1991b). The average total recovery of the dose was 88.9% in males and 85.2% in females. Approximately 69% of the dose was excreted in the first 24 hours by both male and female rats. Urinary excretion accounted for 69.7 to 79% of the dose in both males and females. In both sexes, the feces accounted for 1.9 to 7.3% of the dose, indicating that biliary excretion of the dose also occurred. Expired $^{14}CO_2$ accounted for 1.3% of the dose in male rats, and 0.9% of the dose in female rats. At termination, male rat carcasses contained an average of 4.3% of the dose, and female rats had 4.7% of the administered dose.

The metabolism of $^{14}$C-molinate in the rats used for three studies described above (Ritter *et al.*, 1991; Ritter, 1991a,b) was examined (Ritter, 1991c). Twenty-two radiolabeled urinary metabolites were separated by thin-layer chromatography. Six metabolites (hexamethyleneimine, 4-keto hexamethyleneamine, 3-hydroxy and 4-hydroxy molinate glucuronide, molinate mercapturic acid, and hydroxy molinate mercapturic acid) each accounted for more than 5% of the urinary radioactivity in one or more of the dose groups. Minor metabolites (<5%) were identified as 5-hydroxy and 4-hydroxy hexamethyleneimine, 3-hydroxy and 4-hydroxy molinate, and unchanged molinate. The metabolites found in the feces were similar to those in the urine, with the exception of glucuronides, which were not well represented in the feces. Most fecal metabolites were non-polar. The proposed metabolic pathway in rats is oxidation of the sulfur to form a reactive sulfoxide. The sulfoxide may either hydrolyze to hexamethyleneimine or undergo conjugation with glutathione. The sulfoxide is excreted ultimately as mercapturic acid conjugates. Alternately, hydroxylation occurs at the 3 and 4 positions of the ring, followed by conjugation with glucuronic acid to form 3-hydroxy and 4-hydroxy molinate glucuronides.

Oral and I.V.- monkey

Male Cynomolgus monkeys (4/dose) were given $^{14}$C-molinate (99.9% purity) either by gavage at 6 mg/kg or 60 mg/kg; or intravenously at 6 mg/kg (Lythgoe *et al.*, 1992). Monkeys receiving an i.v. injection of molinate (6 mg/kg) excreted 87.4% of the administered dose in the urine in the first 24 hours, but no detectable amount in the feces. After 8 days, an average 95.8% had been excreted in the urine, and 1.2% in the feces. Total recovery was 97.5% of the administered dose. A total of 8 metabolites, which accounted for 68% of the urinary radioactivity, were identified. The principal metabolites were the glucuronide conjugate of 4-hydroxy molinate, followed by: the cysteine conjugate of molinate; molinate mercapturate, the glucuronide conjugate of 3-hydroxy molinate; the methyl ester of the glucuronide conjugate of 4-hydroxy molinate; the acetic acid conjugate of molinate; the glucuronide conjugate of ring hydroxylated molinate; hexamethyleneimine; and 4-hydroxy molinate. The profile of excretion observed following a single oral dose (6 mg/kg) was very similar to that following i.v. dosing. Total recovery was 51.1% of the administered dose after 8 days. Urinary excretion accounted for 48% of the administered dose in 24 hours. During the following 7 days a further 2.3% was excreted in the urine. Following an oral dose of 60 mg/kg, 83.1% of the administered dose was recovered in 8 days. Approximately 79% of the radiolabel was excreted in the urine, and 0.2% in the feces in the first 24 hours. After 8 days, an average of 80.2% of the radiolabel had been excreted in the urine, and 1.8% in the feces.

Oral- human

Six human volunteers were give a single oral dose (5 mg) of molinate (99.7% purity) in corn oil (Batten *et al.*, 1992). An average of 39±9.7% of the administered dose was excreted in the form of 4-hydroxy molinate in the urine by 24 hours.

Dermal- rat

$^{14}$C-molinate (99% purity) in methanol was applied to the skin of male Sprague-Dawley rats at three dose levels, 10.6, 1.39 and 0.61 mg/kg (Holmes, 1990). Following application, the dose site was occluded for 24 hours. The fraction of the dose absorbed was approximately 55% at all three dose levels.

$^{14}$C-molinate (99% purity) in water was applied to the skin of male Sprague-Dawley rats at three dose levels, 10, 1 and 0.1 mg/kg (Little, 1991). Following application, the dose site was occluded for 24 hours. The fraction of the dose absorbed was approximately 46.6, 37.8 and 38.9% at the 0.1, 1.0 and 10 mg/kg doses, respectively.

Inhalation- rat

Male Wistar rats (5/group) were exposed to atmospheric concentrations of technical molinate (99% purity) as a vapor via whole body (4.05 ± 1.13 *u*g/L) or via nose only (7.72 ± 4.71 *u*g/L), and as a dust via nose only (4.34 ± 0.3 *u*g/L) for six hours (Hext, 1991). The theoretical absorbed dose, assuming all molinate enters via the inhalation route, is calculated using 0.04 $m^3$/kg-hr (respiratory volume of the rat) and the presumption that 100% of molinate in lungs is absorbed. If it were assumed that the absorbed dosages were derived solely from the inhalation route, then the theoretical absorbed doses, based on default inhalation values (Zielhuis and van der Kreek, 1979) were 0.97 mg/kg (whole body vapor exposure), 1.85 mg/kg (nose only vapor exposure), and 0.23 mg/kg (nose only dust exposure). In contrast, the mean absorbed doses for the rats (estimated by extrapolating from measured concentrations of 4-hydroxymolinate in the urine of the rats) were 49.1 mg/kg (whole body vapor exposure), 13.4 mg/kg (nose only vapor exposure), and 1.96 mg/kg (nose only dust exposure). These data indicate that there were major discrepancies between absorbed dosages estimated by default values and those measured directly. Consequently, it was concluded that default values for inhalation could not be used to accurately estimate the absorbed dosage of molinate in rat inhalation studies.

B. ACUTE TOXICITY

Molinate is moderately toxic. The oral lethal dosage for humans was estimated to be 50 to 500 mg/kg (Gosselin *et al.*, 1984). The oral $LD_{50}$ reported in animal studies ranged from 501 to 4640 mg/kg depending on the species and the formulations tested (Table 1). An acute inhalation study performed by the Stauffer Chemical Company showed testicular damage from the gross pathologic examination and histologic evaluation of testes from male rats exposed to technical molinate at 0.28 mg/L for 4 hours (Miller, 1980). It was reported that "the testicular effect was both time-dependent and dose-related".

Table 1. The Acute Toxicity of Technical Molinate

| Species | Sex | Dose | Ref[a] |
|---|---|---|---|
| | | **TECHNICAL GRADE (97-99%)** | |
| Oral $LD_{50}$ | | | |
| Rat | M/F | 501-720 mg/kg | 1 |
| Mouse | M/F | 795-1260 mg/kg | 1 |
| I.V. $LD_{50}$ | | | |
| Rat | | 233 mg/kg | 2 |
| Inhalation $LC_{50}$ | | | |
| Rat | | 2400-2900 mg/$m^3$ | 2 |
| Mouse | | 2100 mg/$m^3$ | 2 |
| Dermal $LD_{50}$ | | | |
| Rabbit | | >2000 mg/kg | 1 |

a/ References: 1. Stauffer, 1968; 2. Saunders and Saylor, undated.

Dermal/Eye Irritation

Technical molinate is a mild to moderate skin and eye irritant in the rabbit. Reversible mild redness in unwashed eyes was observed in the primary eye irritation studies in rabbits given molinate at 100 mg/eye. Primary dermal irritation studies of formulated molinate in rabbits at 0.5 g/animal showed either no effect or slight erythema at 24 hours. The symptom appeared to be transient and subsided by 72 hours (Stauffer, 1968).

Clinical Signs (inhalation)

Rats exposed once to an atmospheric concentration of technical molinate (4.9 mg/L) exhibited severe depression, prostration, ataxia, shallow and audible breathing, salivation, brown or red stains about the face, and hindleg weakness (Miller, 1980). At 0.28 mg/L the study, performed by the Stauffer Chemical Company, showed testicular damage from the gross pathologic examination and histologic evaluation of testes from male rats. It was reported that "the testicular effect was both time-dependent and dose-related".

Table 2. Acute Toxicity of Molinate Formulations

| Species | Sex | Dose | Ref[a] |
|---|---|---|---|
| | | **FORMULATIONS** | |
| | | **Ordram 6E and 8E** | |
| Oral $LD_{50}$ rat | | 794 mg/kg | 1 |
| | | **Ordram 15G** | |
| Oral $LD_{50}$ rat | | 4100-4198 mg/kg | 2 |
| Dermal $LD_{50}$ rabbit | | >2,000 mg/kg | 2 |
| | | **Ordram 10LG (10% granular formulation with Linseed oil)** | |
| Oral $LD_{50}$ rat | | 1100 - 1150 mg/kg | 3,4 |
| Dermal $LD_{50}$ rabbit | | >5000 mg/kg | 3,4 |
| | | **Ordram 5G** | |
| Oral $LD_{50}$ rat | | 4640 mg/kg | 3 |

a/ References: 1. Stauffer, 1968; 2. Morgan, 1987; 3. Dean, 1977; 4. Brown, 1980.

Formulations- Dermal/Eye Irritation

Formulations of molinate were mildly irritating to moist skin (Category III-IV), and severely irritating to the eye (Category II)(Morgan, 1987). However, molinate formulations did not cause sensitization in guinea pigs (Mutter, 1986).

Formulations- Clinical Signs (oral)

Adverse clinical signs for all rats dosed orally with molinate (Ordram 15G) were depression, rough coats, hunched postures, ptosis, ataxia and stained fur (Morgan, 1987).

C. SUBCHRONIC TOXICITY

**Summary.** Most of the subchronic studies were performed to elucidate the effect of molinate on male reproduction in rats. The No-Observed-Effect-Level (NOEL) for acutely appearing clinical signs in orally dosed rats was 15 mg/kg-day. The NOEL (reduced fertility, reduced number of implants) in rats following 5 days of molinate treatment was 11.5 mg/kg-day. The 5-week NOEL was 0.48 mg/kg-day for sperm abnormalities in male rats. Molinate had no effect on serum LH or testosterone levels in treated rats. Five to eight weeks after treatment with molinate, male fertility in rats was only partially restored. The 6-week NOEL for reduced fertility in male mice was 20 mg/kg-day. The reproductive effects of molinate were not limited to rodents. Female rabbits, mated with treated male rabbits, exhibited increased pre-implantation loss, but a NOEL could not be established. Morphological abnormalities were not noted in rabbit sperm, but the 9-week NOEL for abnormally stained sperm was 40 mg/kg-day.

Gavage- rat

Five groups of 8 male Crl:CD (SD) BR rats were dosed by gavage for 10 days with 0 (4 ml corn oil), 15, 75 or 150 mg/kg-day molinate (98.1% purity), or 2,2,4-trimethylpentane as a positive control at 200 mg/kg-day (Horner, 1992a). The study was designed to determine whether molinate caused the accumulation of *a*-2u-globulin in the kidneys of male rats. 2,2,4-Trimethylpentane caused the formation of *a*-2u-globulin in the rat kidney, but molinate did not. All three molinate groups exhibited a statistically significant ($P<0.01$) dose-related decrement in weight gain by day 4 (approximately 20%, 0.9%, and 0.4% of control, respectively). The Lowest-Observed-Effect-Level (LOEL) for decrement in body weight gain was 15 mg/kg-day. Clinical signs were seen at 75 (salivation) and 150 mg/kg-day (salivation, subdued appearance, hunched posture, piloerection, ocular discharge, stained nose and mouth, urinary incontinence, irregular breathing) by day 3. The NOEL for clinical signs was 15 mg/kg-day. The study was considered supplemental, examining a possible cause for male kidney tumors.

Groups of ten female Crl:CD(SD)BR rats were dosed by gavage with molinate (98.1% purity) at 0 (corn oil) 75, 135, or 200 mg/kg-day on days 7-9 of gestation (Horner, 1992b). High mortality (5/10 at 135 mg/kg-day; 9/10 at 200 mg/kg-day) in the two highest dose groups limited the extent of parameters measured, or reduced the precision of available data. Lipid content of adrenals was increased at the two highest doses. Fatty cytoplasmic vacuolation was noted in adrenal zona fasiculata and zona reticularis, and in *corpora lutea* at the two highest doses. No change in plasma progesterone was detected at any dose level. The NOEL was 75 mg for histopathological changes in the adrenal gland and death. The data were considered supplemental, examining possible mechanisms of action of molinate on the female reproductive system.

Molinate (purity not stated) was given to twelve week old male Sprague-Dawley rats (12/group) by gavage at 0 or 20 mg/kg-day for 10 weeks (Killinger, 1982). After the treatment period, the fertility of male rats was tested by mating each male with two untreated females. Dosed animals had a significantly ($P<0.05$) reduced pregnancy index, a marked reduction in the numbers of implants per pregnant dam, and reduced quantity and quality of sperm from the cauda epididymis (Table 3). The data were considered supplemental, examining possible mechanisms of action of molinate on the male reproductive system.

Table 3. The effects of molinate on rat fertility and sperm parameters following ten weeks of exposure by gavage (Killinger, 1982).

| Parameters[a] | Dosage (mg/kg-day) | |
|---|---|---|
| | 0 | 20 |
| Mean # implants/corpora lutea | 12.7/15.6 (81%) | 4.1/13.6* (30%) |
| Viable fetuses | 12.7+3.5 | 3.9±3.3* |
| % motile sperm | 50.4±12.3 | 24.5±3.6* |
| % abnormal sperm | 3.5±1.9 | 33.3±9.8* |
| sperm (x $10^6$/ml) | 51±13 | 23±10* |

a/ Mean±SD
* Significantly different, P<0.05, from the control by Dunnett's two tailed t test.

Male Sprague-Dawley rats (12/group) were dosed with molinate (98.2% purity) at 0, 11.5 or 50 mg/kg-day for 5 days, and then were mated with 1 female per week for 10 weeks (Minor, 1981). Male rats treated with 50 mg/kg molinate for 5 days exhibited a significant (P<0.05) reduction in fertility, and a reduced number of implants per pregnant female in the third week post-treatment (Table 4). There was no increase in fetal resorptions. The NOEL for 5 days of molinate treatment was 11.5 mg/kg-day. In the second part of the study, male rats were dosed with 0, 0.26, 4.8, 11 or 27 mg/kg-day (actual dosages) for 5 or 10 weeks. Males dosed for five weeks at 4.8, 11, or 27 mg/kg-day exhibited decreased sperm motility and viability, decreased sperm numbers, an increased percentage of abnormally appearing sperm, and the females they were mated with had a reduction in the number of implantations (Table 5). No increase in fetal resorptions was observed. Neither serum testosterone levels, nor serum gonadotropin levels (LH and FSH) were significantly (P>0.1) affected by the treatment with molinate. The major types of sperm abnormalities were detached sperm heads and tails, heads and tails bent at angles, and rupture of sperm membranes at the head and midpiece junction and the midpiece-tail junction. The 5-week NOEL was 0.26 mg/kg-day for sperm abnormalities. The data were considered supplemental, examining possible mechanisms of action of molinate on the male reproductive system.

Table 4. The effects of molinate on fertility and pre-implantation loss in rats following five days of exposure by gavage (Minor, 1981).

| Parameters | Dosage (mg/kg-day) 0 | 11.5 | 50 |
|---|---|---|---|
| Implants/corpora lutea[a] | | | |
| Week 1 | 12.9/15.5 (83%) | 10.6/16.6 (64%) | 10.2/13.7 (74%) |
| Week 2 | 13.3/16.4 (81%) | 14.4/15.9 (91%) | 11.1/13.9 (80%) |
| Week 3 | 14.8/17.4 (85%) | 12.5/16.0 (78%) | 5.2/15.8* (33%) |
| Week 4 | 15.1/18.3 (83%) | 14.7/16.6 (89%) | 11.5/16.4* (70%) |
| Week 5 | 15.0/16.2 (93%) | 14.5/18.6 (78%) | 14.8/16.1 (92%) |

a/ Mean number of implants (N=12) divided by the mean number of corpora lutea.
* Significantly different, P<0.05, from the control by the Mann Whitney-U Nonparametric Rank test.

Table 5. The effects of molinate on plasma hormone levels and epididymal sperm parameters in rats following five weeks of exposure by gavage (Minor, 1981).

| Parameters[a] | Dosage (mg/kg-day) 0 | 0.26 | 4.8 | 11 | 27 |
|---|---|---|---|---|---|
| LH (ng/ml) | 12±13 | 20±15 | 15±18 | 26±26 | 18±18 |
| FSH (ng/ml) | 81±42 | 84±46 | 82±46 | 85±44 | 130±51 |
| Testosterone (ng/ml) | 12±8 | 17±11 | 19±15 | 19±7 | 17±9 |
| % Viable Sperm | 93±3 | 94±3 | 64±16* | 68±12* | 32±17* |
| % Motile Sperm | 73±8 | 73±6 | 46±16* | 39±13* | 13±11* |
| % Abnormal Sperm | 7±1 | 10±4 | 35±17* | 46±23* | 73±14* |

a/ Mean ± S.D; 12 animals sampled per parameter.
* Significantly different, P<0.05, from the control by the Mann Whitney-U Nonparametric Rank test.

In a repeat of the second part of an earlier study (Minor, 1981), male Crl:CD(SD)BR rats (12/group) were dosed by gavage with 0, 0.48, 0.98, 1.9, 2.9, 3.8, or 7.7 mg/kg-day molinate (96.8% purity) for 5 weeks (Hodge, 1993a). No clinical signs, or treatment-related effects on body weight gain were reported. Scanning electron microscopic examinations were conducted on portions of sperm (head, mid-piece; mid-piece and tail; and tail) from the epididymides. Statistically significant ($P<0.05$) mid-piece abnormalities were noted at 1.9, 2.9, 3.8 and 7.7 mg/kg-day. Although mid-piece abnormalities were also observed at 0.48 and 0.98 mg/kg-day and not in concurrent controls, they were not significant ($P>0.1$). Significant ($P<0.01$) numbers of head-less sperm were noted at 0.98 mg/kg-day. The NOEL for sperm abnormalities, considered in combination, was 0.48 mg/kg-day. The data were considered supplemental, examining possible mechanisms of action of molinate on the male reproductive system.

Diet- rat

Molinate (purity not stated) was given in the diet at 8 to 32 mg/kg-day for 12 weeks to male and female Charles River rats in a cross-over mating study (Woodard, 1975a). Male rats treated with molinate were found to be responsible for the reduction of fertility. No litters were born from control females mated with males treated at 16 or 32 mg/kg-day. Eight pairs of rats with males treated at 8 mg/kg-day and mated with control females produced only two litters. Control males mated with treated females produced the expected numbers of litters. A NOEL was not established. The data were considered supplemental, examining possible mechanisms of action of molinate on the female and male reproductive systems.

Molinate (98.8% purity) at 0 or 32 mg/kg-day was given in the diets of male Sprague-Dawley CD rats for 7 weeks (Woodard, 1975b). The males were each mated with two females in weeks 5, 6 and 7 of treatment. A final mating of the males was done 5 weeks after termination of treatment. No litters were sired during weeks 5-7. After 5 weeks off treatment, 2 of 5 males successfully sired litters. The data were considered supplemental, examining possible mechanisms of action of molinate on the male reproductive system.

Dermal- rat

Wistar rats (5/sex/group) were given 6-hour dermal applications of 0, 10, 25 or 50 mg/kg molinate (97.6% purity) for 21 consecutive days (Leah, 1989). There were no deaths, and no clinical signs. The major toxicological effects were skin irritation and slight to moderate hydronephrosis at 25 and 50 mg/kg-day. The NOEL for hydronephrosis was 10 mg/kg-day. No examination of male reproductive parameters was reported. The study was considered supplemental.

Inhalation- rat

Male Sprague-Dawley rats were exposed via whole body inhalation for 6 hr/day, 5 days/week for 13 weeks to technical molinate at 0, 0.1, 0.6, 1.8 or 4 mg/m$^3$ (Knapp, 1982a). At four weeks and 12 weeks of dosing, and 7 weeks post-dosing, each male was cohabited with 2 untreated females for a period of 7 nights or until each male had successfully mated with both females. There were no treatment-related changes in body weight, food consumption, hematology, clinical chemistry, brain cholinesterase activity, or histopathology of the brain, liver, kidney, or ocular muscle. No dose related changes were observed in male serum hormone concentrations of LH, FSH, testosterone, thyroxin, triiodothyronine, or TSH at the end of treatment. The pregnancy index (no. pregnant females/no. cohabited) was decreased at doses of 0.6 mg/m$^3$ or above (Table 6). Decreased numbers of implants were noted at 1.8 mg/m$^3$ and above. Necrosis of testicular spermatids and/or spermatocytes was noted at all air concentrations (Table 7). At the end of the treatment period, the severity of the necrosis did not appear to be dose related. Necrotizing rhinitis was also noted at all air concentrations of molinate, and appeared to be dose related increasing in incidence and severity. Some residual effects

(higher incidence and slightly increased severity of paranasal sinusitis in all exposed males) of inhalation exposures was observed in animals kept 2-months post dosing. Only partial reversibility of the effects of molinate on reproduction was observed as well. Following 9 weeks of recovery, there was no significant difference in testicular appearance between controls and the two low dose (0.1 and 0.6 mg/m$^3$) groups (Table 7). Necrosis of spermatids and spermatocytes in the testes of rats at the higher doses (1.8 and 4 mg/m$^3$) was still significantly different from controls and appeared to worsen during the 9 week recovery period. Although the fertility of previously treated rats was not significantly different from controls, the number of animals tested after the recovery phase was only half the number of animals tested during dosing. Consequently, no conclusions can be drawn regarding recovery of fertility. The study was not acceptable under FIFRA requirements, but the data were considered useful for examining possible mechanisms of action of molinate on the male reproductive system.

Table 6. The effects of atmospheric molinate exposure on the fertility of rats (Knapp, 1982a).

| | Atmospheric Exposure Level (mg/m$^3$) | | | | |
|---|---|---|---|---|---|
| Parameter/Time | 0 | 0.1 | 0.6 | 1.8 | 4 |
| Pregnant/Cohabited[a] | | | | | |
| 4 weeks | 42/48$^{++}$ (88%) | 38/48 (79%) | 32/48 (67%) | 31/48* (65%) | 24/48** (50%) |
| 12 weeks | 44/48 (92%) | 42/48 (88%) | 43/48 (90%) | 42/48 (88%) | 36/47* (77%) |
| 7 weeks recovery | 22/24 (92%) | 20/24 (83%) | 20/24 (83%) | 19/24 (79%) | 20/24 (83%) |
| Implants/corpora lutea (%) per gravid female | | | | | |
| 4 weeks | 62±30 | 66±35 | 57±38 | 38±37* | 18±29* |
| 12 weeks | 87±21 | 81±29 | 88±21 | 83±32 | 57±38* |
| 7 weeks recovery | 95±12 | 85±16 | 85±25 | 88±17 | 82±30 |

a/ Two untreated female rats were mated with each treated male rat (24/atmospheric concentration) for a minimum of 7 days at each time point. Half the male rats were terminated at the end of treatment, so only 12/dose were kept for the 7 week post-treatment period.

* Significantly different (P<0.05) from controls by Fisher exact test or Dunnett's two tailed t test.

** Significantly different (P<0.01) from controls by Fisher exact test.

++ Significant (P<0.01) by Peto's dose-weighted chi square trend test.

Table 7. The effects of atmospheric molinate exposure on rat testicular morphology (Knapp, 1982a).

| Parameter/Time | Atmospheric Exposure Level ($mg/m^3$) | | | | |
|---|---|---|---|---|---|
| | 0 | 0.1 | 0.6 | 1.8 | 4 |
| Testicular necrosis, severity grade[a] (N=12) (12 weeks exposure) | 1.00 | 2.00* | 2.00* | 1.92* | 2.08* |
| Testicular necrosis, severity grade (N=12) (9 weeks recovery following 12 week exposure) | 1.00 | 1.00 | 1.25 | 2.17* | 2.45* |

a/ Severity grade is an arithmetic mean based on a scale of 1 to 5 for increasing severity.
* Significantly different, $P<0.05$, from the control by the Mann-Whitney U test.

Male Sprague-Dawley rats were exposed via whole body inhalation for 6 hr/day, 5 days/week for 4 weeks to technical molinate (99% purity) at 0, 0.1, 0.2, 0.3, 0.6 or 1.6 $mg/m^3$ (Knapp, 1982b). At four weeks, each male was cohabited with 2 untreated females for a period of 7 nights or until each male had successfully mated with both females. The major sperm abnormalities observed in epididymal sperm were detached heads and sperm with broken membranes between the head and midpiece, or between the midpiece and tail regions. The NOEL for increased implantation loss, increased numbers of abnormal sperm, and a reduced percentage of motile sperm (cauda epididymis) was 0.3 $mg/m^3$ (Table 8). Chromorhinorrhea was observed in all dose groups and the controls at the same frequency and severity. The data were considered supplemental, examining possible mechanisms of action of molinate on the male reproductive system.

Table 8. The effects of four weeks of atmospheric exposure to molinate on sperm morphology and the fertility of rats (Knapp, 1982b).

| Parameter | Atmospheric Exposure Level ($mg/m^3$) | | | | | |
|---|---|---|---|---|---|---|
| | 0 | 0.1 | 0.2 | 0.3 | 0.6 | 1.6 |
| Pregnant/Cohabited[a] | 40/48 (83%) | 45/48 (94%) | 42/48 (88%) | 45/48 (94%) | 41/48 (85%) | 39/48 (81%) |
| Implants/corpora lutea (%) (per gravid female)[b] | 93±19 | 96±13 | 96±13 | 95±11 | 80±24* | 73±30* |
| Epididymal sperm[b] ($x10^6$/ml) | 34±9 | 41±15 | 38±17 | 27±15 | 29±11 | 23±12 |
| % motile sperm[b] | 73±8 | 71±14 | 78±10 | 72±11 | 68±11 | 58±12* |
| % abnormal sperm[b] | 9±2 | 10±2 | 9±4 | 12±5 | 18±7* | 19±14* |

a/ Two untreated female rats were mated with each treated male rat (24/atmospheric concentration) for a minimum of 7 days.
b/ Mean ± S.D.
* Significantly different, $P<0.05$, from the control by Dunnett's two tailed t test.

Male Sprague-Dawley rats (10/group) were exposed to air concentrations of molinate via whole body at 0, 2.2, 11.1 or 42 $mg/m^3$ for six hours per day, five days a week for 13 weeks (Biodynamics, 1979). Each male was mated with two females at three weeks and at 12 weeks during treatment. Male reproductive performance (mean no. fetuses, implants/corpora lutea) was impaired at all exposure levels (Table 9). Abnormal sperm, and a decrease in epididymal sperm count and implantation rate were detected in some animals at all doses, but the greatest effect (6/10) was at the high dose. Molinate had no affect on post-implantation loss. Testicular degeneration occurred in the treated males with the incidence and severity markedly higher in the high exposure group. In the low and mid-dose groups the change was mild and limited to a few seminiferous tubules and characterized by depletion of spermatozoon. A more severe change was noted especially in the high dose group and characterized by patchy to zonal necrosis of all cells of the germinal epithelium. The degree of testicular degeneration was reflected in the epididymides by a concomitant decrease in the sperm population and an increase in abnormal spermatozoa (deformed sperm cells, giant cells, fragments or dead cells). In the high-dose group, the fertility indices (no. males mating/no. males exposed to females) for the males continued to be depressed at the one-month recovery interval. At the three-month recovery interval, the fertility index improved and was comparable to the control suggesting possible reversibility of the reproductive effect. The data were considered supplemental, examining possible mechanisms of action of molinate on the male reproductive system.

Table 9. The effects of thirteen weeks of atmospheric molinate exposure on testicular pathology and weight, and the fertility of male rats (Biodynamics, 1979).

| Parameter | Atmospheric Exposure Level ($mg/m^3$) | | | |
|---|---|---|---|---|
| | 0 | 2.2 | 11.1 | 42 |
| Three Weeks | | | | |
| No. Pregnant females | 16 | 14 | 7 | 4 |
| Implants/corpora lutea (per gravid female) | 13.1/18.6 (70%) | 6.9/16.5 (42%)** | 5.1/15.9 (32%)** | 1.3/14 (9%)** |
| No. fetuses[a] | 12.6±1.8 | 6.6±3.6** | 4.7±3.8** | --- |
| Twelve Weeks | | | | |
| No. Pregnant females | 17 | 15 | 14 | 0 |
| Implants/corpora lutea (per gravid female) | 12.2/15.4 (79%) | 8.9/15.9 (56%)** | 2.7/12.8 (21%)** | --- |
| No. fetuses[a] | 12.0±2.5 | 8.7±4.5** | 2.5±1.6** | --- |
| Four Weeks Recovery | | | | |
| No. Pregnant females | 19 | 20 | 14 | 11 |
| Implants/corpora lutea (per gravid female) | 11.4/15.3 (75%) | 10.7/15.0 (71%) | 12.1/14.7 (82%) | 10.3/13.4 (77%) |
| No. fetuses[a] | 11±2.8 | 10.4±2.0 | 11.7±1.7 | 0** |
| Twelve Weeks Recovery | | | | |
| No. Pregnant females | 18 | 17 | 17 | 17 |
| Implants/corpora lutea (per gravid female) | 12.1/13.8 (88%) | 12.7/14.0 (86%) | 12.5/14.7 (85%) | 12/16.2ψ (74%) |
| No. fetuses[a] | 11.7±2.1 | 12.1±2.5 | 12.0±2.9 | 11.6±3.7 |
| Testicular wt. (g) N=10 | 3.36 | 3.43 | 3.29 | 2.57** |
| Testicular degeneration | 0/10 (0%) | 4/10ψ (40%) | 3/10 (30%) | 8/10ψ (80%) |

a/ Mean ± SD

ψ Significantly different (P<0.05) from control by Fisher exact test.

** Significantly different (P<0.01) from the control by Dunnett's two tailed t test.

Gavage- mouse

One hundred male, CD-1 mice of proven fertility were randomized into five dose groups; vehicle control (corn oil), 2, 20, 105, or 200 mg/kg-day molinate (98.2% purity) (Killinger, 1980a). The mice were dosed daily by gavage for seven weeks. Fertility was determined by mating each treated and control male with two untreated females after 2, 4, and 6 weeks of treatment. The number of pregnancies, the number of implants, and the number of viable fetuses were significantly reduced in litters fathered by mice treated with 105 and 200 mg/kg-day molinate (Table 10). The 2-week NOEL for these reproductive endpoints was 20 mg/kg-day. The data were considered supplemental, examining possible mechanisms of action of molinate on the male reproductive system.

Table 10. Effects of molinate by gavage on fertility in the mouse (Killinger, 1980a).

| | Dosage (mg/kg-day) | | | | |
|---|---|---|---|---|---|
| Parameter/Time | 0 | 2 | 20 | 105 | 200 |
| Fertility[a] | | | | | |
| 2 weeks | 28/38 (74%) | 31/38 (82%) | 28/40 (70%) | 15/38* (39%) | 17/30 (57%) |
| 4 weeks | 33/38 (87%) | 34/38 (90%) | 38/40 (95%) | 32/38 (84%) | 9/30* (30%) |
| 6 weeks | 33/38 (87%) | 35/38 (92%) | 38/40 (95%) | 27/38* (71%) | 8/30* (27%) |
| Implants/Corpora Lutea | | | | | |
| 2 weeks | 11/12 (92%) | 12/12 (100%) | 11/13 (85%) | 8/11* (73%) | 11/14 (79%) |
| 4 weeks | 12/15 (80%) | 13/13 (100%) | 13/14 (93%) | 10/13 (77%) | 5/10* (50%) |
| 6 weeks | 11/13 (85%) | 11/14 (79%) | 12/14 (86%) | 9/13* (69%) | 5/12* (42%) |
| Viable fetuses[b] | | | | | |
| 2 weeks | 10±4 | 10±3 | 10±4 | 7±4* | 10±3 |
| 4 weeks | 10±4 | 10±3 | 11±2 | 8±4 | 3±3* |
| 6 weeks | 10±2 | 10±3 | 10±3 | 7±4* | 4±3* |

a/ Number of pregnant females/number of females mated.
b/ Mean ± SD
* Significantly different, P<0.05, from control by Fisher's exact test.

Gavage- rabbit

Adult male New Zealand White rabbits (10/group) were dosed by gavage with molinate (99% purity) in corn oil at 0, 10, 100 or 200 mg/kg-day for 12 weeks (Tinston, 1991). Ten females per group were designated for insemination by the ten males in the same group at four different time points (-1, 4, 8, and 12 weeks of exposure). Because of the inconsistency in the results at 100 mg/kg-day, and the low group size in the 200 mg/kg-day (due to five mortalities), the apparent decline in rabbit fertility was considered inconclusive (Table 11). There were no consistent effects on erythrocyte or brain cholinesterase activities at any treatment level. There was no evidence for any consistent pathological changes which could be attributed to treatment with molinate. The data were considered supplemental, examining possible mechanisms of action of molinate on the male reproductive system.

Table 11. The effects of molinate by gavage on rabbit fertility after eight and twelve weeks of exposure (Tinston, 1991).

| | Dosage (mg/kg-day) | | | |
|---|---|---|---|---|
| Parameter/Time | 0 | 10 | 100 | 200 |
| Mean No. implants/corpora lutea[a] | | | | |
| (8 weeks) | 7.3/9.8 (74%) | 7.8/10.4 (75%) | 5.5/9.5 (58%) | 4.0/7.0 (57%) |
| (12 weeks) | 9.4/10.6 (87%) | 8.3/10.3 (81%) | 9.2/11.2 (82%) | 5.8/11.3 (51%) |

a/ N = 10 rabbits in the control, 10 and 100 mg/kg groups. N = 5 rabbits in the 200 mg/kg group.

Adult male New Zealand White rabbits (15/group) were dosed orally by gavage with molinate (99% purity) in corn oil at 0, 40, 80 or 160 mg/kg-day for 13 weeks (Tinston, 1992). Fifteen females per group were designated for insemination by the ten males in the same group at four different time points (7 weeks before dosing and at 5, 9 and 13 weeks of exposure). Seven mortalities occurred during the study (one in the 40 mg/kg-day, two in the 80 mg/kg-day, and four in the 160 mg/kg-day), consequently the high dosage was lowered to 120 mg/kg-day after 5 weeks. Red blood cell cholinesterase activity was inhibited (11 to 23%) in all dose groups at all times. Examination of sperm at the light microscope level indicated a dose-related increased incidence of atypically stained heads and abnormal morphology in ejaculated and epididymal sperm samples from the 80 and 160/120 mg/kg-day groups (Table 12). However, no detectable sperm abnormalities could be discerned at the electron microscope level. In addition pre-implantation losses were observed in females mated with treated males (all treatment doses) in the fifth and ninth week of treatment. A NOEL for pre-implantation loss could not be established. The NOEL for abnormal staining of sperm was 40 mg/kg-day. The data were considered supplemental, examining possible mechanisms of action of molinate on the male reproductive system.

Table 12. The effects of molinate on rabbit fertility and sperm morphology with up to thirteen weeks of dosing by gavage (Tinston, 1992).[a]

| Paramter/Time | Dosage (mg/kg-day) 0 | 40 | 80 | 160/120[b] |
|---|---|---|---|---|
| Mean # implants/corpora lutea | 10.3/11.6 | 8.2/11.7 | 8.2/11.7* | 7.6/11.6* |
| (5 weeks)[c] | (94%) | (70%) | (70%) | (66%) |
| (9 weeks)[d] | 8.9/10.7 (83%) | 6.9/11.1* (62%) | 7.2/9.8 (73%) | 6.6/11* (60%) |
| (13 weeks)[e] | 7.86/11 (71%) | 9.83/11.5 (85%) | 8/10.5 (76%) | 7.5/10.3 (73%) |
| Atypical staining of sperm head[f] | | | | |
| Week 5 | 2.9±4.5 | 5.6±4.9 | 6.6±6.7 | 12.1±13.1* |
| Week 9 | 2.0±2.8 | 3.5±3.8 | 9.0±10.1* | 13.5±21.4* |
| Week 13 | 2.4±2.8 | 5.2±5.6 | 9.7±10.9* | 18.5±22.1* |
| Epididymal (week 13) | 1.9±2.2 | 3.8±3.9 | 10.4±14.4* | 19.8±27.3* |
| Abnormal sperm heads[f,g] | | | | |
| Week 5 | 8.9±7.2 | 12.3±8.2 | 16.3±9.5 | 21.5±13.8 |
| Week 9 | 8.8±6.5 | 12.1±8.8 | 20.6±17.4 | 21.9±21.6 |
| Week 13 | 8.8±6.4 | 14.7±11.6 | 23.5±21.6 | 26.9±21.8 |
| Epididymal (week 13) | 36.0±21.7 | 40.2±19.5 | 48.3±19.7 | 42.6±27.5 |
| Abnormal sperm morphology[f.h] | | | | |
| Week 5 | 10.2±7.9 | 14.6±8.8 | 19.6±12.2 | 23.3±13.7 |
| Week 9 | 10.1±7.1 | 14.4±9.5 | 23.4±18.6 | 23.2±21.3 |
| Week 13 | 10.6±6.9 | 17.2±11.6 | 25.8±22.0 | 28.6±21.8 |
| Epididymal (week 13) | 37.2±21.4 | 42.0±18.6 | 50.8±20.0 | 44.5±26.6 |

* Significantly different (P<.05) from controls by Fisher's exact test of the individual data.

a/ From summary data provided by the registrant in draft report; statistical analyses were performed upon receipt of individual animal data.

b/ Lowered to 120 mg/kg after 5 weeks.

c/ N=14 in controls; N=13 in 40 and 80 mg/kg groups; N=8 in the 160 mg/kg group.

d/ N=15 in controls; N=11 at 40 mg/kg; N=12 at 80 mg/kg; N=10 at 160 mg/kg.

e/ N=14 in controls; N=13 at 40 mg/kg; N=12 at 80 mg/kg; N=9 at 160 mg/kg.

f/ N ≥ 11 (mean %±SD)

g/ Head abnormalities were: detached, missing acrosome, abnormal size regardless of shape, and double heads.

h/ Abnormal sperm morphology- total head and/or tail abnormalities.

Gavage- monkey

Male Cynomolgus monkeys (10/group) were dosed by gavage with 0 (corn oil), 0.2, 10 or 50 mg/kg-day molinate (purity not stated) for 12 weeks (Zuhlke and Bee, 1991). No changes in sperm morphology were identified at either the light or electron microscope levels. The study was of very limited value for evaluating possible effects on sperm morphology due to the tremendous variability in the parameters measured. No attempt was made to examine potential adverse effects on fertility. The data were considered supplemental, examining possible mechanisms of action of molinate on the male reproductive system.

## D. CHRONIC TOXICITY/ONCOGENICITY

**Summary.** Lifetime exposure of male rats to molinate caused kidney tumors at only the highest dose (29 mg/kg-day), implying a potential threshold for oncogenicity. Tumor formation in female rats was not affected by molinate treatment. Neither male nor female mice exhibited increased tumor formation related to molinate treatment. The principal non-oncogenic toxicological endpoints from chronic exposure to molinate were neurotoxicity and reproductive toxicity. The NOEL for neurotoxicity (demyelination of sciatic nerve) in rats at one year was 1.9 mg/kg-day. At two years, the Lowest Observed Effect Level (LOEL) for neurotoxicity (skeletal muscle atrophy, peripheral nerve degeneration, and distal spinal cord changes) was 0.3 mg/kg-day. Indications of reproductive toxicity in rats at two-years were oligospermia (NOEL = 1.8 mg/kg-day), and ovarian thecal/interstitial cell vacuolation/hypertrophy (NOEL = 2 mg/kg-day). In mice, the NOEL for neurotoxicity (increased incidence and severity of sciatic nerve degeneration/demyelination and Schwann cell hyperplasia; increased frequency of eosinophilic bodies in the spinal cord and the medulla of the brain) was 10.4 mg/kg-day and 13.9 mg/kg-day for males and females, respectively; the NOEL for ovarian effects was 13.9 mg/kg-day; and the NOEL for testicular degeneration was 1.0 mg/kg-day. No effects of molinate on testicular function were reported in dogs. The NOEL for neurotoxic effects (clinical signs) in dogs was 1.0 mg/kg-day. The 1-year NOEL for reduced hematocrit in dogs was 1 mg/kg-day.

Diet- Rat

Fischer rats (60/sex/group) were fed technical molinate (98.8% purity) at 0, 8, 16 or 32 mg/kg-day (reduced to 0, 0.63, 2.0 or 6.32 mg/kg-day at week 18) for two years (Woodard, 1977a). Histological examination did not reveal any carcinogenic effect. However, the design and conduct of this study limit any final conclusions with regard to oncogenic effects in this species because the dosages were too low, and there was excessive loss of tissue samples from autolysis (Appendix A). In addition, the histological examinations were inadequate. A NOEL could not be established due to the inadequacy and insufficiency of the sample analysis and data presentation. The study was unacceptable to DPR under the Federal Insecticide Fungicide and Rodenticide Act (FIFRA) guidelines (USEPA, 1984).

In a combined chronic toxicity/oncogenicity study, fifty Crl:CD (SD) BR rats/sex were administered molinate at 0, 7, 40, or 300 ppm (corresponding to 0.3, 1.8, 13 mg/kg-day for males, and 0.4, 2.0, 15 mg/kg-day for females from consumption data) in the diet for up to 2 years (Pettersen and Richter, 1990). Another group for 1-year interim examination had 10 rats/sex administered molinate at 7, 40, and 300 ppm and 20 rats/sex for controls and at 600 ppm (corresponding to 0.3, 1.9, 17, 29 mg/kg-day for males; 0.4, 2.3, 17, 35 mg/kg-day for females). Ovarian thecal/interstitial cell vacuolation/hypertrophy was reported at the 12-month interim examination in rats treated at 300 and 600 ppm (Table 13). Animals being treated at 40 and 300 ppm for 24 months also exhibited the same effect. The 2-year NOEL for ovarian thecal/interstitial cell vacuolation/hypertrophy was initially set at 7 ppm (approximately 0.4 mg/kg-day). However, a "blind" re-examination of the tissue slides by a different pathologist indicated the NOEL for ovarian thecal/interstitial cell vacuolation/hypertrophy was

actually 40 ppm (2 mg/kg-day) (Hodge, 1993b). Modest oligospermia was noted in epididymides at 300 ppm, and testicular degeneration was noted at 600 ppm. The NOEL for male reproductive effects was 40 ppm (approximately 1.8 mg/kg-day in males). Histopathological examination of the sciatic nerve in both males and females at one year revealed degeneration/demyelination at 300 and 600 ppm. The 1-year NOEL for neurotoxicity in males was 1.9 mg/kg-day. At two years, animals at 300 ppm exhibited hind limb ataxia beginning on day 651. Skeletal muscle atrophy, peripheral nerve degeneration, and distal spinal cord changes were observed at all doses (Table 14). There was no NOEL at two years, and the LOEL for neurotoxicity was 7 ppm (approximately 0.3 mg/kg-day). Body weight gain was significantly ($P<0.05$) depressed (14%) in male rats at 300 ppm. Inhibition of red blood cell cholinesterase activity (10 - 39%) without associated clinical signs was observed at 600 ppm. The combined incidence of adenomas and carcinomas (5/48) observed in kidneys of males at 300 ppm was significantly ($P<0.05$) higher than the concurrent controls (0/47), but not the historical controls (maximum of 2 adenomas or carcinomas/60 animals examined occurring in 2/9 studies) (Table 14). The first adenoma was observed at week 106, and the first carcinoma at week 92. As the carcinomas appeared first, this suggested there was no progression from benign towards malignant tumors. None of the tumors were associated with early deaths. No kidney tumors were noted at any other dosage in males, but control females exhibited an adenoma (1/29). The combined incidence of hepatocellular adenomas and carcinomas in male rats exhibited a statistically significant ($P<0.05$) trend, but the occurrence of liver tumors (malignant, benign, or combined) at any treatment level was not significantly different from the incidence in concurrent controls (Table 14). The occurrence of testicular tumors was apparently not treatment related, as the incidence of interstitial cell tumors at any dosage was not significantly different from controls, and there was no significant trend. The study was acceptable to DPR under FIFRA guideline requirements.

Table 13. Effects of molinate on the frequency of thecal/interstitial cell vacuolation/hypertrophy in the ovaries of CD/BR rats (Pettersen and Richter, 1990; Hodge 1993b).

| | Dose (ppm) | | | | |
|---|---|---|---|---|---|
| Termination Time | 0 | 7 | 40 | 300 | 600 |
| 12 month-interim termination | 0/20 (0%) | 0/10 (0%) | 0/10 (0%) | 10/10** (100%) | 20/20** (100%) |
| 24 month-final termination | 1/16 (6%) | 0/20 (0%) | 2/19 (11%) | 27/29** (93%) | --- |
| 13-24 month unscheduled death | 5/30 (17%) | 3/24 (13%) | 8/28 (29%) | 17/20** (85%) | --- |

** Significantly different, $P<0.01$, from control by Fisher's exact test.

Table 14. Histopathologic effects associated with molinate in rats exposed via diet for 2 years (Pettersen and Richter, 1990).

| | Male Dosage (mg/kg-day) | | | | Female Dosage (mg/kg-day) | | | |
|---|---|---|---|---|---|---|---|---|
| Parameter | 0 | 0.3 | 1.8 | 13 | 0 | 0.4 | 2.0 | 15 |
| **Non-Neoplastic Lesions** | | | | | | | | |
| Skel. musc. atrophy | | | | | | | | |
| Thigh | 6/70++ (9%) | 20/60** (33%) | 28/60** (47%) | 43/60** (72%) | 1/70 (1%) | 6/60* (10%) | 12/60** (20%) | 11/60** (18%) |
| Gluteus | 5/17+ (29%) | 12/15** (75%) | 16/19** (84%) | 25/28** (89%) | | | | |
| Sciatic nerve degeneration | 21/69++ (30%) | 32/60** (53%) | 31/60* (52%) | 50/60** (83%) | 11/70++ (16%) | 16/60 (27%) | 23/60** (38%) | 37/60** (62%) |
| Spinal cord inflammation | 0/62++ (0) | 6/48** (13%) | 8/51** (16%) | 17/57** (30%) | | | | |
| **Neoplastic Lesions[a]** | | | | | | | | |
| Kidneys | | | | | | | | |
| cortical adenoma | 0/47 | 0/46 | 0/49 | 2/48 | 1/29 | 0/32 | 0/34 | 0/41 |
| carcinoma | 0/47 | 0/46 | 0/49 | 3/48 | 0/29 | 0/32 | 0/34 | 0/41 |
| combined[b] | 0/47++ (0%) | 0/46 (0%) | 0/49 (0%) | 5/48* (10%) | 1/29 (3%) | 0/32 (0%) | 0/34 (0%) | 0/41 (0%) |
| Liver | | | | | | | | |
| hepatocell. aden. | 1/40 | 1/31 | 1/36 | 3/45 | 0/29 | 2/32 | 1/34 | 1/41 |
| hepatocell. carcin. | 0/40 | 0/33 | 2/37 | 2/46 | 0/29 | 0/32 | 0/34 | 0/41 |
| combined | 1/40+ (3%) | 1/33 (3%) | 3/37 (8%) | 5/46 (11%) | 0/29 (0%) | 2/32 (6%) | 1/34 (3%) | 1/41 (2%) |
| Testes | | | | | | | | |
| Interstitial cell | 3/45 | 5/40 | 5/42 | 7/48 | - | - | - | - |

* Significantly different (P<0.05) from control by Fisher's exact test.
** Significantly different (P<0.01) from control by Fisher's exact test.
\+ Significant (P<0.05) by Peto's dose-weighted chi-square trend test.
++ Significant (P<0.01) by Peto's dose-weighted chi-square trend test.
a/ Expressed as number of incidences per number of animals examined from the time the first tumor was reported.
b/ The highest incidence of kidney tumors (adenomas or carcinomas) in historical controls was 2/60.

A Russian study cited in a California Department of Health Services' review indicated that molinate administered to rats at 6.5 and 13 mg/kg-day for 10 months caused a series of adverse effects (CDHS, 1989). The adverse effects included embryotoxicity, changes in adrenal and oxidative enzyme functions, hypothermia, a decrease in hemoglobin and hematocrit values, and a decrease in erythrocyte and leukocyte counts at the higher dosages. A decrease in the sulfhydryl content of the blood was also reported. CDHS estimated NOEL for hematological effects was 0.65 mg/kg-day using

default assumptions. The study was unacceptable to DPR under FIFRA guidelines as it was in summary form; the duration of the study was too short; an inadequate number of animals were used; inadequate histopathology was performed; and the clinical chemistry and hematological analyses were incomplete.

Dietary - Mouse

A feeding study in male and female mice showed no treatment related adverse effects up to 14.2 mg/kg-day for 99-101 weeks (Woodard, 1977b). A separate experiment was conducted to examine the effects from exposure including the in-utero and nursing periods. The same level of molinate (3.6, 7.2 and 14.2 mg/kg-day) fed to pregnant mice from mid-gestation through weaning, and to their offspring for up to 78 weeks, did not cause any adverse effect except a slight decrease in the survival rate of the offspring treated at the highest dosage of 14.6 mg/kg-day. Histological examination revealed no evidence of carcinogenic effects. A NOEL of 7.2 mg/kg-day was reported based on the decrease in survival rate. The study was considered unacceptable to DPR under FIFRA due to the inadequate histological examination and the lack of data on hematology/blood chemistry.

Groups of 50 CD-1 mice of each sex were fed a constant dietary concentration of molinate (97.6% purity) at 0, 10, 100, 1000, or 2,000 ppm (corresponding to 0, 1.0, 10.4, 105, or 200 mg/kg-day for males and 0, 1.3, 13.9, 133, or 249 mg/kg-day for females) for 18 months (Potrepka and Morrissey, 1991). Administration of molinate to female mice at 2,000 ppm decreased the survival rate. A significant ($P<0.01$) increase in incidence and severity of adrenal degeneration and mineralization in both males and females was observed at 1,000 and 2,000 ppm. Clinical signs indicative of neurological involvement were also observed in mice at 2,000 ppm. Histopathological examination of the nervous system revealed evidence of treatment-related changes at 1,000 and 2,000 ppm. There were: 1) increased incidence and severity of sciatic nerve degeneration/demyelination, 2) increased incidence of Schwann cell hyperplasia, and 3) increased frequency of eosinophilic bodies in the spinal cord and the medulla of the brain (Table 15). Testicular degeneration was increased in a dose dependent manner at 100 ppm and above (Table 16). An increased incidence of thecal/interstitial cell hyperplasia of the ovary was seen in animals fed molinate at 1,000 and 2,000 ppm. The histopathological evaluation revealed no evidence of a treatment-related effect on the incidence of any tumor type in females. The incidence of interstitial cell tumors in the testis was 0/48, 1/32, 1/46, 5/46, 0/49 at the respective doses. In the absence of a dose response, the tumor incidence at 1,000 ppm was not considered a consequence of the treatment. The NOEL for testicular degeneration was 10 ppm (equivalent to 1.0 mg/kg-day for males). The NOEL was 100 ppm (equivalent to 10.4 and 13.9 mg/kg-day for males and females, respectively) based on the effects on the nervous system and ovaries. The study was acceptable to DPR under FIFRA guidelines.

Table 15. Histopathologic effects associated with the neurotoxicity of molinate in mice exposed via diet for 18 months (Potrepka and Morrissey, 1991).

| | Male Dosage (mg/kg-day) | | | | | Female Dosage (mg/kg-day) | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| Parameter[a] | 0 | 1.0 | 10.4 | 105 | 200 | 0 | 1.3 | 13.9 | 133 | 249 |
| **Brain**, medulla eosinophil. bod. | 12/46 (26%) | 14/45 (31%) | 19/47 (40%) | 22/49* (45%) | 37/46** (76%) | 11/49 (22%) | 7/48 (15%) | 11/47 (23%) | 33/49** (67%) | 33/46** (72%) |
| **Sciatic nerve** Schwann cell hyper. | 1/50 (2%) | 2/49 (4%) | 0/48 (0%) | 7/50* (14%) | 24/49** (50%) | 2/50 (4%) | 0/50 (0) | 3/49 (6%) | 25/49** (50%) | 43/49** (88%) |
| demyelination | 15/50 (30%) | 13/49 (27%) | 17/48 (35%) | 37/50** (74%) | 44/49** (90%) | 23/50 (46%) | 20/50 (40%) | 26/49 (53%) | 39/49** (80%) | 49/49** (100%) |
| **Spinal cord,** thoracic eosinophil. bod. | 3/47 (6%) | 1/42 (2%) | 3/47 (6%) | 9/50 (18%) | 24/46** (52%) | 2/47 (4%) | 1/48 (2%) | 0/43 (0) | 37/47** (79%) | 32/45** (71%) |
| **Spinal cord,** sacral eosinophil. bod. | 0/47 (0) | 0/46 (0) | 0/46 (0) | 4/50 (10%) | 8/46** (17%) | 0/47 (0) | 0/49 (0) | 0/47 (0) | 7/47** (15%) | 5/45* (11%) |

a/ All parameters, both male and female, exhibit a significant trend (P<0.01) by Peto's dose-weighted chi-square trend test.
* Significantly different (P<0.05) from control by Fisher's exact test.
** Significantly different (P<0.01) from control by Fisher's exact test.

Table 16. Effects of chronic molinate exposure on reproductive parameters in the mouse (Potrepka and Morrissey, 1991).

| | Male Dosage (mg/kg-day) | | | | |
|---|---|---|---|---|---|
| Parameter | 0 | 1 | 10.4 | 105 | 200 |
| Testes wt. (g)[a] | 0.231 | 0.216 | 0.225 | 0.219 | 0.173* |
| Testes degeneration | 10/50 (20%) | 16/48 (25%) | 24/48* (50%) | 34/49* (69%) | 34/50* (68%) |
| | Female Dosage (mg/kg-day) | | | | |
| Parameter | 0 | 1.3 | 13.9 | 133 | 249 |
| Ovary Thecal/Interstitial Hypertrophy | 1/50 (2%) | 2/48 (4%) | 3/47 (6%) | 9/49* (18%) | 29/49* (59%) |

a/ Mean weight
* Significantly different, P<0.05, from control by Fisher's exact test.

Oral - Dog

In a one-year study, technical molinate (97.6% purity) was administered in gelatin capsules to dogs at 0, 1, 10, 50, or 100 mg/kg-day (Pettersen and Wadsworth, 1990). The 100 mg/kg-day group was taken off treatment on day 106 and administered empty capsules for the remaining duration of the study. Neurological effects included clinical signs of ataxia, splayed hind limbs, reduced locomotor activity, tremor, abnormal voice, and noisy breathing in 50 and 100 mg/kg-day groups. Many of the functional deficits observed in animals at 100 mg/kg-day showed no signs of recovery upon removal from the treatment for 259 days. At 50 mg/kg-day, dogs exhibited hind limb functional deficits (awkwardness to ataxia) beginning at about 3 months. Intermittent clinical signs (abnormal postural reactions) were noted in male (1/4) and female (2/4) dogs dosed with molinate at 10 mg/kg-day for 9 months. Microscopic findings indicated eosinophilic bodies or vacuolation in the brain, significant ($P<0.05$) reduction in brain weight (20%), demyelination in the periphery at 50 mg/kg-day, and demyelination of the sciatic nerve at all doses (Table 17). However, considering the sciatic and tibial nerves together, there was no clear dose response in incidence or severity in the histological evidence of neuropathies at the lower doses compared to the controls. The NOEL for neurotoxicity, based on clinical signs, was 1 mg/kg-day. Evidence of a mild hemolytic anemia included: a decrease in red blood cells, hemoglobin, and hematocrit; an increase in reticulocyte counts and red blood cell fragility; an increase in the degree and/or incidence of splenic hemosiderosis and/or extramedullary hematopoiesis; an increase in hemosiderin-laden Kupffer cells; an increase in platelet counts. Reduction in hematocrit was statistically significant in males at 10, 50, 100 mg/kg-day for 3 and 6 months. The NOEL for mild hemolytic anemia was 1 mg/kg-day based on the reduction in hematocrit. The study was acceptable to DPR under FIFRA guidelines.

Table 17. Histopathological evidence of neurotoxic effects of molinate in the dog after oral exposure for one year (Pettersen and Wadsworth, 1990).

| | Male Dosage (mg/kg-day) | | | | Female Dosage (mg/kg-day) | | | |
|---|---|---|---|---|---|---|---|---|
| Parameter | 0 | 1 | 10 | 50 | 0 | 1 | 10 | 50 |
| Brain vacuolation (medulla) | 0/4 | 0/4 | 1/4 | 2/4 | 1/4 | 0/4 | 0/4 | 2/4 |
| Brain eosinophilic bodies (medulla) | 0/4 | 0/4 | 0/4 | 2/4 | 0/4+ | 0/4 | 0/4 | 3/4 |
| Sciatic nerve demyelination | 0/4+ | 3/4 | 3/4 | 4/4* | 2/4 | 1/4 | 4/4 | 2/4 |
| Tibial nerve demyelination | 2/4 | 2/4 | 0/4 | 4/4 | 1/4 | 0/4 | 1/4 | 3/4 |
| Recurrent laryngeal nerve demyelination | 0/4+ | 0/4 | 0/4 | 4/4* | 0/4+ | 0/4 | 0/4 | 4/4* |

* Significantly different ($P<0.05$) from control by Fischer's exact test.
\+ Significant ($P<0.05$) by Peto's dose-weighted chi-square trend test.

## E. GENOTOXICITY

Summary. The genotoxicity of molinate was examined in several assay systems. One study with mouse lymphoma cells *in vitro* demonstrated mutagenicity with metabolic activation. Molinate was positive in one bone marrow micronucleus test, and negative in another. Elevated frequencies of both chromosome aberrations and sister chromatid exchanges with activation were noted. However, these cytogenetic effects were not consistent in repeat assays, and were not dose-related (none occurring at the highest dose). Molinate did not cause unscheduled DNA synthesis. No mutagenic activity was indicated in microbial systems, with or without metabolic activation.

### Gene Mutation

Molinate (97.6% purity) was tested with *Salmonella typhimurium* strains TA 1535, TA1537, TA1538, TA98, and TA100 at 0 (DMSO), 1.6, 4, 8, 40, 200, 1000 or 5000 *u*g/plate, with and without activation for 64-68 hours incubation (Callander, 1988). A third trial involved strains TA1535 and TA1537 with activation only at 0.32, 0.8, 1.6, 4, 8, or 20 *u*g/plate. There was no consistent increase in revertants. The study was acceptable to DPR. The acceptability of the genotoxicity studies is based on the Toxic Substances Control Act guidelines (Federal Register, 1985).

Molinate (98.8% purity) was tested in a series of assays in mouse L5178Y +/- cells with doses ranging from 0.0125 to 0.28 *u*l/ml without activation and from 0.01 to 0.1 *u*l/ml with activation using 48 hour or 96 hour expression times (Majeska, 1984a). No mutagenicity was observed in the absence of activation, but activation, either mouse or rat liver S9 extract, induced a 2 to 5 fold increase in mutation frequency. The results were reproducible, and not due to artifacts of selection or toxicity. The study was acceptable to DPR.

Molinate was tested in a *Salmonella* assay and found negative for mutagenicity as tested with 8 strains of *Salmonella typhimurium* (Anderson *et al.*, 1972). The study was incomplete and unacceptable to DPR.

Molinate (purity unstated) at 0, 0.005, 0.5 and 50 ppm was tested with strains TA1535, TA1537, TA1538, and TA100, with and without activation (Piper, 1975). No adverse effect was reported. The study was unacceptable to DPR because no individual plate counts were reported; positive controls were inadequate; strain TA98 was not tested; too few dose levels were used; the S9 protocol was inadequate; and the test material was inadequately characterized.

Molinate (purity unstated) was tested at 6 dose levels ranging from 0.01 to 500 *u*l/plate using *Salmonella* strains TA98, TA100, TA1535, TA1537 and TA1538, and yeast strain D4, with and without activation (Brusick, 1975). Toxicity was found at 100 and 500 *u*l/plate. No adverse effect was reported. The study was unacceptable to DPR because there was only one plate per dose level; the negative control values with activation were high; and the test material was inadequately characterized.

Molinate (99.8% purity) was tested at 0, 10, 50, 100, 500, 1000, or 3000 *u*g/plate with *Salmonella* strains TA98, TA100, TA1535, TA1537, and TA1538 and *Escherichia coli* strain WP2 *hcr*, with and without activation (Shirasu *et al.*, 1977). Toxicity was observed at 3000 ug/plate, but no adverse effects. The study was unacceptable to DPR due to a lack of quality assurance; only duplicate plates; lack of lot number identification.

Structural Chromosomal Aberration

Molinate (97.6% purity) was incubated at 0 (DMSO), 24, 95 or 190 *u*g/ml with human lymphocytes from two donors (1 male and 1 female) *in vitro* for 3.25 to 3.75 hours in the presence or absence of S9 (rat liver) activation (Howard and Richardson, 1988). No adverse clastogenic effect was noted. This study was acceptable to DPR.

Molinate (98.8% purity) was given by gavage to male mice (15 mice/group) at 0, 200, 400, or 600 mg/kg, and to female mice (15 mice/group) at 0, 100, 200, or 400 mg/kg (Majeska, 1983). Five thousand bone marrow cells were sampled from 5 mice/sex/time point at 24, 48 and 72 hours. No adverse effects were noted. The study was acceptable to DPR.

Molinate (98.8% purity) was tested in a series of assays in L5178Y mouse lymphoma cells with doses ranging from 0.0125 to 0.2 *u*l/ml without activation, and from 0.0025 to 0.04 ul/ml with activation (Majeska, 1984b). Neither chromosome aberration frequency, nor sister chromatid exchange frequency was increased in the absence of activation. Activation with rat liver S-9 extract resulted in some statistically significant increases in both chromosome aberration and SCE frequencies, but the effects were not dose-related, and not repeatable. The study was acceptable to DPR.

CFLP mice (5/sex/group) were given molinate (97.4% purity) by gavage at 175, 350, 525, or 700 mg/kg (Pinter *et al.,* 1990). The dose at 700 mg/kg was too toxic for evaluation. A statistically significant increase in the incidence of micronucleated polychromatic erythrocytes (MPCE) was found at 525 mg/kg at 48 hours but not at 24 or 72 hours after dosing. Pronounced chromosome damage and moderated myelotoxic effects were observed at 350 mg/kg in the absence of general toxicity. At 175 mg/kg there was no difference in the mean MPCE frequency between the controls and the treated animals. The positive control compound, cyclophosphamide, caused a high incidence of MPCE in all experiments. The study was not acceptable to DPR due to the lack of individual data and information on the conduct of the study.

Other Genotoxic Effects

Rat hepatocytes were exposed to molinate (97.6% purity) at concentrations of $10^{-9}$ to $10^{-2}$ molar for 17 to 20 hours in 2 independent tests (Trueman, 1989). Doses of $10^{-4}$ and $10^{-5}$ were selected as the high concentration experiments 1 and 2, respectively. The three subsequent lower concentrations from each experiment were also selected for unscheduled DNA synthesis evaluation. Net nuclear grain counts were less than zero for all molinate treatments examined. Thus, no adverse effects were indicated. The study was acceptable to DPR.

Molinate (99.8% purity) was tested at 0, 1, 5, 10, 25, 50 and 100% (v/v) on the paired *B. subtilis* strains H17 (repair competent) and M45 (repair deficient) in a disc diffusion assay using the streak technique (Shirasu *et al.*, 1977). There was no indication of recombination. The study was considered acceptable to DPR.

F. REPRODUCTIVE TOXICITY

**Summary.** In addition to causing decreased male fertility and increased sperm abnormalities, molinate induced reduced fertility in female rats. The NOEL for reduced fertility in the female was 50 ppm (approximately 3.7 mg/kg-day). The NOEL for histopathological changes in the rat ovary was 0.44 mg/kg-day. Human epidemiological data from production workers were not utilizable because of inadequate quality assurance/quality control and poor study design.

Diet- rat

Molinate (97.6% purity) was administered in the diet to female Sprague Dawley rats (25/dose) at dosages of 0 (0.1% corn oil), 6, 50, or 450 ppm (approximately 0, 0.44, 3.7 or 33.3 mg/kg-day) for two generations (Gilles and Richter, 1989). No clinical signs or necropsy findings suggestive of toxicity were observed at any dosage. Significant ($P<0.05$) reductions in the litter size were observed at 450 ppm, and vacuolation/hypertrophy of ovarian thecal/interstitial cells occurred at dosages of 50 and 450 ppm in both generations (Table 18). The NOEL for reduced fertility was 50 ppm (approximately 3.7 mg/kg-day). The NOEL for histopathological changes in the ovary was 0.44 mg/kg-day. The study was acceptable to DPR as a supplemental study under FIFRA testing guidelines.

Table 18. Effects of molinate on fecundity and ovarian function in rats (Gilles and Richter, 1989).

| Parameter | Dosage (mg/kg-day) 0 | 0.44 | 3.7 | 33 |
|---|---|---|---|---|
| Thecal/Interstitial cell vacuolation/hypertrophy | | | | |
| ($P_0$) | 0/25 (0%) | 0/25 (0%) | 2/25 (8%) | 25/25* (100%) |
| ($P_1$) | 0/25 (0%) | 0/25 (0%) | 4/25 (16%) | 25/25* (100%) |
| Litter Size[a] | | | | |
| $P_0$ | 13.3±2.1 | 13.3±2.1 | 13.7±3.6 | 11.5±2.3** |
| $P_1$ | 13.3±3.7 | 13.2±3.0 | 12.7±2.5 | 11.8±2.4* |

a/ Mean ± SD; mean litter size is the number of live pups on day 4 plus all pups either dead or missing from day 0 to day 4.
* Significantly different, $P<0.05$, from control by Dunnett's two tailed t test.
** Significantly different, $P<0.01$, from control by Dunnett's two tailed t test.

Sprague-Dawley rats (25/sex/group) were treated with molinate (purity not stated) at 0, 0.063, 0.2 or 0.63 mg/kg-day for three generations (Woodard, 1977c). Decreased fertility was observed at the highest dose. No teratogenic effects or abnormal histopathology were observed. The LOEL could not be determined. This study was considered unacceptable to DPR under FIFRA guidelines due to its lack of characterization of the active ingredient, lack of dietary analysis, and insufficient numbers of female $F_1$ breeders.

Epidemiological Studies- human

Male workers from three molinate production plants were evaluated for adverse reproductive effects from working with Ordram® (Taves, *et al*., 1984a,b,c,d). The "absorbed dose" was estimated to range from 0.07 to 0.26 mg/kg-day. No significant adverse effects were reported regarding sperm count, proportion of normal appearing sperm, and fertility history. The number of workers from different plants participating in the studies ranged from 18 to 107. The studies suffered from major deficiencies in their data/sample collection and analyses. Retrospective fertility data collected from the men were found to be unreliable. Sperm samples could not be proven to be obtained from the individual submitting the samples. [In two instances, individuals with vasectomies submitted semen samples with sperm in the sample.] The samples were analyzed for sperm count and percent normal sperm, as measured by light microscopy. Comparison of groups by change in exposure hours with change in sperm count or percent normal sperm gave equivocal results. Multivariate regression analyses erased any association between Ordram® exposure and altered sperm parameters. The report indicated that the sensitivity of the study was too limited to detect a decrease in sperm count of less than 40-50% and a decrease in normal appearing sperm of less than 25%. Documentation of the exposure concentration/dose was not adequate to allow quantitative assessment. The power of the study, along with the validity of the conclusions, was severely limited by the procedure used to collect the reproductive histories, the methodology used for determining natality, the methodology used for estimating exposure levels, the validity of the sperm count data, the statistical procedures for analyzing sperm count and percent abnormal sperm, the lack of data on sperm motility, and the absence of electron microscopic evaluation of sperm morphology. Therefore, the information provided was unsuitable to characterize the potential of molinate to affect male reproduction in humans.

## G. DEVELOPMENTAL TOXICITY

**Summary.** Molinate was not teratogenic in rats or rabbits. In rats, the NOEL for maternal toxicity (cholinergic signs, decrement in food consumption and weight gain) and developmental toxicity (increased resorptions, intrauterine growth retardation) was 35 mg/kg-day. In rabbits, the NOEL for maternal toxicity (decrement in weight gain) and developmental toxicity (increased resorptions, intrauterine growth retardation) was 20 mg/kg-day.

Gavage- Rat

Female Crl:CD (SD) BRVAF/Plus rats (26/dose) were given molinate (97.6% purity) by gavage at 0 (corn oil), 2.2, 35 or 140 mg/kg-day on days 6-15 of gestation (Minor, 1990). Maternal food consumption and weight gain were reduced (17% and 68%, respectively), and cholinergic signs (salivation) were observed at 140 mg/kg-day (day 14). Also at 140 mg, fetal resorptions were significantly ($P<0.01$) increased (9%), litter size decreased (42%), and there was evidence of intrauterine growth retardation (decreased fetal weight, dilated brain ventricles, incomplete ossification of the sternebrae). Both the maternal and developmental NOELs were 35 mg/kg-day. This study was acceptable to DPR under FIFRA guidelines.

Gavage - Mouse

No teratogenic effects were observed when molinate (96.5% purity) was given in the diet to pregnant mice at 8 or 24 mg/kg-day from day 6 to day 18 of gestation (Woodard, 1967). This study was not acceptable to DPR as a FIFRA Guideline study because there were insufficient treatment groups, and the dosages were too low. At day 18, there were insufficient numbers of pregnant females and fetuses to draw any conclusions.

Gavage- Rabbit

Molinate (98.8% purity) at 0, 2, 20 or 200 mg/kg-day administered by gavage to pregnant New Zealand white rabbits caused toxic effects in both the dams and the pups (Minor, 1985). Maternal toxicity included a significant ($P<0.05$) decrement in body weight gain (360%), and an increase in relative liver weight (119%). Fetal toxic effects which may or may not be attributable to the maternal toxicity were: increased occurrence of aborted and resorbed litters; a slight delay in sternebral ossification; and a reduction in extra paired short ribs. The NOEL for maternal and developmental toxicity was 20 mg/kg-day. This study was considered acceptable to DPR under FIFRA Guideline requirements.

## H. NEUROTOXICITY

**Summary.** Both chickens and rats were used to characterize the neurotoxicity of molinate. The NOEL for acute neurotoxicity in chickens was 200 mg/kg based on observations of microscopic lesions, walking behavior deficits, and other neuromuscular weakness. The 1-day LOEL in rats for clinical signs and performance decline in the functional observational battery tests was 25 mg/kg. The 1-day NOEL for neuronal cell necrosis of the pyramidal neurons in the pyriform cortex in rats was 100 mg/kg.

Gavage- hens

An acute neurotoxicity study was conducted in adult hens administered 2 dosages (3 weeks apart) at 20 or 2,000 mg/kg or four dosages (3 weeks apart) at 63, 200, 630 or 2,000 mg/kg of technical Ordram® by gavage (Sprague, 1983). Acute neurotoxicity was observed at dosages of 630 mg/kg and 2,000 mg/kg. There was striking degeneration in cerebellar peduncles and dorsal funiculi of the cervical spinal cord. More distal regions of the spinal cord and peripheral nerves were affected to a lesser degree. Molinate-elicited toxicity appeared shortly after the dosing. After a 120-day recovery, there was no definitive evidence of residual insult in the central or peripheral nervous systems of the treated hens although the incidence of "neuronal swelling and chromatolysis" was somewhat higher in the brain, and in the cervical and thoracic spinal cord of the 2,000 mg/kg group. The NOEL was 200 mg/kg based on the neurotoxicity with additional observations of microscopic lesions, walking behavior deficits, and other neuromuscular weakness. DPR considered the study unacceptable, but upgradeable upon receipt of additional "time to effect" data for the clinical observations.

Gavage- rat

Alpk:APfSD rats (12/sex/dose) were given single oral doses of molinate (96.8% purity) at 0, 25, 100, or 350 mg/kg by gavage (Horner, 1994). At four hours post dosing, decreased activity was noted in all females and 11/12 males given doses of 350 mg/kg. Other observations were hunched posture, lachrymation, upward curvature of the spine, and urinary incontinence (females only). At 100 mg/kg, 7/12 animals of each sex exhibited decreased activity, urinary incontinence (1 female), and upward curvature of the spine (2 males). At 25 mg/kg, one female exhibited decreased activity, upward curvature of the spine and increased response to touch. There was a statistically significant ($P<0.05$) increase in the time to tail flick in both sexes at all dose levels, which was dose-related. Likewise, these was a significant ($P<0.05$) reduction in overall motor activity for both sexes at all dose levels, which was dose-related. There was no 1-day NOEL for clinical signs and performance decline in the functional observational battery tests. At 16 days, histopathological examination revealed an increase in the incidence of neuronal cell necrosis of the pyramidal neurons in the pyriform cortex of females given 350 mg/kg. The 1-day NOEL for histological evidence of neuropathies was 100 mg/kg. The study was not acceptable to DPR under FIFRA guidelines because some groups were too small for meaningful evaluation (e.g. n=3 for glial fibrillary acidic protein assays); the functional observational

battery was not performed in the systematic manner recommended in the guidelines; and findings were typically not graded as to severity.

Dietary- rat

Alpk:APfSD rats (12/sex/group) were dosed with molinate (96.8% purity) at 0, 50, 150, or 450 ppm for 90 days (Horner, 1994). There was a statistically significant ($P<0.01$) decrease in absolute (but not relative) brain weight in males (2%) and females (4%) at 450 ppm. No other significant histopathological changes were observed. No clinical signs or effects of motor activity and limited functional observational battery measures were reported. Brain cholinesterase was significantly ($P<0.01$) reduced in males dosed with 150 and 450 ppm (15% and 42%, respectively); and significantly ($P<0.05$) reduced in females at 50, 150, and 450 ppm (7%, 26%, and 48%, respectively). Significant ($P<0.01$) inhibition of brain neuropathy target esterase (NTE) activity was noted at all doses in both sexes (from means of 20 to 59% in males, and 25 to 61% in females). The inhibition appeared to be dose related. Plasma cholinesterase activity was not inhibited, but red blood cell cholinesterase activity was significantly ($P<0.01$) in males (27% at 450 ppm) and females (22 and 32% at 150 and 450 ppm). No NOEL exists for inhibition of brain cholinesterase activity or NTE activity. The study was unacceptable to DPR under FIFRA guidelines due to deficiencies in the design and conduct of the Functional Observation Battery tests.

# IV. RISK ASSESSMENT

## A. HAZARD IDENTIFICATION

A review of the toxicological information summarized in Table 19 indicates the most critical/sensitive toxicological endpoints of concern from repeated exposure to molinate were adverse reproductive effects, neurotoxicity, and possible oncogenicity. With regard to reproductive effects, the results consistently demonstrated that exposure of male laboratory animals to molinate via the oral route, or via inhalation caused a decrease in fertility, abnormal sperm morphology, decreased epididymal sperm numbers, and/or testicular degeneration. Decreased fertility, as evidenced by significant ($P<0.05$) pre-implantation loss, was also observed in females mated with male rats, mice and rabbits exposed to molinate by gavage. The pre-implantation loss may have been an indication of the inability of sperm to fertilize ovulated ova. Female rats, administered molinate in the diet, exhibited significantly ($P<0.05$) reduced litter sizes and histopathological abnormalities in ovaries (vacuolation/hypertrophy of the thecal/interstitial cells). Female mice, exposed to molinate in the diet, displayed the similar histopathological abnormalities in the ovaries.

Studies on male rats suggest that the adverse effect of molinate is on the Sertoli cells. Neither serum gonadotropin levels, nor serum androgen levels were affected by exposure to molinate through the oral (Minor, 1981), or inhalation routes (Knapp, 1982a,b). Therefore, molinate does not exert its antifertility effects through alteration of hormonal levels. This tends to rule out the Leydig cells as targets of molinate, as their function in the spermatogenic process appears to be limited to androgen production (Setchel, 1978).

Spermatogenesis occurs in the seminiferous tubules of the testis, and maturation of the sperm is completed in the epididymis (Ewing *et al.*, 1979). If the epididymis were the target of molinate, the effects of molinate would have been observed almost immediately. Yet, antifertility effects in male rats were not observed until the third week following exposure (Minor, 1981). The 3-week latency in observed effects led to the suggestion that molinate affected the later stages of spermatogenesis (Minor, 1981). In addition, histopathological examination of the testes revealed necrosis of spermatids and spermatocytes in the seminiferous tubules (Woodard, 1975b; Knapp, 1982a; Potrepka and Morrissey, 1991). The data do not, however, support the notion that testicular germinal cells are themselves the targets of molinate.

The production of spermatozoa from round spermatids in rats requires approximately 3 weeks (LeBlond and Clermont, 1952; Perey *et al.*, 1961). Yet, studies indicated that the effects of molinate on male reproduction persisted for a period longer than three weeks after dosing had stopped (Woodard, 1975b; Biodynamics, 1979; Knapp, 1982a). The necrosis of spermatids and spermatocytes observed in the testes 9 weeks after dosing had ceased (Knapp, 1982a) was not due to a residual body burden of molinate. Molinate did not concentrate in rats (Ritter, 1991a,b,c), and 75% of a molinate dose was excreted within 24 hours (Peffer, 1991). Nor can the persistent necrosis of spermatocytes and spermatids be due to effects on spermatogonia.

The process of spermatogenesis in rats requires approximately 56 days (LeBlond and Clermont, 1952; Perey *et al.*, 1961). Yet, at 63 days post-dosing, damaged spermatocytes and spermatids were still apparent in the rat testes (Knapp, 1982a). Spermatogonia damaged by molinate would have already completed the process of spermatogenesis by 63 days. In the testes, the only other cell type intimately involved in spermatogenesis is the Sertoli cell (Wright, 1991). Therefore, the Sertoli cells are probably the targets of molinate. .

The fact that molinate did not cause the same type of abnormalities in primate sperm morphology (Zuhlke and Bee, 1991) that had appeared in rodents (Biodynamics, 1979; Minor, 1981; Killinger, 1982; Knapp, 1982a,b), provides insufficient evidence to conclude that the adverse male reproductive effect is a rodent-specific phenomenon. No measurements of Sertoli cell function were conducted, such as production of transferrin, androgen binding protein, and SGP2 (Wright, 1991). Further, the great variability of the measured sperm parameters, and lack of fertility testing also contributed to the study's inconclusive nature.

Human epidemiological studies indicating a lack of effect of molinate on workers in production facilities were not convincing (Taves, *et al*., 1984a,b,c,d). The poor quality of the epidemiological data, insensitive nature of the analyses, and the inadequate documentation of the exposure dose rendered the studies ineffective as qualitative proof that molinate does not have the potential to affect human reproductive systems.

In addition to the effects on male reproduction, molinate also caused decreased fertility in female rats (Gilles and Richter, 1989). No data were available to suggest a possible mechanism of action of molinate on female reproduction. The physiological significance of the increased incidence of vacuolation/hypertrophy of the interstitial/thecal cells in the ovaries of mice and rats is unknown (Gilles and Richter, 1989; Pettersen and Richter, 1990; Potrepka and Morrissey, 1991). Normally, vacuolation/hypertrophy of the interstitial/thecal cells only occurs in rodents towards the end of their lifespan (Cotchin and Roe, 1967). Although not directly correlated, an increase in the phenomenon appears to presage infertility caused by higher dosages of molinate in female animals (Gilles and Richter, 1989).

The weight of evidence suggests molinate should be considered a potential human reproductive toxicant. The risk assessment for potential human exposure uses data from the most critical/sensitive toxicity endpoints, the most sensitive species, and the more sensitive sex. It also takes into consideration the exposure duration and the relevance of the adverse effect.

The non-reproductive effects of molinate can be summarized as follows: Molinate was oncogenic in male rats but not in female rats or mice (either sex) (Pettersen and Richter, 1990; Potrepka and Morrissey, 1991). A mutagenicity study with mouse lymphoma cells demonstrated mutagenicity with metabolic activation (Majeska, 1984a), and a published mouse micronucleus assay was positive for MPCE (Pinter *et al.,* 1990). However, all other genotoxicity studies were negative. Peripheral nerve degeneration was observed in dogs, mice and rats as a result of long-term exposure to molinate (Pettersen and Wadsworth, 1990; Pettersen and Richter, 1990; Potrepka and Morrissey, 1991). Molinate caused developmental toxicity in rabbits (an increased occurrence of aborted and resorbed litters, a slight delay in sternebral ossification, and a reduction in extra paired short ribs) and intrauterine growth retardation in rats (decreased fetal weight, dilated brain ventricles, incomplete ossification of the sternebrae), which may or may not be related to maternal toxicity (Minor, 1985, 1990).

Examination of the application practices for molinate indicates that it is used only during a six week period in a given year. Therefore, the principal types of exposure, both occupational and non-occupational, will be short-term and seasonal. Even though the use-season for molinate is limited to a 6-week period, data suggest the neurotoxic effects of molinate may not be reversible. Dogs dosed by gavage exhibited ataxia, splayed hind limbs, reduced locomotor activity, tremor, abnormal voice, and noisy breathing. Many of the functional deficits observed in animals after 106 days of treatment showed no signs of recovery upon removal from the treatment for 259 days (Pettersen and Wadsworth, 1990). Consequently, potential annual occupational exposure was considered as well.

Short-term Toxicity

A small number of studies examined the toxic effects of short-term exposure to molinate. These effects occurred in developmental and reproductive toxicity studies after repetitive daily dosing. In rabbits, the 13-day No-Observed-Effect-Level (NOEL) for maternal toxicity (decrement in weight gain) and developmental toxicity (increased resorptions, intrauterine growth retardation) was 20 mg/kg-day (Minor, 1985). In rats, the 9-day NOEL for maternal toxicity (cholinergic signs, decrement in food consumption and weight gain) and developmental toxicity (increased resorptions, intrauterine growth retardation) was 35 mg/kg-day (Minor, 1990). The 5-day NOEL for reduced fertility was 11.5 mg/kg-day in male rats (Minor, 1981). A significant ($P<0.01$) decrement in maternal body weight gain was seen at 4 days in rats receiving oral doses of 150, 75 or 15 mg/kg-day (there was no NOEL) in a range finding study (Horner, 1992b). The toxicological significance of the decrement in body weight gain, though, is unclear. Clinical signs (salivation, subdued appearance, hunched posture, piloerection, ocular discharge, stained nose and mouth, urinary incontinence, irregular breathing) were noted in rats at 3 days after being dosed with 150 mg molinate/kg-day (Horner, 1992a). The 7-day NOEL for clinical signs (salivation) in the rat in the same study was 15 mg/kg-day. Rats given 350, 100 or 25 mg/kg of molinate in a single dose exhibited clinical signs (including upward curvature of the spine; hypersensitivity) and a significant, dose-related decline in performance on functional observational battery tests at 4 hours after molinate was administered (Horner, 1994). There was no NOEL.

The NOEL used to calculate a margin of safety for potential acute occupational exposure to molinate was 11.5 mg/kg-day for reduced fertility in rats (Minor, 1981). Although the effect was noted after five daily doses, this is the lowest short-term NOEL. In addition, this NOEL addresses one of the overall toxicological endpoints of concern (reproduction). No single dose studies which examined this effect were available. As oral absorption of molinate was effectively 100% (Ritter 1991a,b,c), the NOELs for oral administration represent the NOEL for an absorbed dose.

Seasonal Toxicity

Molinate, an herbicide associated with rice planting, is only used during a 6 week period in May and June each year. Consequently, the principal long term exposure to molinate is seasonal.

Several subchronic studies investigated the effects of atmospheric concentrations of molinate (Biodynamics, 1979; Knapp, 1982a,b). However, the dosage of molinate absorbed by rats in the whole-body exposure inhalation studies could not be determined accurately. Ordinarily, a theoretical absorbed dosage (mg/kg-day) would be calculated assuming: 1) all molinate entered the body through the respiratory route, and 2) default respiratory values (ventilation frequency and percentage absorbed) could be used to calculate the dosage in mg/kg-day. However, the body burden of rats exposed to atmospheric concentrations of molinate in whole-body exposure studies was derived from more than one route. The absorbed dosage, based on the amount of 4-hydroxy molinate in the urine, of rats exposed via whole body to atmospheric concentrations of molinate was seven times the absorbed dosage of rats exposed via nose-only (Hext, 1991). This was consistent with the scientific literature which indicated that rats, exposed to dusts or chemical vapors via whole-body, absorbed 5-8 times more material than rats exposed via nose-only (Blair *et al.*, 1974; Langard and Nordhagen, 1980; Wolff *et al.*, 1982; Iwasaki *et al.*, 1987; Jaskot and Costa, 1994; Tyl *et al.*, 1995). Further, the absorbed dosage in the nose only portion of the experiment was more than seven times greater than that predicted by the use of default inhalation values (Zielhuis and van der Kreek, 1979). Chamber leakage reported in the nose-only exposures to molinate (Hext, 1991) may have contributed an unquantifiable oral component (possibly due to grooming behavior). The net effect was that the absorbed dosage from a single, six-hour, whole-body exposure of rats was nearly 50 times greater than predicted by calculations using default inhalation values. There are no data on absorbed dosages from repetitive whole-body inhalation exposures. Consequently, the absorbed dosages which caused the toxic effects observed in the repetitive-dose, whole-body exposure inhalation studies cannot be estimated.

Exposure of rats to atmospheric concentrations of molinate for 13 weeks was shown to cause necrotizing rhinitis in one study (Knapp, 1982a), but not in another (Biodynamics, 1979). As the histopathology in either study was not done until the end of the 13 week exposure period, the effect of exposure to atmospheric concentrations of molinate for 6 weeks could not be ascertained.

For the purposes of estimating the risks to humans from seasonal exposure to molinate, the applicable initial lowest NOEL for the oral route was 0.26 mg/kg-day (Lowest-Observed-Effect-Level [LOEL] = 4.6 mg/kg-day) based on sperm abnormalities observed at the light microscope level in a 5-week gavage study in male rats (Minor, 1981). A subsequent 5-week gavage study in male rats attempted to better define the NOEL for sperm abnormalities (Hodge, 1993a). Based on this study, the 5-week NOEL for sperm abnormalities (by scanning electron microscopy) was 0.48 mg/kg-day. The lowest oral NOEL (0.44 mg/kg-day) was for female reproductive effects (histopathological changes in the ovary), reported in a two-generation study in female rats (Gilles and Richter, 1989). This subchronic toxic endpoint was consistent with the histopathological changes in the ovary reported in a two-year combined toxicity/oncogenicity feeding study in rats (Pettersen and Richter, 1990). However, the toxicological significance of this endpoint was not obvious as the histopathological changes in the ovary did not correlate with observed female infertility in the same study (Wickramaratne, 1993). As the subchronic NOEL (0.44 mg/kg-day for histopathological changes in the ovary) was not substantially different from the 5-week NOEL of 0.48 mg/kg-day for sperm abnormalities in males, and the toxicological significance of the latter was readily apparent, the subchronic NOEL (0.48 mg/kg-day) for sperm abnormalities was used as the basis to calculate margins of safety for potential seasonal occupational exposures to molinate.

Annual Toxicity

As the neurotoxic effects of molinate may not be reversible, an appropriate neurotoxic NOEL was sought to serve as the basis for estimating margins of safety for potential annual exposures. Acute neurotoxicity, as indicated by behavioral and histopathological changes, were caused in the hen as the result of a single dose (Sprague, 1983). Although clinical signs were not reported in the mouse, histopathological examination of the nervous system revealed an increased incidence and severity of sciatic nerve degeneration/demyelination; an increased incidence of Schwann cell hyperplasia, and an increased frequency of eosinophilic bodies in the spinal cord and the medulla of the brain (Potrepka and Morrissey, 1991). The 2-year NOEL for histopathological indications of peripheral neuropathies in the mouse was 10.4 mg/kg-day. In rats, the 1-year NOEL for peripheral nerve degeneration (demyelination of the sciatic nerve in both male and female) was 1.9 mg/kg-day. At two years, skeletal muscle atrophy, peripheral nerve degeneration, and distal spinal cord changes were observed at all dosages (Pettersen and Richter, 1990). The 2-year LOEL was 0.3 mg/kg-day. Because there was no NOEL, an Estimated-No-Effect-Level (ENEL) was calculated by dividing the LOEL of 0.3 mg/kg-day by an uncertainty factor of 10 (Dourson and Stara, 1985; USEPA, 1987). This default procedure yielded a 2-year ENEL of 0.03 mg/kg-day for neurotoxicity. However, this 2-year ENEL in rats is derived from a lifetime of continuous exposure to molinate, and is not relevant in calculating margins of safety for potential annual exposures experienced by humans.

The nervous system of dogs appeared to be more sensitive than that of rats to chronic exposure to molinate. Intermittent clinical signs (abnormal postural reactions) were noted in male (1/4) and female (2/4) dogs dosed for several months with molinate at 10 mg/kg-day (Pettersen and Wadsworth, 1990). The 1-year NOEL for neurotoxicity in the dog was 1 mg/kg-day. This NOEL (1mg/kg-day for intermittent clinical signs in dogs) was used to establish margins of safety for theoretical annual occupational exposure to molinate.

Lifetime Toxicity

A mutagenicity study with mouse lymphoma cells demonstrated mutagenicity with activation (Majeska, 1984a), and a published study indicated that molinate caused micronucleus formation in mice (Pinter *et al.,* 1990). However, all other genotoxicity studies were negative. Molinate was oncogenic in male rats, causing kidney tumors only at the highest dose (Pettersen and Richter, 1990). However, female rats did not exhibit any kidney tumors associated with molinate exposure. Nor was molinate oncogenic in either male or female mice (Potrepka and Morrissey, 1991). The possibility that the tumors in the male rats may have been the result of *a*-2u-globulin formation, and thus not relevant to human oncogenicity, was explored. However, molinate did not cause the accumulation of *a*-2u-globulin in the kidneys of male rats (Horner, 1992a). As the kidney tumors produced in the study were rare tumors (Potrepka and Morrissey, 1991), USEPA considered molinate a candidate for regulation based on its oncogenic potential in male rats (Taylor and Rinde, 1992). The $q_1^*$ derived by USEPA is 0.11 $[mg/kg\text{-}day]^{-1}$, which is an estimate of the upper bound on potency.

The weight of evidence suggesting an oncogenic potential for molinate was weak. 1) The evidence for oncogenicity in rats was equivocal as the incidence of hepatocellular adenomas and carcinomas in treated male rats exhibited a statistically significant trend, but the occurrence of adenomas or carcinomas (separately) at any dose was not significantly different from that in concurrent controls. Only male rats developed a significant incidence of kidney tumors (combined adenomas and carcinomas) associated with molinate exposure, and only at the high dose. The kidney tumors appeared at a dose which may have exceeded the maximum tolerated dose, as indicated by a 14% decrement in body weight gain and clear evidence of systemic toxicity (peripheral neuropathies). 2) There was no indication of substance-related oncogenicity in the mouse. 3) Molinate was mutagenic in mouse lymphoma cells *in vitro* with metabolic activation, positive in one bone marrow micronucleus test (negative in another), and caused non-reproducible (and non-dose related) elevation of both chromosome aberrations and sister chromatid exchanges with activation. However, molinate did not cause unscheduled DNA synthesis, nor was mutagenic activity indicated in microbial systems, with or without metabolic activation.

The slope of the dose-response curve for the combined incidence of kidney adenomas and carcinomas was zero between the control animals and the animals at the next two doses. When the slope in the low-dose portion of the dose response curve is effectively zero, mathematical models which assume that there is no threshold (e.g. the linearized multistage model) are not applicable for estimating the slope of the curve. Indeed, when Global 86 was used, the maximum likelihood estimate of the slope was effectively zero [$Q(6) = 2.3 \times 10^{-8}$]. The Chi square goodness of fit value for the calculation was $3.8 \times 10^{-5}$, and the P-value for the Monte Carlo test was 0.58 (Appendix C).

In this circumstance, where the linear multistage model does not fit and a toxicological threshold appears to exist, a margin of safety approach for lifetime exposure may be applied. When USEPA has applied this procedure to a potentially oncogenic endpoint, an additional uncertainty factor of 10 has been used (USEPA, 1986b). The LOEL for kidney tumors in male rats was 13 mg/kg-day with a NOEL of 1.8 mg/kg-day (Petterson and Richter, 1990).

Table 19. Summary of selected molinate toxicity studies

| STUDY | SPECIES | ROUTE | EFFECT | LOEL (mg/kg-day) | NOEL (mg/kg-day) | GENOTOXIC | REF[a] |
|---|---|---|---|---|---|---|---|
| acute (3d)[b] | rat | oral | clinical signs (salivation) | 75 | 15 | | 1 |
| subchron. (5d) | rat | oral | infertility, reduced # implants | 50 | 11.5 | | 2 |
| subchron. (5wk) | rat | oral | decr. sperm motil.,incr sperm abnorm. | 0.98 | 0.48 | | 2 |
| subchron. (10wk) | rat | oral | preimp. loss, sperm abnormalities | 20 | - | | 3 |
| subchron. (12wk) | rat | diet | red. litter number | 8 | - | | 4 |
| subchron. (5wk) | rat | diet | red. litter number | 32 | - | | 5 |
| subchron. (13wk) | rat | inhal. | sperm abnormalities | 0.1 mg/m$^3$ | | | 6 |
| subchron. (4wk) | rat | inhal. | sperm abnormalities | 0.6 | 0.3 mg/m$^3$ | | 7 |
| subchron. (13wk) | rat | inhal. | sperm abnormalities, red. implants. | 2.2 mg/m$^3$ | | | 8 |
| subchron. (4wk) | mouse | oral | fert., pre-implantation loss | 105 | 20 | | 9 |
| subchron. (5wk) | rabbit | oral | abnormal staining sperm | 80 | 40 | | 10 |
| combined | rat | diet | thecal/interstitial cell vacuolation | 15 | 2 | | 11* |
| combined | rat | diet | epididymal oligospermia | 13 | 1.8 | | 11* |
| combined | rat | diet | nerve degeneration | 0.3 | - | | 11* |
| combined | rat | diet | male kidney tumors | 13 | 1.8 | | 11* |
| oncogenicity | mouse | diet | testicular degeneration | 10.4 | 1 | | 12* |
| oncogenicity | mouse | diet | thecal/interstitial cell vacuolation | 133 | 13.9 | | 12* |
| oncogenicity | mouse | diet | neural toxicity | 105 | 10.4 | | 12* |
| chronic | dog | diet | reduced hematocrit | 10 | 1 | | 13* |
| chronic | dog | diet | clinical signs | 10 | 1 | | 13* |
| reproduction | rat | diet | red. fertility | 33.3 | 3.7 | | 14 |
| reproduction | rat | diet | thecal/interstial cell vacuolation | 3.7 | 0.44 | | 14 |
| developmental | rat | gavage | clinical signs; intrauterine growth retard. | 140 | 35 | | 15* |
| developmental | rabbit | gavage | mat. decr. wt. gain; intra. growth retard. | 200 | 20 | | 16* |
| gene mutation | bacteria | *in vitro* | | | | - | 17* |
| gene mutation | mammal | *in vitro* | | | | + | 18* |
| chromosome | mammal | *in vitro* | | | | - | 19* |
| chromosome | mammal | *in vivo* | | | | - | 20* |
| chromosome | mammal | *in vivo* | | | | - | 21* |
| DNA damage | mammal | *in vitro* | | | | - | 22* |

a/ References- 1. Horner, 1992; 2. Minor, 1981 and Hodge, 1993a; 3. Killinger, 1982; 4. Woodard, 1975a; 5. Woodard, 1975b; 6. Knapp, 1982a; 7. Knapp, 1982b; 8. Biodynamics, 1979; 9. Killinger, 1980; 10. Tinston, 1992; 11. Pettersen and Richter, 1990; 12. Potrepka and Morrissey, 1991; 13. Pettersen and Wadsworth, 1990; 14. Gilles and Richter, 1989; 15. Minor, 1990; 16. Minor, 1985; 17. Callander, 1983; 18. Majeska, 1984a; 19. Howard and Richardson, 1988; 20. Majeska, 1983; 21. Majeska, 1984b; 22. Trueman, 1989.

b/ The number in parenthesis is the time when the listed effect was first reported.

* Acceptable to DPR under FIFRA guidelines.

## B. EXPOSURE ASSESSMENT

DPR evaluated the potential adverse effects to workers (farmers, mixers/loaders, flaggers, and pilots) and the general public from exposure to molinate. Members of the general population with potential exposure to molinate include those who regularly eat rice and those served by the water utilities using the Sacramento River as the source of drinking water. The Sacramento River receives water drained from the Sacramento Valley rice fields treated with molinate. Potential routes of exposure to molinate in the drinking water supply include ingestion as well as inhalation and dermal contact from general household uses. Potential exposure for workers was via inhalation and skin contact. Exposure to molinate in the ambient air shortly after application is also a concern for farmers who enter the fields shortly after use, and for the general public residing in communities in the proximate vicinity where molinate is applied by aerial spraying.

### Dietary Exposure

#### *1. Anticipated Residues*

Water seeded rice is the only food crop recommended for the use of molinate to control watergrass. Currently, the tolerance level (maximum residue legally allowed) set for molinate in or on rice grains is 0.1 ppm. The tolerance is for "negligible residues", as the USEPA does not expect that any residues of molinate will be found on rice (CFR, 1992a). Consequently, none of the state or federal commodity monitoring programs test for molinate residues.

Residues of molinate and its degradation products were measured under the "glasshouse" condition (Imai and Kuwatsuka, 1988). Molinate was not detected (<0.4 ppb), but degradation products were found (concentration was not presented). Monitoring results presented by the Stauffer Chemical Company (1985) showed non-detectable concentrations of molinate in/on rice grains. The analytical detection limit used by the Stauffer Chemical Company was 0.05 ppm. In the absence of residue data from monitoring programs, it was assumed that the anticipated residue level of molinate in or on rice grain for the acute exposure was at the minimum detection limit of 0.05 ppm and that for the chronic exposure was 0.025 ppm (50% of the minimum detection limit).

There are no tolerances for molinate in fish, so commercial fish tainted with molinate should be removed prior to sale and cannot be included in the dietary exposure assessment for commercial foodstuffs. Exposure to molinate through consumption of sport fish, though, is theoretically possible as 1) molinate has been found in the Sacramento River and adjacent waterways (Cornacchia *et al.*, 1984; Ross *et al*, 1990; Harrington and Lew, 1992), and 2) fish readily absorb molinate (Lay *et al.*, 1979; Tjeerdema and Crosby, 1987, 1988a,b; Martin *et al.*, 1992). However, a number of factors, including declining concentrations of molinate in the waterways, rapid depuration and metabolism of molinate by fish, and the effect of chemical volatization and degradation during the cooking of fish, would combine to reduce the theoretical exposure to molinate through consumption of sport fish to negligible levels (see Risk Appraisal section).

#### *2. Dietary Exposure Analyses*

Dietary exposure analyses were conducted using the Technical Assessment System (TAS) software programs (TAS, 1992a,b). The dietary consumption estimates were based on data from the 1987-88 Nationwide Food Consumption Survey conducted by the U.S. Department of Agriculture (USDA). The USDA survey was a probability survey of respondents who were representative of the U.S. population. It was conducted in all four seasons of the year and in all regions of the continental United States. Respondents were surveyed for three days in their homes. The analyses for both acute and chronic exposure assumed that residues in the rice actually consumed were at the anticipated residue levels.

*a. Acute Dietary Exposure*

The following assumptions are used to estimate potential acute dietary exposure from measured residues: (1) the residue does not change over time, (2) the concentration of residue does not decrease when the raw agricultural commodity (RAC) is washed, (3) processing of RACs into various food forms does not reduce or increase the residue concentration, and (4) all foods that are consumed will contain the highest reported residue.

Acute dietary exposure analyses were conducted using the Exposure-4® software program developed by Technical Assessment Systems, Inc (TAS). The Exposure-4® software program estimates the distribution of user-day (consumer-day) exposures for the overall U.S. population and specific population subgroups (TAS, 1992a). A user-day is any day in which at least one food from the specific commodity list is consumed. The consumption analysis uses individual food consumption data as reported in the 1987-88 USDA Nationwide Food Consumption Survey (USDA, 1987-88). Based on the 95% percentile of user-days exposures for all specific population subgroups, the potential acute dietary exposure of the population subgroup of non-Hispanics other than black and white had the highest potential acute exposure of 0.40 *u*g/kg-day (Table 20). Other population subgroups had the potential exposure of less than 0.2 *u*g/kg-day ranging from 0.09 to 0.19 *u*g/kg-day.

*b. Chronic Dietary Exposure*

The following assumptions were used to estimate potential chronic dietary exposures from measured residues: 1) the residue level does not change over time, 2) residues are not reduced by washing the RAC, 3) processing into various food forms does not reduce or increase the residue concentration, and 4) exposures to a commodity at all reported residue levels do occur, *i.e.* a commodity with the average calculated residue is consumed every day at an annual average level (dosage).

The potential chronic dietary exposure was calculated using the Exposure-1® software (TAS, 1992b). The food consumption data for the chronic analysis were also derived from the USDA 1987-88 Nationwide Food Consumption Survey. The potential exposure dosage from ingesting rice for non-nursing infants less than one year old was 0.03 *u*g/kg-day (Table 20). Nursing infants less than one year old had the potential exposure dosage of 0.01 *u*g/kg-day. The potential exposure for non-Hispanics other than black or white was 0.03 *u*g/kg-day. The general population in the western region of the United States had the potential exposure dosage of 0.01 *u*g/kg-day.

Table 20. Potential dietary exposure to molinate

| Population Subgroup | Potential Exposure (*u*g/kg-day) | |
|---|---|---|
| | Acute[a] | Chronic[b] |
| U. S. - Overall | 0.13 | 0.01 |
| Western Region | 0.13 | 0.01 |
| Non-Hispanic other than black or white | 0.40 | 0.03 |
| Infants < 1 yr (Nursing) | 0.18 | 0.01 |
| Infants < 1 yr (Non-nursing) | 0.18 | 0.03 |
| Children (1-6 yrs) | 0.19 | 0.01 |
| Children (7-12 yrs) | 0.17 | 0.01 |
| Males (13-19 yrs) | 0.11 | 0.01 |
| Males (20+ yrs) | 0.12 | 0.003 |
| Females (13+/pregnant/not nursing) | 0.07 | 0.002 |
| Females (13+/Nursing) | 0.12 | 0.01 |
| Females (13-19 yrs/NP/NN) | 0.09 | 0.004 |
| Females (20+ yrs/NP/NN) | 0.11 | 0.003 |

a/ Acute exposure assumes residue at the minimum detection limit of 0.05 ppm and represents potential exposure at the 95$^{th}$ percentile.
b/ Chronic exposure assumes residue at half of the minimum detection limit (0.025 ppm).
NP Not Pregnant
NN Not Nursing

Residential Exposure

1. Water Supplies (Sacramento/West Sacramento)

*a. Oral Route*

Table 21 shows the peak concentrations of molinate detected at various monitoring sites from 1986 to 1990. Detailed data were presented in a series of reports: O'Brian, 1989; CDFA, 1986, 1987, 1989, 1991b. The peak concentrations at all sites were found around mid-May through mid-June of the year. Concentrations detected at the Colusa Basin Drains ranged from 16 to 59 ppb during the peak period of 1990 with an average concentration of about 35 ppb. The Sacramento River water had concentrations ranging from 2 to 9 ppb and an average concentration of 4 ppb during the same period.

Table 21. Water concentrations of molinate detected at various monitoring locations in 1986-1990*

| Locations | Concentration (ppb) | | | | |
|---|---|---|---|---|---|
| | 1986 | 1987 | 1988 | 1989 | 1990 |
| CBD1 Maximum | 77 | 43 | 67 | 51 | 51 |
| Average** | 52 | 35 | 47 | 37 | 35 |
| | (7.4) | (2.8) | (5.1) | (3.1) | (5.0) |
| CDB5 Maximum | 88 | 53 | 89 | 60 | 59 |
| Average** | 67 | 37 | 55 | 40 | 34 |
| | (9.4) | (3.3) | (7.5) | (3.6) | (6.3) |
| SR1 Maximum | 11 | 8 | 8 | 6 | 9 |
| Average** | 6 | 5 | 6 | 4 | 4 |
| | (0.7) | (0.8) | (0.5) | (0.5) | (0.9) |
| SRR Maximum | 14 | 6 | 5 | 5 | 7 |
| Average** | 5 | 3 | 3 | 2 | 4 |
| | (0.6) | (0.4) | (0.2) | (0.3) | (0.3) |

* Samples taken by either the City of Sacramento or the California Department of Fish and Game.

** Data represent the arithmetic mean concentrations detected during the peak period from mid-May to mid-June. Numbers in the parenthesis are standard errors. Only concentrations ≥ 20 ppb for CBD1 and CBD5 and ≥ 2 ppb for SR1 and SRR are included in the calculations unless they are within the peak period.

CBD1: Colusa Basin Drain at Roads 109 and 99E near Knight's Landing in Yolo County.

CBD5: Colusa Basin Drain at Highway 20 in Colusa County.

SR1 : Sacramento River at Village Marina in Sacramento County considered to be representative of the intake for the West Sacramento.

SRR : Sacramento River at the intake to the City of Sacramento water treatment facility.

Molinate is oxidized to its sulfoxides by the chlorination process employed in the water treatment facilities (Ross, 1983). Analyses performed by the City of Sacramento from 1983 to 1986 indicated that molinate was not detected in the tap water (CDFA, 1984; Myers, 1983-89). On the other hand, molinate sulfoxide was found in the finished tap water at a level comparable to the level of the parent molinate observed in the pretreated Sacramento River water. DPR concurred with the opinion of the CDHS that measurements of the molinate concentration in Sacramento River water intake for the water treatment facilities could be used as a surrogate to evaluate potential human exposure to molinate and its by-products in drinking water supplies (Berteau, 1984).

CDHS set a MCL of 20 *u*g/L for molinate in drinking water (CDHS, 1989). Based on the 1990 data, molinate concentrations of 2 to 9 ppb (*u*g/L) detected in the Sacramento River water were below the MCL of 20 *u*g/L. The potential daily exposure dosages of molinate and its by-products from ingesting water from Sacramento River are presented in Table 22. Young children residing in West Sacramento could potentially ingest an average of 0.43 *u*g/kg-day to a maximum of 0.89 *u*g/kg-day during mid-May to mid-June. The potential average daily dosage and maximum daily dosage for adults was 0.12 *u*g/kg and 0.25 *u*g/kg, respectively. The potential daily dosage of molinate and its by-products from drinking water for residents in the City of Sacramento was about 80% of that observed for residents in West Sacramento.

Table 22. Potential daily exposure to molinate from drinking water*

| Sources | Exposure Dosage (*u*g/kg-day) | | | |
|---|---|---|---|---|
| | Adult | | Child | |
| | Average | Maximum | Average | Maximum |
| SR1 | 0.12 | 0.25 | 0.43 | 0.89 |
| SRR | 0.10 | 0.19 | 0.35 | 0.65 |

* Assumes 70 kg body weight and daily water consumption of 2 liters for adults. Assumes 10 kg body weight and daily water consumption of 1 liter for the child.

SR1 Sacramento River at Village Marina in Sacramento County considered to be representative of the intake for West Sacramento.

SRR Sacramento River at the intake to the City of Sacramento water treatment facility.

Molinate concentrations in the Sacramento River peaked from mid-May to mid-June for about a month, coinciding with the seasonal application of the herbicide in rice fields and the subsequent drainage of the contaminated water into the Sacramento River after a holding period of up to 19 days. The duration of exposure to detectable concentrations of molinate in drinking water is approximately one month per year. The potential reproductive effects, based on studies in rats, are short-term and seasonal in nature. Thus, the arithmetic average water concentration during the peak period is used to estimate the potential exposure level.

Even though the use-season for molinate is limited to a 6-week period, data suggest the neurotoxic effects of molinate may not be reversible. Consequently, the potential annual exposure to molinate was also calculated. As detectable levels of molinate have been measured in the river water for approximately 30 days each year, the annual exposure would equal the seasonal exposure multiplied by 30 days/365 days per year. Thus, the annual exposure from drinking water would range from 0.01 *u*g/kg-day for adults to 0.04 *u*g/kg-day for children in West Sacramento.

The combined potential exposure dosage from daily ingestion of rice and water from the Sacramento River ranges from 0.13 *u*g/kg-day for the adult population to 0.46 *u*g/kg-day for non-nursing infants less than one year old, with major contribution being from the water (Table 23).

Table 23. Combined potential exposure of West Sacramento residents to molinate from drinking water and diet (rice).

| | Exposure Dosage (*u*g/kg-day) | | |
|---|---|---|---|
| Population | Rice | Water* | Combined |
| Adults | 0.005 | 0.12 | 0.13 |
| Non-Hispanics Other Than Black and White | 0.029 | 0.12 | 0.15 |
| Nursing Infants (<1 year old) | 0.007 | 0.43 | 0.44 |
| Non-Nursing Infants (<1 year old) | 0.029 | 0.43 | 0.46 |

* Data is taken from Table 22, the Average Daily Dosage from SR1, Sacramento River water intake for West Sacramento.

*b. Inhalation Route*

Molinate is very volatile, and evaporation is expected to be the major route of dissipation from water. In general, volatility also increases with the increase in temperature when water is used for showering, laundry, and washing purposes. It is suggested that the contribution from inhalation of volatile organic compounds, such as tetrachloroethene and trichloroethene, from domestic uses of the water supply could be as much as that from the ingestion of two liters of water (Andelman, 1985). A comparison of the calculated Henry's Law Constant (Table 24) shows that the volatility of molinate is about 0.007% to 0.015% of that for tetrachloroethene and trichloroethene. Therefore, the potential exposure to molinate via inhalation from household uses of the water supply is expected to be negligible.

Table 24. Comparison of Henry's Law Constant at 20°C

| Compounds | Henry's Law Constant |
|---|---|
| Molinate | 0.000065 |
| Tetrachloroethene | 0.847 |
| Trichloroethene | 0.393 |

$$\text{Henry's Law Constant H} = \frac{\text{Concentration in Air (}u\text{g/L)}}{\text{Concentration in Water (}u\text{g/L)}}$$

$$= \frac{16.04\ P^*M}{T^*S}$$

Where: P is the equilibrium vapor pressure in torr.
M is the gram molecular weight per mole.
T is the temperature in °K. °K = 273 + °C.
S is the solubility in water in mg/L.

*c. Dermal Route*

Dermal contact with contaminated water while swimming or bathing is a potential route of exposure to molinate. However, as environmental factors (*e.g*. water temperature and flow volume) limit swimming in the Sacramento River and adjacent waterways during May, and no molinate was measured in domestic waters supplies, it is believed that the dermal route is insignificant compared to the oral and inhalation routes.

2. Ambient Air (Maxwell and Williams)

*a. Inhalation*

In 1986, the ambient air concentrations of molinate were measured on the rooftops of the public buildings in four Sacramento Valley towns (Seiber *et al*., 1989). Sampling was carried out for four 24 hour intervals (Monday a.m. through Friday a.m.) for four weeks during the period selected to represent the highest uses of molinate. The measurement of ambient air concentration did not discriminate between fine particulate aerosol and the vapor phase of molinate (Mischke, 1989). The maximum concentration and the highest arithmetic average concentration were detected at Maxwell at 1.7 *u*g/m$^3$ and 0.65 *u*g/m$^3$, respectively. After adjusting for the collection efficiency of 67%, the arithmetic average ambient air concentration of molinate encountered by residents of Maxwell during the season was 0.28 *u*g/m$^3$.

The arithmetic average ambient air concentration of Molinate in 1992 at Maxwell according to measurements taken by the California Air Resources Board was **0.72** *u*g/m$^3$ (range = 0.4 to 1.17 *u*g/m$^3$) and at Williams it was **0.39** *u*g/m$^3$ (range = 0.16 to 0.50 *u*g/m$^3$) (EMPM, 1992).

Using the standard default value for human inhalation of 0.29 m$^3$/kg-day for adults and 0.6 m$^3$/kg-day for infants (Anderson *et al*., 1983), and assuming a 50% retention and 100% absorption (Raabe, 1986, 1988), the estimated seasonal dosage for an individual in Maxwell in 1986 was:

Adults- (0.28 *u*g/$m^3$)x(0.29 $m^3$/kg-day)/2 = 0.04 *u*g/kg-day

Infants- (0.28 *u*g/$m^3$)x(0.6 $m^3$/kg-day)/2 = 0.09 *u*g/kg-day

The estimated seasonal dosage for an individual in Maxwell in 1992 was:

Adults- (0.72 *u*g/$m^3$)x(0.29 $m^3$/kg-day)/2 = 0.10 *u*g/kg-day

Infants- (0.72 *u*g/$m^3$)x(0.6 $m^3$/kg-day)/2 = 0.22 *u*g/kg-day

The estimated seasonal dosage for an individual in Williams in 1992 was:

Adults- (0.39 *u*g/$m^3$)x(0.29 $m^3$/kg-day)/2 = 0.06 *u*g/kg-day

Infants- (0.39 *u*g/$m^3$)x(0.6 $m^3$/kg-day)/2 = 0.12 *u*g/kg-day

Potential annual exposures may be estimated by amortizing the seasonal dosage (which occurs during a 35 day period) over the entire year (365 days). Thus, the arithmetic average annual daily dosage would be equivalent to the seasonal dosage multiplied by 35/365:

| Maxwell | 1986 | 1992 |
|---|---|---|
| Adults | 0.004 *u*g/kg-day | 0.01 *u*g/kg-day |
| Infants | 0.009 *u*g/kg-day | 0.021 *u*g/kg-day |
| Williams | | |
| Adults | not measured | 0.006 *u*g/kg-day |
| Infants | not measured | 0.012 *u*g/kg-day |

A theoretical worst case scenario can be derived from the supposition that a family could live in a home adjacent to a treated rice field. Rice fields are only treated once during the rice growing season, so the exposure would follow the pattern described by Ross and Sava (1986). They reported that the highest measured air concentration of molinate, 48 cm above the surface of a treated rice paddy, was 48 *u*g/$m^3$ on the day of application. However, the air concentrations dropped precipitously with time, theoretically reaching non-detectable levels in a week. The 5 day arithmetic average was estimated to be 18.7 *u*g/$m^3$. Theoretically, individuals living in a home next to the rice paddy would receive the following short-term daily dosages through the inhalation route:

Adults- (18.7 *u*g/$m^3$)x(0.29 $m^3$/kg-day)/2 = 2.71 *u*g/kg-day
Infants- (18.7 *u*g/$m^3$)x(0.6 $m^3$/kg-day)/2 = 5.61 *u*g/kg-day

As a rice paddy is treated only once during the year with molinate, there would not be a seasonal, or annual exposure to consider.

*b. Combined Inhalation and Dietary Exposure*

Because a computerized method for estimating seasonal dietary exposure was not available, theoretical seasonal dietary exposure to molinate was calculated using EX1 (assumes some individuals do not consume rice), with residues assumed to be 50% of the MDL (0.025 ppm) representing an average residue level. The combined potential seasonal exposures to molinate in the ambient air and by ingestion of rice for the people living in the city of Maxwell were calculated to be: general adult population (0.11 *u*g/kg-day), non-hispanics other than black and white (0.13 *u*g/kg-day), nursing infants less than 1 year old (0.23 *u*g/kg-day), and non-nursing infants less than 1 year old (0.24 *u*g/kg-day). The combined potential annual exposures to molinate in the ambient air and by ingestion of rice for the people living in the city of Maxwell were calculated to be: adults (0.015 *u*g/kg-day), non-hispanics other than black and white (0.039 *u*g/kg-day), nursing infants less than 1 year old (0.028 *u*g/kg-day), and non-nursing infants less than 1 year old (0.047 *u*g/kg-day).

Occupational Exposure

Primary routes of exposure for workers were from inhalation and via body and hand contact for mixers/loaders, flaggers, and pilots. The work period was estimated to last 35 days per year (Donahue, 1993).

Handlers of the Ordram 8E formulation (liquid), which is little used in California, must wear work clothing under chemical-resistant coveralls (including disposable coveralls such as Tyvec QC , Tyvek laminated with Saranex , Polypropylene laminated with polyethylene, Encase II , or a similar approved brand), chemical resistant gloves and foot coverings, tightly woven head covering, and a full face respirator. If the loader uses a closed system, he is not required to wear chemical resistant coveralls (the outer layer) or a full face respirator. However, thye must wear a chemical resistant apron over their work clothing.

*1. Farmers*

Farmers entering the rice field shortly after molinate application also had potential exposure to the airborne herbicide. The potential exposure duration for farmers was estimated to be one hour per day. Measurement at 48 cm above the water surface of the rice field showed an air concentration of 48 *u*g/$m^3$ immediately after the application of molinate in 1985 (Ross and Sava, 1986). Under the study conditions, the concentration decreased to 8.3 *u*g/$m^3$ three days after the treatment. The arithmetic average concentration was 21.95 *u*g/$m^3$ within three days after molinate application. Assuming a respiratory rate of 0.84 $m^3$/hr a body weight of 75.9 kg (Appendix B), a daily exposure of one hour, and 50% retention and 100% absorption, the potential arithmetic average exposure dosage was 0.12 *u*g/kg-day for farmers entering the rice field within three days after molinate application.

*2. Agricultural Workers*

Theoretical exposures associated with the use of Ordram 8E, based on surrogate exposure data from EPTC (Ross *et al.,* 1989), are indicated in Table 25. The use permit issued for the liquid formulation of molinate (Ordram 8E) precludes any use other than irrigation (Ross, 1991c). Consequently, Ordram 8E cannot be applied by air in the State of California.

Table 25. Theoretical exposure dosages for workers associated with the use of Ordram 8E.

| Workers | Absorbed Exposure Dosage[a] (*u*g/kg-day) Dermal[b] | Inhalation[c] |
|---|---|---|
| Ground M/L[d] | 0.05 | 0.78 |
| Ground App.[d] | 0.02 | 0.34 |
| Comb. M/L/A[d] | 0.10 | 1.63 |
| Water Run[e] | 1.05 | - |

a/ Based on 75.9 kg body weights (Ross, 1991a,b)
b/ Mitigation includes long-sleeved shirt, long pants, and rainsuit; dermal absorption estimated at 53%.
c/ Calculated from 100% absorption, 50% retention; assumes no ingestible particulate matter
d/ Derived from surrogate data, as cited from Ross, *et al*: 1989. "EPTC, mixer/loader/applicator studies for the reevaluation of EPTC". DPR Vol. 117-063.
e/ Based on surrogate data derived from using EPTC (Formoli, 1996).

Ordram 10G (granular formulation) is applied from the air. Ordram is not used every day during the five-week season that it could potentially be used (Donahue, 1993). Some days the wind is in excess of 7 mph, and the material cannot be applied from the air. Intermittent mechanical difficulties further limit flying days. On those days, there is no exposure to the work crews. Examination of the pilots' logs indicates that aerial applications take place on only 74-80% of the available days.

The estimated exposures of pilots and flaggers associated with the application of the new granular formulation are shown in Table 26. Loaders were identified as having the highest potential exposure among the workers involved in the application of molinate. The exposure assessment for loaders has been revised based on the new data submitted by the registrant (Zeneca, 1993). Use of granular molinate is currently regulated under the 1995 Permit Conditions (Andrews, 1995) which requires work clothing under or over several types of disposable coveralls (including chemical resistant types), chemical resistant gloves and boots, a tightly woven head coveing and full facer respirator.

The absorbed dosage was estimated from measured levels of 4-hydroxymolinate in the urine of workers handling 1200 lb bags during applications in California (Zeneca, 1993; Formoli, 1993d). The measured absorbed dosage includes any potential dietary, drinking water, or other non-occupational exposures as well. It was assumed that the absorbed dosage was directly proportional to the amount of formulation handled. To calculate the maximum permissible exposure to molinate, the measured geometric mean of the absorbed daily dosage was multiplied by the proportion of molinate handled, as well as the range of absorbed dosages, are presented in Table 26. Drivers involved in direct loading remained in the closed cab at all times when the bags were being loaded into the airplane. Drivers involved in transfer-loading would often vacate the cab to assist the loader on the bucket, after hoisting the bag up. Loaders involved in direct loading, emptied the bag directly into the airplane hopper. Loaders involved in transfer loading, emptied the bag into the bucket and then into the airplane hopper.

The maximum number of bags which could be loaded directly by an individual in a given day would be 14, based on physical limitations, not permit conditions (Ross, 1994). An individual doing direct loading for the season would be limited to loading 228,000 pounds based on the seasonal margin of safety. The weight limit per individual doing a combination of direct and indirect loading for the season would be 135,000 pounds, also based on the seasonal margin of safety. The estimated maximal mean absorbed daily dosages of molinate for direct loaders using the 10-G formulation being loaded from fourteen 1200 lb bags (Zeneca, 1993) are shown in Table 26. Use of 50 lb bags of

Ordram 10G is prohibited in California (Ross, 1991). Mean absorbed daily dosages for drivers and workers who are involved in both direct and indirect loading are also included in Table 26.

Pilots must also wear work clothing. If they become involved in the loading operation, they, too, must wear the same personal protective equipment as the loaders. Flaggers must wear work clothing and be either in an enclosed cab, or wear cloth or disposable coveralls over their work clothing, a NIOSH approved half-face organic vapor (pesticide) respirator, eye protection, tightly woven head covering, and chemical resistant gloves.

Table 26 - Absorbed Daily Dosages for Short-Term, Seasonal, and Annual Exposure to Molinate for Workers in Various Job Classifications.

| Work Task | (N) | ADD[a] (*u*g/kg-day) | SADD[b] (*u*g/kg-day) | AADD[c] (*u*g/kg-day) |
|---|---|---|---|---|
| Driver (no suit) | 5 | 0.76±2.31 | 0.59 | 0.06 |
| Driver (carbon suit) | 5 | 0.56±2.20 | 0.43 | 0.04 |
| Direct Loader (Tyvek) | 10 | 10.58±2.70 | 4.10 | 0.39 |
| Direct Loader (Carbon) | 9 | 6.89±2.54 | 2.66 | 0.25 |
| Direct + Trans. (Tyvek) | 9 | 3.70±2.02 | 2.85 | 0.27 |
| Direct + Trans. (Carbon) | 6 | 4.85±1.70 | 3.74 | 0.36 |
| Flagger[d] | 8 | 1.1±1.3 | 0.8 | 0.08 |
| Pilot[d] | 5 | 3.5+1.4 | 2.7 | 0.26 |

a/ Geometric mean Absorbed Daily Dosage ± standard deviation for short-term exposure; assumes direct loading of 16,800 lb Ordram 10G, or direct and transfer loading of 5,000 lb.

b/ Geometric mean Seasonal Absorbed Daily Dosage, assumes applications occur for approximately 27 days during the 35 day use season, and that the weight limits imposed by permit conditions will be observed (Ross, 1994).

c/ Geometric mean Annual Absorbed Daily Dosage, assumes that molinate is used only 27 days in each 365 day year.

d/ From Appendix B.

C. RISK CHARACTERIZATION

The reproductive effects observed in animals exposed to molinate are considered to have a biological threshold. Exposure below a certain level is not expected to cause adverse effects. The margin of safety (MOS) for exposure to molinate is calculated as the ratio of an appropriate NOEL established in animal studies to the potential exposure dosage estimated for human population.

$$\text{Margin of Safety} = \frac{\text{NOEL}}{\text{Exposure Dosage}}$$

Dietary Exposure

*1. Acute Dietary Exposure*

The greatest calculated theoretical acute dietary exposure to molinate for various population subgroups having user-days at the 95$^{th}$ percentile of consumption was 0.4 *u*g/kg-day (Table 20). Thus, the lowest MOS for theoretical acute dietary exposure during a single day was approximately 29,000 (Table 27), based on a NOEL of 11.5 mg/kg-day for sperm abnormalities observed in rats exposed to molinate daily for 5 days (Minor, 1981).

*2. Chronic Dietary Exposure*

The highest calculated theoretical chronic dietary exposure to molinate from rice consumption was 0.03 *u*g/kg-day (Table 20). Consequently, the lowest MOS for theoretical chronic dietary exposure was 16,000 (Table 27), based on a NOEL of 0.48 mg/kg-day for sperm abnormalities in rats (Hodge, 1993a).

Residential Exposure

1. Water Supplies

*a. Seasonal And Annual Exposure via Drinking Water and Diet*

The seasonal and annual MOS-oral route calculated for the different subpopulations residing in West Sacramento are presented in Table 27. Drinking water was the primary contributor to the potential exposure to molinate via the oral route, assuming the by-products of molinate are as toxic as the parent compound. In order to examine the worst case situation, the seasonal MOSs for ingesting rice, water, or the combined exposure from both were calculated. These MOSs ranged from 1,000 to 96,000 for the population subgroups. Concentrations of molinate and its by-products in the water distributed to the Sacramento residents were approximately 80% of that distributed to the West Sacramento residents. The combined theoretical annual MOS for the Sacramento residents would range from approximately 2,000 to 200,000.

Table 27. Margins of safety for potential seasonal and annual oral exposure to molinate by residents in West Sacramento

| | Seasonal | | | Annual Margin of Safety[2] | | |
|---|---|---|---|---|---|---|
| Populations | Rice | Water | Combined | Rice | Water | Combined |
| Adults | 96,000* | 4,000 | 3,000 | 200,000 | 8,000 | 8,000 |
| Non-Hispanics Other Than Black and White | 17,000 | 4,000 | 3,000 | 50,000 | 8,000 | 7,000 |
| Nursing Infants (<1 year old) | 69,000 | 1,000 | 1,000 | 143,000 | 2,000 | 2,000 |
| Non-Nursing Infants (<1 year old) | 17,000 | 1,000 | 1,000 | 34,000 | 2,000 | 2,000 |

1/ For seasonal exposures, the margins of safety were based on subchronic NOEL of 480 *u*g/kg-day for sperm abnormalities in rats (Hodge, 1993a).
2/ For annual exposures, the margins of safety were based on an NOEL of 1000 *u*g/kg-day for clinical signs of neurotoxicity in the dog (Pettersen and Wadsworth, 1990).
* All values greater than 1,000 were rounded to the nearest 1,000.

2. Ambient Air

*a. Inhalation route*

For the worst case exposure, a family living in a home adjacent to a treated field, a different NOEL would have to be used to calculate the margins of safety as fields are treated one-time only. The NOEL for short term exposure (5 days) is 11.5 mg/kg-day for infertility in rats (Minor, 1981). The margins of safety (MOSs) for short term ambient air exposure would be as follows:

Adults- (11,500 *u*g/kg-day/2.71 *u*g/kg-day) = **4,000**
Infants- (11,500 *u*g/kg-day/5.61 *u*g/kg-day) = **2,000**

From the seasonal exposure data, it would appear that infants are the population subgroup with the greatest potential exposure to atmospheric concentrations of molinate. The NOEL for sperm abnormalities (480 *u*g/kg-day), a reflection of Sertoli cell toxicity, was used as the basis for calculating the potential risk to infants from ambient exposure to molinate. The margins of safety (MOSs) for seasonal average ambient air exposure in Maxwell and Williams were:

Maxwell, 1986.
Adults- (480 *u*g/kg-day/0.04 *u*g/kg-day) = **12,000**
Infants- (480 *u*g/kg-day/0.09 *u*g/kg-day) = **5,000**

Maxwell, 1992.

Adults- (480 *u*g/kg-day/0.10 *u*g/kg-day) = **4,800**
Infants- (480 *u*g/kg-day/0.22 *u*g/kg-day) = **2,000**

Williams, 1992.

Adults- (480 *u*g/kg-day/0.06 *u*g/kg-day) = **8,000**
Infants- (480 *u*g/kg-day/0.12 *u*g/kg-day) = **4,000**

The NOEL of 1,000 *u*g/kg-day for neurotoxicity (clinical signs) in the dog was used to estimate the margins of safety (MOSs) for annual average ambient air exposure in Maxwell and Williams:

Maxwell, 1986.

Adults- (1,000 *u*g/kg-day/0.004 *u*g/kg-day) = **250,000**
Infants- (1,000 *u*g/kg-day/0.009 *u*g/kg-day) = **111,000**

Maxwell, 1992.

Adults- (1,000 *u*g/kg-day/0.010 *u*g/kg-day) = **100,000**
Infants- (1,000 *u*g/kg-day/0.021 *u*g/kg-day) = **48,000**

Williams, 1992.

Adults- (1,000 *u*g/kg-day/0.006 *u*g/kg-day) = **167,000**
Infants- (1,000 *u*g/kg-day/0.012 *u*g/kg-day) = **83,000**

*b. Combined Inhalation and Dietary Exposure*

The MOSs for combined potential seasonal and annual exposure to molinate in the ambient air and by ingestion of rice for the people living in the city of Maxwell are presented in Table 28. Comparison of Table 27 and Table 28 shows that the MOS was essentially the same with or without the additional theoretical exposure from ingestion of rice.

Table 28. Margins of safety for Maxwell residents from potential seasonal and annual exposure to molinate from inhalation and rice consumption.

| Populations | Seasonal MOS[1] | Annual MOS[2] |
|---|---|---|
| Adults | 4,000* | 10,000 |
| Non-Hispanics Other Than Black and White | 2,000 | 8,000 |
| Nursing Infants (<1 year old) | 2,000 | 4,000 |
| Non-Nursing Infants (<1 year old) | 1,000 | 4,000 |

1/ Margins of safety based on subchronic NOEL of 480 *u*g/kg-day for sperm abnormalities in the rat (Hodge, 1993a).

2/ Margins of safety for all population subgroups were based on a chronic NOEL of 1,000 *u*g/kg-day for neurotoxicity in dogs (Pettersen and Wadsworth, 1990). Annual dietary exposure to molinate did not change, however, absorbed dosages through the inhalation route were modified by 30/365 to account for the limited seasonal exposure during the course of one year.

* All values greater than 1,000 were rounded to the nearest 1,000.

Occupational Exposure

Margins of safety for the geometric mean potential occupational exposures of agricultural workers to molinate ranged from 1,000 to 32,000 for short-term; from 117 to 1,700 for seasonal, and from 3,000 to 33,000 for annual exposures (Table 29). If the 95$^{th}$ percentile of short-term exposure [geometric mean x (standard deviation)$^{1.645}$] were considered for these same workers, the MOSs would range from 214 for direct loaders (wearing Tyvek) to 6,765 for flaggers. The MOS for farmers entering the fields for 1 hour a day immediately after molinate application was 96,000.

Table 29- Margins of Safety for Short-Term, Seasonal, and Annual Exposures Associated with Handling Molinate in Various Job Classifications.

**Ordram 10G (Granular Formulation)**

| Work Task | Short-term MOS[a] | Seasonal MOS[b] | Annual MOS[c] |
|---|---|---|---|
| Driver (no suit) | 15,000 | 813 | 17,000 |
| Driver (carbon suit) | 21,000 | 1,000 | 25,000 |
| Direct Loader (Tyvek) | 1,000 | 117 | 3,000 |
| Direct Loader (Carbon) | 2,000 | 178 | 4,000 |
| Direct + Trans. (Tyvek) | 3,000 | 168 | 4,000 |
| Direct + Trans. (Carbon) | 2,000 | 128 | 3,000 |
| Flagger | 10,000 | 600 | 13,000 |
| Pilot | 3,000 | 178 | 4,000 |

**Ordram 8E (Liquid Formulation)**

| Workers | Short-term MOS | Seasonal[d] MOS | Annual MOS |
|---|---|---|---|
| Ground M/L | 14,000 | 750 | 17,000 |
| Ground App. | 32,000 | 1,700 | 33,000 |
| Comb. M/L/A | 7,000 | 380 | 8,000 |
| Water Run | 11,000 | 593 | 13,000 |

a/ Based on the geometric mean ADD (Table 26) and a NOEL of 11.5 mg/kg-day for reduced fertility in rats (Minor, 1981).
b/ Based on a NOEL of 480 *u*g/kg-day for sperm abnormalities in the rat (Hodge, 1993a).
c/ Based on an NOEL of 1,000 *u*g/kg-day for clinical signs in the dog (Pettersen and Wadsworth, 1990).
d/ The duration of application of Ordram 8E during the 35 day season is unknown. Consequently, it was assumed that the formulation would be used by the same workers on all 35 days.
* All values greater than 1,000 were rounded to the nearest 1,000

The oncogenic potential of molinate was considered equivocal, and the data could not be properly described by the linearized multistage model. Nevertheless, public concern regarding potential carcinogens compels some examination of this endpoint. The greatest Lifetime Average Daily Dose [LADD = AADD x (40 years of work/70 years of life)] for occupational or non-occupational exposure to molinate was 0.22 *u*g/kg-day for direct loaders wearing Tyvek suits. Therefore, the MOS for potential lifetime exposure for direct loaders wearing Tyvek suits, based on an apparent NOEL of 1.8 mg/kg-day for kidney tumors in the rat (Pettersen and Richter, 1990), would be 8,182.

# V. RISK APPRAISAL

Risk assessment is a process used to evaluate the potential for exposure and the likelihood that the toxic effects of a substance may occur in humans under the specific exposure conditions. Every risk assessment has inherent limitations on the application of existing data to estimate the potential risk to human health. Therefore, certain assumptions and extrapolations are incorporated into the hazard identification, dose-response assessment, and exposure assessment processes. This, in turn, results in uncertainty in the risk characterization, which integrates all the information from the previous three processes. Qualitatively, risk assessment for all chemicals has similar types of uncertainty. However, the degree or magnitude of the uncertainty varies depending on the availability of the data and the exposure scenarios being assessed. Specific areas of uncertainty associated with this risk assessment for molinate are delineated in the following discussion.

A margin of safety calculated to be 100 or greater would generally be considered adequate for protection against the potential toxicity of a chemical. However, the number 100 is only a benchmark. This benchmark of 100 includes an uncertainty factor of 10 for intraspecies variability in responsiveness, and assumes that humans are 10 times more sensitive to molinate than are laboratory animals (Davidson *et al.*, 1986; Dourson and Stara, 1983,1985; USEPA, 1986A). These uncertainty factors may not be mutually independent (Calabrese and Gilbert, 1993). In the absence of scientific evidence to the contrary, the adverse reproductive effects and neurotoxicity observed in laboratory animals were expected to occur in humans at similar doses in similar time frames. Specific areas of uncertainty associated with this risk assessment for molinate are delineated in the following discussion.

Toxicology

The NOEL (11.5 mg/kg-day) used to assess the reproductive risk of workers to a single dose of molinate comes from a study in which rats were dosed for 5 consecutive days (Minor, 1981). It is likely that the NOEL for a single dose of molinate would be greater than 11.5 mg/kg.

As a result of study design, the LOEL for adverse effects on male reproduction following repeated dosing for 5 weeks was 1.0 mg/kg-day, with a NOEL of 0.48 mg/kg-day (Hodge, 1993a). Most laboratory data indicate that the dose of a toxin required to elicit a specific effect declines with the duration of continuous administration of that toxin (USEPA, 1986A). Consequently, a 27-day NOEL for reproductive effects in male humans may be greater than the 35-day NOEL (0.48 mg/kg-day) for those same reproductive effects in rats, which was selected for calculating the MOSs for potential seasonal exposures.

A NOEL for neurotoxicity from a one year exposure study in dogs (Petterson and Wadsworth, 1990) was used as the basis for calculating MOSs for annual exposure. Several assumptions are inherent in this selection. 1) It was assumed that the neurotoxic effects of molinate are not reversible, based on the results reported in the chronic dog study (Petterson and Wadsworth, 1990). If these neurotoxic effects are reversible, then the MOSs for annual exposures, based on amortized seasonal exposures, may be greater. 2) As a result of dose selection, the LOEL in the dog study was 10 mg/kg-day. The true NOEL is likely to be higher, somewhere between 1 mg/kg-day and 10 mg/kg-day. 3) The transient clinical signs at the LOEL did not appear until the 9th month. As stated above, the dose of a toxin required to elicit a specific effect declines with the duration of continuous administration that toxin. Consequently, the MOSs for potential annual occupational exposure may be greater.

Consideration also needs to be given to the possibility that the neurotoxic effects of molinate are not reversible. Over a lifetime of exposure to molinate, the rat was the most sensitive species examined- with an ENEL of 30 *u*g/kg-day for histopathological evidence of neuropathies (Potrepka and Morrissey, 1991). The greatest Lifetime Average Daily Dose [AADD x (40 years of work/70 years of life)] for occupational or non-occupational exposure to molinate was 0.22 *u*g/kg-day for direct loaders wearing Tyvek suits. The corresponding lifetime MOS for this group of workers would be 136.

By convention, when the "margin of safety" approach has been applied to a potentially carcinogenic endpoint, an additional uncertainty factor of 10 has been used (USEPA, 1986b). The MOS for potential lifetime exposure for direct loaders wearing Tyvek suits was 8,182, which exceeded the MOS of 1,000 recommended by USEPA.

Residential Exposure

Molinate sulfoxide was found in Sacramento tap water, but the parent compound was not detected. It was assumed that degradation products of molinate detected in the water are as toxic as the parent compound. Thus, the risk may be overestimated if the degradation products are less toxic, and underestimated if they are more toxic.

Molinate was not detected (<0.4 ppb) in or on rice grain in a study conducted under "glasshouse" conditions, but unquantified degradation products of molinate were found. Under field conditions, neither molinate nor degradation products were found. As rice is not harvested until 5 months after molinate application, and the half-life of molinate in the field was 3 days (Curry *et al*, 1989), there is probably no dietary exposure to molinate in rice. Nonetheless, theoretical exposure via ingestion of rice was estimated because a tolerance (for "negligible residues") exists. The calculation assumed the residue level of molinate in/on the rice for the acute exposure was at the minimum detection limit (0.05 ppm) and that for the annual exposure was half of the minimum detection limit (0.025 ppm) used in the monitoring.

Calculation of inhalation exposures for the residents of the towns Maxwell and Williams depended upon a few air samples collected in less than one week's time. This short-term sampling protocol may result in an overestimate of potential non-occupational seasonal exposure (USEPA, 1992). Further, it was assumed that residents remained in the towns 24 hours a day, each day, for the entire season.

Theoretically, it is possible to have dietary exposure to molinate through consumption of contaminated sportfish. However, contaminated water is held in rice paddies for a minimum of 30 days before being released into waterways where fish are located. Consequently, game fish would only be exposed to molinate in the water for about 3 weeks or less. Although fish readily take up molinate (Lay *et al.*, 1979; Tjeerdema and Crosby, 1987, 1988a,b; Martin *et al.*, 1992), 98% is either metabolized or depurated in a 24 hour period (Lay *et al.*, 1979; Tjeerdema and Crosby, 1987, 1988a,b). Thus, as molinate concentrations decline in the waterways, the level of contamination in the fish goes down. Monitoring data indicated that in the first week of June, 1990 the average concentration of molinate in edible tissue in catfish, collected from the Colusa Basin Drain, was 0.37 *u*g/g (Harrington and Lew, 1992). If it is assumed 1) the fish would not taste bad (Martin *et al.*, 1992), and 2) that the concentration of molinate in the fish would remain unchanged during cooking (Harrington and Lew, 1992; Martin *et al*., 1992), then a sports fisherman might consume 240 g (approximately 1/2 pound) of fish in a meal (USEPA, 1990). This individual would ingest 88.8 *u*g of molinate. Assuming the individual weighs 75.9 kg, the absorbed dosage would be 1.2 *u*g/kg. The 5-day NOEL for short-term exposure to molinate, based on sperm abnormalities in male rats (Minor, 1981), was 11,500 *u*g/kg. Thus, in this theoretical situation, the MOS for consuming contaminated fish would be approximately 10,000.

Occupational Exposure

The estimate of absorbed dosage was based on the assumption that the 4-hydroxymolinate in the collected urine represented 39% of the absorbed dosage (Formoli *et al.*, 1993c). The metabolic study on which this assumption was based indicated there was a degree of variability (± 11%) in the amount of absorbed material converted to the metabolite in humans (Gloxhuber *et al.*, 1989). Consequently, the actual exposure may be somewhat higher or lower than the estimated exposure. The small number of workers included in the study also contributes to the uncertainty of the short-term exposure estimates. To protect for possible underestimation of short-term exposure, the MOSs for the 95% upper confidence limit on potential short-term exposures were calculated. These MOSs were also greater than the value conventionally recommended to protect people from the toxic effects of molinate.

It was assumed that the absorbed dosage of molinate was linearly related to the amount of molinate handled by the loaders and drivers. In the absence of confirmatory data, this adds to the uncertainty of the exposure estimate. Finally, for the purposes of assessing the average exposure during the six week use season, it was assumed that molinate was handled approximately 74% of the available days due to restricted flying conditions (Donahue, 1993). Even on days when flying was possible, the logs indicated that not all aircraft flew. Thus, actual seasonal exposure to molinate may be less than the estimate presented in this document.

Mitigation measures included the wearing of protective clothing, chemical resistant clothing and respiratory devices, and limiting the amount of molinate handled.

## VI. TOLERANCE ASSESSMENT

### A. BACKGROUND

A tolerance is the maximum amount of pesticide residue that may remain in or on a food, or animal feed (USEPA, 1991). The USEPA tolerance program was developed as an enforcement mechanism to identify illegal residue concentrations resulting from potential non-compliance with the product label requirements (e.g. improper application rates or methods, inadequate pre-harvest intervals, direct or indirect application to unproved commodities). Tolerances are enforced by the FDA, USDA, and state enforcement agencies (e.g. Enforcement Branch of DPR)

The data requirements established by USEPA for tolerances include: 1) residue chemistry which includes measured residue levels from field studies, 2) environmental fate studies, 3) toxicology studies which evaluate the hazards to humans, domestic animals, and non-target organism, 4) product performance such as efficacy, and 5) product chemistry which includes physical-chemical characteristics and analytical method (CFR, 1992b). The field studies must reflect the proposed use with respect to the rate and mode of application, number and timing of applications, and the proposed formulations (USEPA, 1982).

Currently, the tolerances set by the USEPA are at levels necessary for the maximum application rate and frequency, and not expected to produce deleterious health effects in humans from annual dietary exposure (USEPA, 1991). USEPA uses the Reference Dose for non-cancer risks, and negligible level (generally defined as a lifetime probability of tumor occurrence at one in a million) for cancer risks as guides to determine the appropriate levels for dietary exposure.

Assembly Bill 2161 (Bronzan and Jones, 1989) requires the DPR to "conduct an assessment of dietary risks associated with the consumption of produce and processed food treated with pesticides". In the situation where "any pesticide use represents a dietary risk that is deleterious to the health of humans, the DPR shall prohibit or take action to modify that use or modify the tolerance....". As part of the tolerance assessment, a theoretical dietary exposure for a specific commodity and specific population subgroups can be calculated from the product of the tolerance and the daily consumption rate.

### B. ACUTE EXPOSURE

An acute exposure assessment using the residue level equal to the tolerance is conducted for each individual label-approved commodity. The TAS Exposure-4 software program and the USDA National Food Consumption Survey (1987-88) are used in this assessment. The acute tolerance assessment does not routinely address multiple commodities at the tolerance levels as the probability of consuming multiple commodities at the tolerance decreases as the number of commodities included in the assessment increases. Ordinarily, residue levels are set equal to the tolerance, and the MOS, based on the upper 95th percentile for user-day exposures for each population subgroup is examined for the most highly consumed commodities (FDA, 1991). In this instance, the only tolerance for molinate is on rice (0.1 ppm), and it is for "negligible residues". This term is used by the USEPA when it does not expect that any residues of the compound will be found on raw agricultural commodities (CFR, 1992b). As the acute MOS was based on a NOEL (11.5 mg/kg-day) for sperm abnormalities in rats, a MOS of at least 100 is generally considered adequate to protect people from the toxic effects of molinate. The MOSs for theoretical acute dietary exposure for all population subgroups ranged from 10,000 to 77,000.

C. CHRONIC EXPOSURE

A chronic exposure assessment using residues equal to the established tolerances for individual or combinations of commodities has not been conducted because it is highly improbable that an individual would chronically consume single or multiple commodities with pesticide residues at the tolerance levels. Support for this conclusion comes from FDA and DPR (formerly California Department of Food and Agriculture) pesticide monitoring programs which indicate that less than one percent of all sampled commodities have residue levels at or above the established tolerance (CDFA, 1990).

## VI. CONCLUSION

Margins of safety for potential short-term, seasonal, annual, and lifetime exposures to workers associated with handling and application of molinate, the general public, and farmers to molinate were greater than the values conventionally recommended to protect people from the toxic effects of a chemical.

Implementation of specific mitigation measures in the 1991, 1992, 1993, 1994 application seasons resulted in reductions of exposure. The mitigation measures included: the requirement that most handlers wear two layers of protective clothing (one of which may be chemical resistant), chemical resistant hand and foot coverings; a full or half face respirator (depending on the activity); and head covering (if not chemical resistant, then or tightly woven fabric). As contact with molinate increases, so does the personal protective equipment requirement. In addition, the total number of pounds of molinate handled by mixer/loaders during a season was limited.

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# APPENDICES

# APPENDIX A

## Summary of Toxicology Information

CALIFORNIA ENVIRONMENTAL PROTECTION AGENCY
DEPARTMENT OF PESTICIDE REGULATION
MEDICAL TOXICOLOGY BRANCH

# SUMMARY OF TOXICOLOGY DATA

## MOLINATE (ORDRAM)

Chemical Code # 000449, Tolerance # 00228
SB 950 # 208

April 7, 1987
Revised 6/17/88, 7/27/89, 12/19/89, 1/25/90, 4/10/90, 5/9/90, 7/26/90, 4/29/91, 8/01/91, 10/15/91, 3/13/92, 5/4/92, and 6/30/94

## I. DATA GAP STATUS

| | |
|---|---|
| Chronic toxicity, rat: | No data gap, possible adverse effect |
| Chronic toxicity, dog: | No data gap, possible adverse effect |
| Oncogenicity, rat: | No data gap, possible adverse effect |
| Oncogenicity, mouse: | No data gap, possible adverse effect |
| Reproduction, rat: | No data gap, possible adverse effect |
| Teratology, rat: | No data gap, possible adverse effect |
| Teratology, rabbit: | No data gap, no adverse effect |
| Gene mutation: | No data gap, possible adverse effect |
| Chromosome effects: | No data gap, no adverse effect |
| DNA damage: | No data gap, no adverse effect |
| Neurotoxicity: | Study type is not required at this time, however two inadequate studies have been submitted, and possible adverse effects are indicated in both. |

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Toxicology one-liners are attached.

In the one-liners below:
****** indicates an acceptable study.
**Bold face** indicates a possible adverse effect.

Revised by G. Chernoff 7/26/90, and by C. Aldous, 4/29/91, 8/01/91, 10/15/91, 3/13/92, 5/4/92, and 6/30/94.

All relevant records on file with DPR as of 5/5/94 have been included in the Toxicology Summary. These include record numbers through 129725 (in Document No. 228-147). Also, there are older record numbers of the 900000+ series. Aldous, 6/30/94.

Note: these pages contain summaries only. Individual worksheets may identify additional effects.

## II. TOXICOLOGY ONE-LINERS AND CONCLUSIONS

### COMBINED, RAT

****228-104 092157** Pettersen, J.C. and Richter, A.G. "Two-year chronic toxicity/oncogenicity study with R-4572 in rats" [Report No. T-13023]. CIBA-GEIGY Corp. Environmental Health Center, Farmington, Connecticut, 11/30/90. Fifty Crl: CD* (SD) BR rats/sex were dosed for up to 2 yr with 0, 7, 40, or 300 ppm molinate (Lot No. EHC-0886-30/WRC-4921-8-22) in diet. Another group for 1-year interim sacrifice had 10/sex at 7, 40, and 300 ppm and 20/sex for controls and 600 ppm. Original review placed the NOAEL = 7 ppm, based on ovarian thecal/interstitial cell vacuolation and/or hypertrophy, which appeared slightly, but statistically significantly elevated at 40 ppm, and markedly increased in incidence and severity at 300 ppm. Later, a "blind" re-evaluation of ovarian slides (DPR Record No. 127478) by M.C.E. Hodge led to a higher NOEL of 40 ppm for ovarian thecal cell hypertrophy (see 1-liner below). Common findings at 300 ppm included: hindlimb ataxia and adduction, skeletal muscle atrophy (these signs were seen in both sexes but were more frequent in males); peripheral nerve (sciatic) degeneration in both sexes; distal spinal cord changes, especially eosinophilic bodies (presumed to be degenerating axons) in lumbar and sacral regions (both sexes) [eosinophilic bodies were also noted in brain (medulla) of several 600 ppm females]; modest cholinesterase (ChE) inhibition (of RBC ChE in both sexes, and of brain ChE in 300 to 600 ppm females: no associated clinical signs, even at 600 ppm); and modestly reduced hematocrits in both sexes. Oligospermia was noted in epididymides at 300 ppm, and testicular degeneration was noted at 600 ppm. There were adenomas or carcinomas in kidneys of five 300 ppm males vs. none in other groups: this was presumed to be treatment related. The neurological effects, the marked ovarian changes, and male kidney tumors are "**possible adverse effects**". Decreased body weights and decreased food consumption were noted, along with <u>increased</u> survival, at 300 ppm in both sexes. This dose may therefore have exceeded the MTD. **Acceptable**, Aldous, 4/5/91, one-liner updated 6/21/94. (See follow-up studies below).

228-120 113132 (supplement to 228-104:092157). Horner, S.A., "Molinate: 10 day oral dosing study in rats", ICI Central Toxicology Laboratory, Cheshire, UK, Jan. 3, 1992. Five groups of 8 male Crl:CD(SD)BR rats were dosed by gavage for 10 days with either 4 ml/kg/day of corn oil vehicle, or with 15, 75, or 150 mg/kg/day molinate, or with 200 mg/kg/day 2,2,4-trimethylpentane (positive control). 150 mg/kg/day molinate led to some treatment-related deaths, associated with clinical signs such as subdued appearance, "hunched position", "sides pinched in", piloerection, ocular discharge, stains around nose and mouth, urinary incontinence, irregular breathing. Both 75 and 150 mg/kg/day molinate led to salivation. All 3 molinate groups had dose-related weight gain decrements. The 150 mg/kg/day group had reduced sperm in urine sediment (possibly treatment-related). Surviving 150 mg/kg/day rats had gross appearance of "reduced testis". All molinate groups had kidneys without visible hyaline droplets and without elevated α-2u-globulin upon microscopic examination. Assay for α-2u-globulin by immunoelectrophoresis found no significant increases in molinate groups, compared with marked elevation in positive controls. Thus the study does <u>not</u> indicate that male-rat-specific elevation of α-2u-globulin is related to the increase in kidney tumors noted in the referenced combined study. Aldous, 3/13/92.

071 085338, 072 090067, 086 088604, interim reports for 104:092157, above. No worksheet provided (G. Chernoff, 7/26/90).

228-144 127478 Hodge, M.C.E., "Two-year chronic toxicity/oncogenicity study with R-4572 in rats. Supplement to Stauffer Chemical Company Report Number T-13023. Histopathological re-evaluation of the ovarian thecal/interstitial cell vacuolation/hypertrophy", Zeneca Central Toxicology Laboratory, 9/22/93. This is a re-evaluation of ovarian slides of the two-year study, Report No. T-13023, DPR Record No. 092157. From the original report, it appeared that there might have been a treatment-related effect on incidence of ovarian thecal/interstitial cell vacuolation/hypertrophy as low as the 40 ppm level, therefore a re-examination was performed. The reviewing pathologist first re-examined all 600 ppm slides, finding that the original grading of ovarian changes was not applied uniformly. He made more specific sets of criteria to define three grades of ovarian thecal/interstitial cell vacuolation/hypertrophy. He then did a "blind" review of all available ovary slides (including 12-month interim sacrifice ovaries of all groups). The only incidences of ovarian thecal/interstitial cell vacuolation/hypertrophy discovered in this re-examination below 300 ppm were of "minimal" grade, and there were 6/69 controls, 3/59 in the 7 ppm group, and 10/60 in the 40 ppm group (see p. 22). There were clear responses in the 300 to 600 ppm groups, both in terms of incidence and degree. The pathologist made a valid conclusion that the NOEL for this effect was 40 ppm. Aldous, 6/21/94.

## CHRONIC TOXICITY, RAT

004 945346, "Ordram: Safety Evaluation by Repeated Oral Administration to Rats for 104 Weeks", (Woodard Research Corp., 6/3/77). Ordram tech. (98.8%). Initial dosages of 0, 8, 16, and 32 mg/kg/day, reduced at week 18 to 0, 0.63, 2.0, and 6.32 mg/kg/day in diets of Fischer rats. Insufficient information to assess chronic or oncogenic potential. [Report cites dose-related increase in testicular weights in mid-dose and high-dose males, however this difference is apparently due primarily to differences in sizes of interstitial cell (ISC) tumors in the last interim and term sacrifice groups. ISC tumors are very common in aged Fischer rats, and there is no evidence of compound effect on time to tumor in this study - C. Aldous, 3/5/87]. Study **UNACCEPTABLE,** not upgradeable: Far too little histology, too much loss of animals to autolysis, etc., dose levels apparently far lower than justifiable. No further information required of this study. (J. Christopher, 3/5/85).

## CHRONIC TOXICITY, DOG

****228-097 095888** Pettersen, J.C. and Wadsworth, P.F., "One-year toxicity study with R-4572 in beagle dogs", Report No. T13236, CIBA-GEIGY CORP., Environmental Health Center, Farmington, CT, 12/17/90. Technical molinate, 97.6% purity, was administered in gelatin capsules daily at dosages of 0, 1, 10, 50, and 100 mg/kg/day. Duration of dosing was 1 year for all but the 100 mg/kg/day group, which was taken off treatment on day 106 and was administered empty capsules for the balance of the study. NOEL = 1 mg/kg/day (infrequent tremors and/or awkward gait in one or more dogs at 10 mg/kg/day, also very slight reduction in hematocrit in 10 mg/kg/day males at 3 and 6 months: see Section V.B. of review for details). Principal findings were neurological, and were the basis for identifying a **"possible adverse effect"**. Common findings included clinical signs of ataxia, splayed hindlimbs, reduced locomotor activity, tremor, abnormal voice, and noisy breathing in 50 and 100 mg/kg/day - recovery groups. Postural reactions, particularly of hindlimbs, were slightly depressed in these groups. These groups were generally hyperreflexic in patellar reflex tests. Many of the functional deficits observed in 100 mg/kg/day - recovery groups showed no signs of remission.

Microscopic findings in brain included eosinophilic bodies or vacuolation, particularly in localized areas of medulla, in several 50 and 100 mg/kg/day - recovery dogs. Minimal to slight degrees of demyelination were observed in various levels of spinal cord, particularly at 50 mg/kg/day. There was slight demyelination of some peripheral nerves, particularly in 50 mg/kg/day dogs. Evidences of mild anemia included consistent reductions of RBC parameters (RBC count, Hgb, HCT), noted in both sexes at 3 months in the 100 mg/kg/day - recovery dogs, decreased HCT values in 50 mg/kg/day males at 3 and 6 months, slightly increased RBC fragility in 50 mg/kg/day dogs, slight increases in reticulocyte counts in 50 mg/kg/day females, and modest evidences of RBC pigment accumulation in spleen and liver. Liver weights were elevated in 50 mg/kg/day dogs, and serum alkaline phosphatase and serum cholesterol were elevated in 50 mg/kg/day males, however liver microscopic findings were limited to slightly elevated Kupffer cell hemosiderin. **Acceptable**. Aldous, 4/18/91.

071 085340, 086 088606 interim reports for 097:095888, above. No worksheet provided (G. Chernoff, 7/26/90).

## CHRONIC TOXICITY, MOUSE

005 945347, "Ordram: Repeated Oral Administration to Mice for Lifetime", (Woodard Research Corp., 6/3/77). Ordram test article not characterized. Dosages of 0, 3.6, 7.2, and 14.4 mg/kg/day in diets of CAF1 mice. Insufficient data to qualify as a meaningful chronic study: "no toxicity of any kind observed", according to JPC [report indicates diminished survival in 14.4 mg/kg/day females near to scheduled term kill, however results not significant, nor were there other data to support treatment effects in any group]. Thus dose levels do not appear to be justified. Only 6 tissues routinely examined microscopically. Hematology/blood chemistry not done because of technical problems. No microscopic pathology of animals dying on study. **UNACCEPTABLE**, not upgradeable. No adverse effects indicated. (J. Christopher, 6/6/85).

005 945348 (Pathology report to 005 945347, above).

## ONCOGENICITY, RAT

See Combined Rat above.

## ONCOGENICITY, MOUSE

****228-107 096396** Potrepka, R.F., and Morrissey, R.L."18-month dietary mouse oncogenicity study with R-4572" [Report No. T-13211]. CIBA-GEIGY Corp. Environmental Health Center, Farmington, CT, 1/14/91. Fifty CD-1 mice per sex per dose were administered tech. molinate (97.6% purity, Lot No. EHC-0886-30/WRC-4921-8-22) in diet for 18 months. Doses were 0, 10, 100, 1000, and 2000 ppm. NOEL for males = 10 ppm (testicular degeneration). The NOEL for other effects was 100 ppm: this was based partially on neurological lesions [peripheral nerve demyelination and Schwann cell hyperplasia, and eosinophilic bodies (considered to be swollen, degenerated axons) in medulla and spinal cord]. Associated clinical effects such as hindlimb adduction, ataxia, hindlimb muscle weakness, splayed hindlimb, and hindlimb atrophy were generally limited to 2000 ppm mice: females were particularly affected. Both sexes had adrenal degeneration at 1000 to 2000 ppm: this was most pronounced in females. Females had ovarian thecal/interstitial cell hyperplasia at 1000 and 2000 ppm: uterine atrophy, amyloidosis, or dilatation, as well as mammary

gland atrophy were seen at 2000 ppm. Dose-related hematology changes, common at 12 and 18 months in both sexes, were decreases in R.B.C. counts, Hb, and HCT. There was no treatment effect on neoplasia. The neurological effects and testicular lesions, and to a lesser extent, ovarian changes, are **possible adverse effects**. **Acceptable.** Aldous, 4/18/91.

071 085339, 088 088605 Interim reports for 096396, above.

## REPRODUCTION SUMMARY

Many reproduction studies with molinate have been conducted in a total of 5 species. The most extensive data base is in rats, where a decrease in fertility, determined by decreased numbers of litters and implants, has been a consistent finding. EM studies of sperm preparations indicate that the primary lesion responsible for the adverse effect in male rats is associated with a disruption of the plasma membrane in the mid-piece region of maturing sperm. From this it has been reasoned that the decreased sperm count, motility, and viability observed in this species are all attributable to the membrane disruption.

In contrast to the findings in rodents, exposing rabbits to capsules of molinate had no adverse effect on fertility, or gross gonadal histology. In the monkey studies, only sperm parameters were measured. Using sperm collected by electroejaculation at weekly intervals over a 12 week period, no adverse effects from molinate exposure were seen on sperm count, motility, or percent normal. The individual variability of these parameters was extremely large from sample to sample in both monkey studies, and posed a major limitation in interpreting the results. This large variability can be attributed, in part, to the use of electroejaculation, a technique which can compromise sperm quantity, concentration, and quality.

The three human studies were conducted as part of a large epidemiologic study on male workers at three molinate production plants. Fertility, calculated from retrospective male questionnaire data, and sperm parameters (count and percent normal) were unaffected by molinate exposure. The methods of questionnaire data collection, natality calculations, sperm collection, and sperm data analyses were severely flawed, thereby eliminating this study for use in a scientific risk assesment.

The argument has been made that the adverse reproductive effects of molinate are restricted to rodents, and are not applicable to other species. This argument is based on a reasonable assumption followed by some questionable logic. The assumption is that the primary defect, leading to disruption of the sperm mid-piece membrane, causes a decrease in sperm count and motility, ultimately resulting in decreased fertility. From this it is argued that since fertility was not decreased (except at doses demonstrably toxic to adults) in the rabbit and human studies, and sperm parameters were not altered in the monkey study, the sperm abnormality must be restricted to the rodent species. This reasoning is based on the questionable assumption that the rabbit, monkey, and human studies are equivalent to the rat studies in scientific validity and power of detection. In the case of fertility, measurements were limited to two rabbit reproduction studies and three human epidemiology studies. As mentioned above, the flawed methods of data collection and analyses in the human studies eliminates their use for further consideration. This leaves only the rabbit studies as non-rodent fertility studies, and these studies were inconclusive and not as well controlled as the rat studies. Various sperm parameters were measured in the three human

studies, and the monkey studies. As mentioned before, the human studies must be deleted from consideration. In the monkey studies, meaningful analyses of the data were compromised by the large amount of individual variability, which could have been reduced by using a different technique of sperm collection. Neither rabbit nor monkey studies involved sufficient animals at all dose levels to meaningfully compare groups for possible treatment effects on sperm morphology. Taking the non-rodent studies as a group, it is quite clear that they do not provide a scientifically acceptable basis for disregarding the results of the extensive body of rat studies. After careful review and consideration, it is the opinion of this reviewer (G. Chernoff), that the weight of evidence provided in the studies submitted to date supports the conclusion that molinate is a reproductive toxicant, with adverse effects not necessarily limited to the rat species (G. Chernoff, 12/19/89). (The above summary was most recently updated by G. Chernoff, 4/10/90; and subsequently by Aldous on 4/29/91 to reflect two additional non-rodent studies).

REPRODUCTION, RAT

Many SD rat reproduction studies have been submitted for review and evaluation. In the one multigeneration study where both sexes were treated (DPR Record No. 945351), decreased fertility was noted at 0.63 mg/kg/day. CDFA (now DPR) review noted that the study provided insufficient information to determine whether the NOEL was the next lower dose of 0.2 mg/kg/day. In studies focusing on male fertility, sperm abnormalities and/or decreased fertility were noted at doses at or above 4 mg/kg/day in oral gavage studies (DPR Record No. 945355), and at inhalation exposures at or above 0.6 mg/$m^3$ (DPR Record No. 041546). In a 2-generation study where treatment was restricted to females, abnormal ovarian histology was observed at 50 ppm (3.7 mg/kg/day), yielding a NOEL of 6 ppm (0.44 mg/kg/day). That study identified reduced fertility at 450 ppm (DPR Record No. 087658). A recently reported re-evaluation of the ovarian histopathology under **chronic** conditions (12 to 24 months) found a NOEL of 40 ppm and an LEL of 300 ppm for such changes. The recent DPR review by Aldous for Record No. 119319 (see below) considered 50 ppm in this **reproduction** study to be a marginal effect level, so that the NOEL for thecal cell hypertrophy under conditions of a reproduction study remains at 6 ppm (as stated in Dr. Chernoff's 1-liner for the study). While none of these studies individually satisfies SB-950 requirements, collectively the results demonstrate a consistent adverse effect on fertility which can be attributed to treatment related dysfunctions in oogenesis and spermatogenesis. Based on these findings, **THE COLLECTIVE STUDIES ARE CONSIDERED ACCEPTABLE**, and the data gap for Rat Reproduction is satisfied.

The essential NOELs are as follows: for females, the dietary reproductive systemic NOEL = 6 ppm (0.44 mg/kg/day), based on the abnormal ovarian histology data in DPR Record Nos. 087658, and the reproductive functional NOEL = 50 ppm (3.7 mg/kg/day), based on the decreased number of litters and reduced litter size in the same record. For males, the dietary reproductive NOEL cannot be firmly established, but is likely to be on the order of 0.5 mg/kg/day. Additional data relating to the recent sperm morphology study (DPR Record No. 127595, Zeneca Study No. KR1189) will help to clarify this NOEL for male reproductive effects. An inhalation (4 week exposure) male reproductive effects NOEL = 0.3 mg/$m^3$† (DPR Record No. 041547). A **POSSIBLE ADVERSE HEALTH EFFECT** (decreased fertility, sperm, and ovarian abnormalities) is noted (G. Chernoff, 12/19/89 and 1/25/90; and C. Aldous, 6/21/94).

†NOTE: According to 228-134:119319, a dose of 0.3 mg/$m^3$ is equivalent to about 3.6 mg/kg/day. The DPR review of that report noted, however, that such equating should be interpreted with caution (Aldous, 6/16/94).

(Rat Multi-generation studies: both sexes treated)

**003 945351**, "Ordram Safety Evaluation by Repeated Oral Administration to Rats: Three Generation Reproduction Study", (Woodard Research Corporation, 6/3/77). Test article was Ordram, not further characterized. 0, 0.063, 0.2, and 0.63 mg/kg/day to males and females in 3-generation, 2 litter/gen. study. Reduction in fertility at 0.63 mg/kg/day. Investigators concluded "survival of pups at the highest dose level may have been adversely affected" (not all reproduction performance data was provided., conclusion found on p. 5). Reviewer (Christopher) noted several serious deficiencies in this study. Not sufficient info. to determine whether LEL was below 0.63 mg/kg/day. **UNACCEPTABLE**, not upgradeable. No further data needed from this study. (J. Christopher, 6/4/85).

003 945338. Pilot reproduction/fertility study. Possibly to set dose levels for study 003:945351 above. No review needed.

228-134 119319 (This is not a study, but is a discussion of appropriate NOELs for reproductive effects). "Derivation of molinate NOELs based on fertility effects", 11/30/92. Discussion states that ICI believes that the male fertility effects seen in rats are rodent-specific, and not relevant to humans, nevertheless bases for NOELs for both sexes are presented. They estimated that 1 $mg/m^3$ in inhalation studies was equivalent to 12 mg/kg/day absorbed dose. They determined that toxicity was strictly a function of absorbed dose, whether by oral or inhalation route. Studies on male rat reproduction by either route were thus combined to determine the overall male rat reproductive effects NOEL. DPR review notes, however, that calculated equivalent doses by different routes did not yield comparable male reproductive toxicity. Investigators suggested that the male rat reproductive NOEL should be 3.6 mg/kg/day, based on their analysis of several LELs and NOELs. This appears to be too high for a derived NOEL, since definitive male rat reproductive effects were observed at 4 mg/kg/day in study T-10421 (DPR Record No. 945355). In addition, Study KR1189 (DPR Record No. 127495) subsequently found a NOEL for male reproductive effects to be on the order of 0.5 mg/kg/day. Regarding female reproductive toxicity, the discussion considered the NOEL for ovarian thecal cell hypertrophy to be 50 ppm, but data suggest that 50 ppm is an LEL, and a conservative NOEL is 6 ppm (same as previous reviews). Aldous, 6/20/94.

228-144 127471 Wickramaratne, G.A., "Report No: CTL/L/5531. First revision to Molinate: The no-observed effect levels for male and female reproductive effects in rats; an overview", 11/18/93. This is a presentation of studies which have been previously reviewed. Items discussed included, among other things, (1) evidence that male reproductive effects resulted from failure of Sertoli cells to properly process sperm cells, (2) evidence that the ovarian thecal cell hypertrophy incidence increases found in the chronic study at doses up to 40 ppm were within historical control range. Investigators concluded that the reproductive effects NOELs should be at least 3.7 mg/kg/day for females and 1 mg/kg/day for males. There were no new data needing changes in DPR conclusions. Aldous, 6/21/94.

(Crossover rat reproduction)

**003 945353**, "Ordram: Experiment to Show Whether the Male or the Female is Responsible for Reduced Fertility in Ordram Fed Rats", (Woodard Research Corp., 5/13/75). Ordram (not further characterized) at doses of 8, 16, or 32 mg/kg/day to either males or females. Only fertility studied. No litters

sired by 16 or 32 mg/kg/day males, and only 2/8 litters in 8 mg/kg/day males. All treated females delivered litters. Infertility concluded to be a male treatment effect. **UNACCEPTABLE** to fill data requirement, but useful data. (J. Christopher, 6/4/85).

(2-generation study with treatment restricted to females)

**070 087658** "Two-Generation Reproduction Study in Female Rats with R-4572", (Ciba-Geigy Environmental Health Center, Report No. T-13218, 11/3/89). R-4572 Technical (Molinate), Lot #EHC-0866-30, 97.6%, was administered in the diet to groups of 25 female Sprague Dawley rats at dose levels of 0 (vehicle control of Purina Rat Chow and 0.1% corn oil), 6, 50, and 450 ppm for two generations. No clinical signs or necropsy findings suggestive of toxicity were observed at any of the dose levels tested and reduced food consumption at 450 ppm and weight gain at 50 and 450 ppm were attributed to poor palatability. Significant reductions in the fertility index and litter size were observed at 450 ppm, and vacuolation/hypertrophy of ovarian thecal/interstitial cells was reported at 50 and 450 ppm. A separate analysis of these data, provided in record no. 090134, reported a lack of association between the abnormal ovarian histopathology and a reproductive deficit, thereby necessitating the establishment of two separate NOEL's, one for systemic effects (abnormal ovarian histology), and one for functional effects (decreased fertility and reduced litter size). Reproductive systemic NOEL = 6 ppm (0.44 mg/kg) based on abnormal ovarian histology which is considered a **POSSIBLE ADVERSE HEALTH EFFECT**. Reproductive functional NOEL = 50 ppm (3.7 mg/kg/day) based on decreased fertility and reduced litter size. The study is acceptable as a supplemental study (G. Chernoff, 12/19/89; 1/24/90).

074 090134, "Evaluation of Two-Generation Study in Female Rats with R-4572", (G.A. Wickramaratne, ICI Americas Inc., Ref. T-13218, December 15, 1989). A supplemental report to 087658, consisting of a detailed re-appraisal of the histopathology data. The suggestion is made that since there is no association between the abnormal ovarian histopathology and the observed functional reproductive deficits, the NOEL for functional reproductive toxicity should be 50 ppm (G. Chernoff, 1/24/90).

228-138 121113 Nearly identical submission to 090134, above, with a few editing changes. Dr. Chernoff had already accepted the major argument of this evaluation, namely that there was no direct association between females with ovarian histopathological changes and females with fertility deficits (see 1-liner for Record No. 087658, above). No new DPR worksheet. Aldous, 6/16/94.

228-121 113585 Horner, J.M., "Molinate: Mechanistic study in the pregnant rat", Report No. CTL/T/2769, ICI Central Toxicology Laboratory, Cheshire, UK, March 3, 1992. Reproduction ancillary study, rat [relates to 228-003: 945351 and several ancillary studies]. Groups of 10 female Crl:CD(SD)BR rats were dosed with 0, 75, 135, or 200 mg/kg/day molinate (98.1%) by gavage (in 10 mg/kg corn oil vehicle) on days 7-9 of gestation. Investigators evaluated clinical observations, microscopic changes in ovaries and adrenals (with particular attention to changes in lipid content), and progesterone levels were measured at termination. High mortality in the higher two dosage groups limited the extent of parameters measured or reduced the precision of available data. Lipid content of adrenals was increased. Fatty cytoplasmic vacuolation was noted in adrenal zona fasciculata and zona reticularis, and in corpora lutea. No change in plasma progesterone was detected. The study did <u>not</u> confirm specific treatment responses which would account for female-mediated reproductive toxicity. Aldous, 5/1/92.

(Male effect ancillary reproduction studies, oral dosing)

**046 041544**. Study T-10715, "A Comparison of the Effects of Benthiocarb, Benthiocarb Sulfoxide, and Ordram← on Male Rat Fertility", (Stauffer, 4/14/82). Molinate, presumed technical, 0 and 20 mg/kg/day by gavage. No NOEL determined (mechanism study). Marked decrease in pregnancy index, and marked reduction in numbers of implants per pregnant dam. Sperm was damaged and reduced in number on maturation. Sperm cell membrane breaks were considered as possible cause of noted reproductive findings. **NOT APPLICABLE** to fill reproductive effects data gap, but useful data. Additional data requested. Confirms previously cited **ADVERSE EFFECTS** in male reproduction in rats. (C. Aldous, 7/28/86).

**018 945355**, "Ordram Fertility Study in Male Rats: Mechanism/Site of Action: T-10421", (Stauffer, 5/1/81). Molinate, tech. (98.2%). Dosages varied between segments of study, but males were dosed with 0, 12, or 60 mg/kg/day for 5 days, or with 0, 0.2, 4, 12, or 30 mg/kg for 5 or 10 weeks. NOEL for 5-day treatment = 12 mg/kg/day (Reduction in fertility significant at week 3 post-treatment; also substantially reduced implants/pregnant female at week 3 and to a lesser extent at week 4). LEL for 10 week treatment with 0 or 12 mg/kg/day was 12 mg/kg/day (reduced fertility and reduced implantation). LEL for 5 week treatment with 0, 12, or 30 mg/kg/day was 12 mg/kg/day (reduced implantation). NOEL for 5-week treatment with 0, 0.2, and 4.0 mg/kg/day = 0.2 mg/kg/day (based on non-significant reduction in implantation; also decreased percentages of viable sperm, motile sperm; increased % abnormal sperm, decreased sperm cell count. All these findings down to 4 mg/kg/day). Serum hormone levels did not explain reproductive toxicity. **UNACCEPTABLE** as an independent study (not a guideline reproduction study), however useful data. No further data required for this study. (J. Christopher, 6/6/85).

228-144 127495 Hodge, M.C.E., "Molinate: Sperm morphology study in the rat", Zeneca Central Toxicology Laboratory, Alderley Park, 9/23/93. Study No. KR1189. Twelve Crl:CD(SD)BR males/group were dosed by gavage with 0, 0.5, 1, 2, 3, 4, or 8 mg/kg/day molinate (96.8%) daily for 35 days. Animals were killed, and sperm samples were taken from the right cauda epididymis for scanning EM evaluation. 100 sperm/rat were examined for various sperm abnormalities. Only incidence of midpiece abnormalities was remarkable: incidence with at least one mid-piece abnormality was 0, 1, 2, 6, 5, 10, and 12 for dose levels of 0, 0.5, 1, 2, 3, 4, or 8 mg/kg/day molinate. The higher 4 dose levels had multiple mid-piece abnormalities in 1, 3, 8, and 11 rats, respectively. Since there was one case of a mid-piece abnormality in the lowest dose group, but none in the concurrent controls, investigators re-examined unspecified numbers of sperm from all the rats in three studies, eventually finding one male having 3 sperm with similar lesions (Record No. 127496, this volume, Report No. CTL/L/5587 by F.M. Smith). Investigators considered 1 mg/kg/day to be the NOEL. This reviewer, however, has insufficient information to determine whether there is a NOEL at all, since existing data do not allow DPR to evaluate how unusual sperm mid-piece abnormalities are in control males. It is requested that registrants provide (1) the identities of the three molinate studies cited in CTL/L/5587 in which the sperm abnormalities have been examined by EM (the CTL Study Nos. do not appear to correlate with any recent male rat reproduction studies on file except for the present one), and (2) how many sperm/rat were examined in this search for abnormal sperm. Study is now **unacceptable**, but will provide useful information when requested data are received. Aldous, 6/21/94.

228-141 121889 Proposed protocol for study 228-144 127495, above.

**003 945350**, "Suppression of fertility in male rats: Ordram Tech", (Litton Bionetics, 10/29/76, LBI Project No. 2621). Ordram technical, lot RCK 0701. Feeding (or gavage during mating concurrent with treatment) of male rats with 0, 0.2, 1.0, or 5.0 mg/kg/day for two weeks. Matings on days 10-14 of treatment, also 2 and 4 weeks post-treatment. Apparent treatment effects at 5.0 mg/kg/day: decreased fertility, decreased # viable pups/litter, and slight but consistent increase in sperm agglutination. NOEL cannot be determined: numerous errors in study conduct, unusually high variability in control data. **UNACCEPTABLE** study, not very useful data, and superceded by other later studies. No more data required from this study. (J. Christopher, 6/4/85).

**003 945352**, "Ordram: Screening Study of Male Fertility and General Reproductive Performance in Rats Using Seven Thiocarbamate compounds", (Woodard Research Corp., 5/12/75). Ordram tech., lot RCK 0701. 0 or 32 mg/kg/day in diets of both males and females, 3 days prior to first mating of males, continuing treatment for 7 weeks, with second, third, and fourth matings in weeks 5, 6, and 7 of treatment. A final mating of males 5 weeks after termination of treatment. Results: Some litters sired in group mated on third day of treatment, however no litters sired with ongoing treatment during weeks 5-7. Apparent partial recovery at week 12 (5 weeks off treatment): 2 of 5 males sired litters. **UNACCEPTABLE** study with limited useful data and superceded by other later studies. No more data required from this study. (J. Christopher, 6/4/85).

(Ancillary rat reproduction studies - male effects: Inhalation)

**046 041546** Study T-10189, "Evaluation of Male Fertility Following Inhalation Exposure to Ordram® Technical in Rats", (Stauffer, 8/13/82). Molinate, tech. 0, 0.1, 0.6, 1.8, and 4.0 mg/$m^3$, 6 hr/day, 5 days/wk, 13 week exposure by inhalation. No NOEL was observed for reproductive nor for non-reproductive effects (necrosis in spermatids and/or spermatocytes, also necrotizing rhinitis, both at all dosages after termination of treatment. Decreased pregnancy index in 0.6 mg/$m^3$ group and above, and decreased implantations at 1.8 mg/$m^3$ and above at termination of treatment. Full recovery from rhinitis and partial reversibility of reproductive effects in 2-month recovery animals.) **UNACCEPTABLE** and not upgradeable to fill data requirements, but useful data. (C. Aldous, July 30, 1986).

**046 041547** Study T-10494, "Evaluation of Male Fertility Following Four-Week Inhalation Exposure to Ordram Technical in Rats", (Stauffer, 1982). Molinate, tech. 0, 0.1, 0.2, 0.3, 0.6, and 1.6, mg/$m^3$, 6 hr/day, 5 days/wk, 4 week exposure by inhalation. Apparent NOEL = 0.3 mg/$m^3$ (higher percentage of "abnormal sperm" and reduced percentage of motile sperm at 0.6 and and 1.6 mg/$m^3$. Reduced numbers of implants at same levels.) **UNACCEPTABLE** and not upgradable to fill data requirements, but useful data. (C. Aldous, July 30, 1988).

**003 028492**, "A 13 Week Inhalation Toxicity Study and Reproduction-Fertility Study of R-4572 in the Rat", (Biodynamics, 12/12/79, Project Nos. 78-7153 & 78-2346, also designated T-10003). Inhalation exposure ( respirable size) to 0, 2.2, 11.1, or 42 mg/$m^3$, 6 hr/day, 5 days/wk. No NOEL observed (decreased sperm count and abnormal sperm, also decreased implantation rate at LDT of 2.2 mg/$m^3$). **UNACCEPTABLE** study to fill data requirements, but useful data. (J. Christopher, 6/4/85).

(Male reproductive effects mechanism studies)

003 945349. Summaries of studies to attempt to identify mechanism of testicular effects in rats. No definitive, documented studies found in this review. No worksheet generated.

REPRODUCTION, MOUSE

**018 945354**, "Ordram Antifertility Study in Mice: T-10121", (Stauffer, 12/80). Molinate, tech. (98.2%). 7-week gavage treatment of male CD-1 mice with 0, 2, 20, 100, or 200 mg/kg/day. NOEL = 20 mg/kg/day (decreased implantations per pregnant female and decreased fertility at 100 mg/kg/day). **NOT ACCEPTABLE** as an independent study (not a guideline reproduction study), however useful data. No further data required from this study. (J. Christopher, 6/6/85).

REPRODUCTION, MONKEY

228-098 095839 Zu"hlke, U., and Bee, W.; "Molinate: Evaluation of sperm morphology in the Cynomolgus monkey". Hazleton Laboratories Deutschland GmbH, Jan. 7, 1991. Ten monkeys per group were dosed with 0, 0.2, 10, or 50 mg/kg/day of molinate (Batch BJB 2605) by gavage (corn oil vehicle) for 12 weeks. There were no changes in sperm morphology identified, nor were any "adverse effects" indicated. The study is of very limited value for evaluating possible effects on sperm morphology, due to small numbers of animals and great variability in measured parameters. There is no apparent reason to submit additional information regarding this study. Aldous, 4/22/91. NOTE: A new submission was received in May, 1991: Document No. 228-111, Record No. 097046. This is presumably the information requested and examined by Dr. Chernoff, who had left this Branch prior to receipt of the photographs, and who is currently not available for comment. He did not prepare a written review for CDFA/DPR Branch records. Aldous, 5/4/92.

228-105 096314 Statement by Dr. Themann that notches in sperm head-neck area are normal in Cynomolgus monkeys and in man, followed by title page to text: Ultrastructure of Human Gametogenesis and Early Embryogenesis, Blerkom, J.V. and Motta, P.M., eds., Kluwer Academic Publishers, Boston, 1989. A photocopy of two human sperm is included, submitted to address the issue of notches; nevertheless the captions do not mention notches, nor could any be seen in this reproduction. C. Aldous, 4/19/91 (no worksheet).

228-110 096794 A single page of EM summary tables of sperm morphology, qualitatively similar to p. 119 of study 095839, above, from which study these data came. Electron micrographs corresponding to these tables have been submitted to Dr. G. Chernoff of Calif. DHS. No additional review is relevant at this time. Aldous, 4/22/91.

228-092 088921 Interim report for 098:095839, above (no worksheet).

228-105 096315 The only data in this 2-page submission are historical data for Cynomolgus monkeys: sperm count and ejaculate weights. C. Aldous, 4/19/91 (no worksheet).

228-105 096316 SOP for "Ejaculate collection and evaluation in primates", with light microscopic analysis. C. Aldous, 4/19/91 (no worksheet).

228-105 096319 SOP for "Preparation of spermatozoa for scanning electron microscopy". C. Aldous, 4/19/91 (no worksheet).

228-105 096320 SOP for "Investigation of spermatoza morphology by light microscopy". Criteria for counting abnormal spermatozoa. C. Aldous, 4/19/91 (no worksheet).

228-105 096321 Copy of part of chapter 2, "Collection and examination of human semen", from WHO laboratory manual for the examination of human semen and semen-cervical mucus interaction, Cambridge University Press, New York, 1987. Included are rough photocopies of several plates showing various sperm abnormalities. C. Aldous, 4/19/91 (no worksheet).

019 945356, "The Effect of Ordram← on Nonhuman Primate Sperm Production", (Stauffer, 12/16/81). T-10714. Ordram, tech. (98.6%), 0, 0.2, 10.0, and 50.0 mg/kg/day, 5 days/week, for 12 weeks. Admin. by oral gavage (with nasal gastric tube) in corn oil vehicle to male monkeys (*Macaca fascicularis*). No evidence of untoward reproductive effects, based on sperm analyses and hormonal levels. High dose sufficient to substantially reduce plasma cholinesterase levels. **NOT ACCEPTABLE** in lieu of a full reproduction study, however useful data. (J. Christopher, 6/4/85).

## REPRODUCTION, RABBIT

228-022 945357, "Ordram Antifertility Study in Rabbits", (Stauffer, 11/80). [T-10176]. Ordram tech. (98.2%). 0, 2, 20, and 200 mg/ml daily to males only for 6 weeks in gelatin capsules. Corn oil vehicle used except in undiluted high dose group. "NOEL" = 200 mg/kg/day (HDT). No effects on parameters studied: mating and fertility indices, litter size, pup weight, gestation length, pup viability. **NOT ACCEPTABLE** (not a complete reproductive study), but useful data. No more data required from this study. (J. Christopher, 6/4/85).

228-128 118022 Tinston, D.J., "Molinate: Fertility study in male rabbits". Report No. CTL/P/3225. This was the first of three efforts to conduct a full-scale evaluation of fertility effects of molinate in male rabbits. This study was terminated prematurely due to high losses of rabbits in the two higher dose groups. Study began with 10 NZW males/group dosed by gavage with 0, 10, 100, or 200 mg/kg/day. The 200 mg/kg/day group was terminated before the first semen evaluation and fertility trial, which was scheduled at week 4. Three were found dead by day 12 and 3 more were killed in extremis by day 13. The 100 mg/kg/day group was maintained through the week 4 trial, however 4 of that group died or were killed in extremis by that time. Females inseminated by the 100 mg/kg/day group survivors had a statistically significantly elevated pre-implantation loss compared to controls (46% vs. 19%). Incidence of sperm mid-piece abnormalities was also elevated in that group. The latter finding was based on light microscopy, whereas EM did not confirm any increase in abnormal sperm. This study gave an apparent NOEL of 10 mg/kg/day, however later studies (particularly the third of this series: DPR Record No. 118042, ICI Report No. CTL/P/3684) were more successful in assessing male rabbit reproductive effects. Study is **not acceptable**, with limited useful data. Since fertility and apparent sperm morphology change occurred only at a relatively high and systemically toxic dose, no "adverse effects" are indicated. Aldous, 6/15/94.

228-108 092643, updated as complete report in 228-113 089637. (ancillary rabbit reproduction) "Molinate: Second fertility study in male rabbits". CTL

Study No: RB0533. ICI Central Toxicology Laboratory (Cheshire, UK). The record in Vol. 108 was the March 18, 1991 INTERIM SUMMARY REPORT (NOT QA-APPROVED). The complete, QA-approved report is in 228-113 089637 (dated 6/6/91). There were no essential changes in the final report from the preliminary report, which was initially reviewed by Medical Toxicology. Ten NZW male rabbits/dose were administered 0, 10, 100, or 200 mg/kg/day daily by gavage. Males were mated 1/1 with untreated does on weeks -1 (pretreatment), 4, 8, and 12. Does were C-sectioned on gestation day 18 to evaluate fertility. At comparable intervals, semen samples from the males were collected for sperm numbers, morphology, motility, and scanning electron microscopy. Males were killed at week 13, and epididymal semen was collected for similar analyses. Blood was sampled at weeks 4, 8, and 12 for RBC cholinesterase (ChE) inhibition, and brains were tested for brain ChE at termination of males. There were no apparent clinical abnormalities. Body weights of 200 mg/kg/day males trailed slightly behind other groups (not definitive evidence of a treatment effect). There was no apparent ChE effect. There were a few mortalities: 3 of the 200 mg/kg/day males and 1 of the 100 mg/kg/day males were presumed by investigators to have died due to molinate. Deaths of 5/10 200 mg/kg/day males by week 12 limited the statistical power of this study, however mean numbers of live fetuses in the 200 mg/kg/day group at week 12 were significantly lower than controls. Apparent (non-significant) increases in pre-implantation and post-implantation losses seemed to account for the reduced numbers of live fetuses at that dose. Plasma membrane abnormalities of the mid-piece region of epididymal sperm were quantified, and there were no significant differences, although mean numbers of mid-piece abnormalities were somewhat elevated in the 200 mg/kg/day group. Study is inconclusive: neither proving nor disproving putative male fertility (including sperm morphology) effects in rabbits. No new "possible adverse effect" is indicated. **Not acceptable** (ancillary study by design: the data gap for reproduction study is already filled). A repeat study is being planned, and a protocol summary (228-115 092997) was submitted on 7/22/91 for Medical Toxicology Branch comment. Aldous, 4/19/91, 8/01/91. (See also 116:098509, below).

228-116 098509, 098510 (photographic supplements to ancillary rabbit reproduction to study 108:092643), above. Supplement provides representative light micrographs and scanning electron micrographs of normal and abnormal sperm, as examined in the ancillary study. This makes the report "complete" (no more data are requested relating to record 092643). The study is not upgradeable, nor is it required to fill a data gap. An elective replacement study in this species is currently being proposed. Aldous, 10/15/91.

228-106 096313 less complete report in comparison to 092643, above. No review is needed for this report. Aldous, 4/9/91.

228-100 092018 Interim report for 092643, above. No review is needed for this report. Aldous, 4/22/91.

085 086900, Protocol for study 108:092643, above.

228-113 089637 This is the complete report for the "Second Fertility Study" in rabbits, which was reviewed as an interim report in 228-108:092643 (see above). There were no substantive changes in this report over what has been previously reviewed. Investigators continue to determine that study is inconclusive, and recommend a replacement study. No new worksheet is needed. Aldous, 7/24/91.

228-092 088922 Rose, P.H., "Molinate: Overview of three studies in male rabbits conducted at CTL". Three studies, taken together, appear to support the choice of dose levels used in study 228-108:092643, above. Four of 5 male NZW rabbits administered 300 mg/kg/day molinate in corn oil died or were killed in extremis after extensive body weight losses were noted during a 28-day study. A subsequent 28-day study found no mortalities and no clinical signs of toxicity up to the highest dose tested of 250 mg/kg/day. A sharp dose-response curve was inferred, and 200 mg/kg/day was considered to be the highest dose likely to be sustainable for a 12-week study. Aldous, 4/22/91 (no worksheet).

228-113 089612 Tinston, D.J. "Molinate: Preliminary study in male rabbits". ICI Central Toxicology Laboratory, 5/31/91. This is one of the 3 studies discussed 092:088922, above. Five male NZW rabbits/group were dosed with 0, 100, 200, or 300 mg/kg/day molinate for 28 days. High dose was not tolerated: 4/5 of 300 mg/kg/day males died or were killed in extremis. Doses up to 200 mg/kg/day seemed tolerable. No worksheet. Aldous, 7/24/91.

228-113 089636 Tinston, D.J. "Molinate: Preliminary study in male rabbits". ICI Central Toxicology Laboratory, 5/31/91. This is one of the 3 studies discussed 092:088922, above. Similar in design to study 089612: this study employed dose levels of 0, 40, 100, and 250 mg/kg/day. The only mortality was one 100 mg/kg/day rabbit. There were no indications that these doses exceeded tolerated range. No worksheet. Aldous, 7/24/91.

228-130 118042 Tinston, D.J., "Molinate: Third fertility study in male rabbits", ICI Central Toxicology Laboratory, Oct. 1, 1992. Report No. CTL/P/3684. Males were dosed by gavage with 0, 40, 80, or 160 mg/kg/day molinate. (The high dose was reduced to 120 mg/kg/day after week 5 due to unacceptable mortality). Study endpoints included fertility and examination of sperm morphology. Males were evaluated pre-dose (week -7), and at weeks 5, 9, and 13. Evaluations included sperm number and motility (light microscopy), sperm morphology of fixed samples (light microscopy), sperm morphology of fixed samples (electron microscopy), and fertility after insemination of untreated females. There was no systemic toxicity NOEL for treated males. Apparently deaths due to treatment were dose-related at all dose levels. Also, RBC cholinesterase was inhibited slightly but statistically significantly at all dose levels. There was a NOEL of 40 mg/kg/day for sperm morphology, based on atypical staining of sperm heads under light microscopy (no corresponding changes under EM). No NOEL could be established for transient pre-implantation loss (evident without dose-response relationship in all treatment groups at weeks 5 and 9, but not at week 13). There was a serious confounding effect due to the choice of gavage dosing, leading to a substantial period of inappetence irrespective of treatment. Thus, a largely inconclusive study provides some useful information, since data suggest that rabbits do not suffer sperm mid-piece abnormalities comparable to rats. Data quality is insufficient to establish an "adverse effect". Aldous, 6/30/94.

228-135 119909 Addendum to Record No. 118042, above (providing standard deviations for litter data). New statistical information does not impact study interpretation. No DPR worksheet. Aldous, 6/15/94.

228-123 113612 Interim report to Record No. 118042. No worksheet needed.

228-124 115786 Interim report to Record No. 118042. No worksheet needed.

228-131 118004 Wickramaratne, G.A., "Molinate: Overview of fertility studies in male rabbits", Oct. 1, 1992. A discussion of major findings of the

four principal rabbit reproduction studies: DPR Record Nos. 945357, 118022, 092643, and 118042. Inconsistencies in degrees of toxicity to treated males between studies, as well as differences in the types of sperm abnormalities (including lack of agreement between light microscopic evaluation and EM evaluation) or differences in reproductive outcomes (such as pre-implantation losses) were offered as evidence that "A rigorous and unique investigation into the potential effects on reproduction in rabbits of molinate has failed to demonstrate any consistent adverse effect". Not a study, nor was new information presented, hence no DPR review. Aldous, 6/15/94.

## REPRODUCTION, RABBIT AND NON-HUMAN PRIMATE

077 (no record number), "Molinate-Further Investigations of Male Fertility in Rabbits and Non-human Primates. Rationale for ICI Modifications of CDFA-Proposed Designs", (ICI Central Toxicology Laboratory, 2/7/90). This brief report presents the rationale for modifying the designs of rabbit and non-human primate studies, proposed by CDFA to further test the hypothesis that the adverse reproductive effect induced by molinate is a rodent specific phenomenon. CDFA agrees with the rationale and accepts the modifications with one exception. The analysis of epididymal sperm (motility, viability, count, and morphology by LM and SEM) is still considered desirable, since it will allow for a comparison with similar data obtained in previous rat studies (G. Chernoff, 3/23/90).

082 090585, "Molinate: Preliminary Study in Male Rabbits", (ICI Central Toxicology Laboratory, Study Number RB0509, 4/17/90). A brief summary of the pilot study used to justify the doses for rabbit study 108:092643. No worksheet provided (G. Chernoff, 5/9/90).

## REPRODUCTION, HUMAN

001 19849-19852, "Epidemiologic Assessment of Fertility in Male Workers Exposed to Ordram at the Stauffer Chemical Co.", (University of Rochester, April 20, 1984). Male workers from three plants were evaluated for adverse reproductive effects from working with Ordram. Retrospective fertility data collected from the men were used to calculate natality (observed/expected birth rate), which did not differ between the various exposure groups. Exposure dosage was estimated by multiplying the number of hours exposure by monitoring data on breathing zone concentrations. Monetary incentives were used in soliciting sperm samples which were analyzed for sperm count and percent normal sperm as measured by light microscopy. Comparison of groups by change in exposure hours with change in sperm count or percent normal sperm gave equivocal results. Multivariate regression analyses erased any association between Ordram exposure and altered sperm parameters. The power of this study, along with the validity of the conclusions, is severely limited by the procedure used to collect the reproductive histories, the methodology used for determining natality, the methodology used for estimating exposure levels, the validity of the sperm count data, the statistical procedures for analyzing sperm count and percent abnormal sperm, the lack of data on sperm motility, and the absence of electron microscopic evaluation of sperm morphology. Because of these limitations, the study is considered inadequate for risk assessment purposes. A review and consideration of the rebuttal material in Record No. 086074 failed to satisfy concerns about the limitations of this study which continues to be considered inadequate for risk assessment purposes (G. Chernoff, 3/22/90).

228-035 000913 Earlier version of 001:019849, cited above. No worksheet. Aldous 4/22/91.

228-037 023535, also -028 945372 Earlier versions of 001:019851, cited above. No worksheet. Aldous 4/22/91.

077 086074, "Reply of Donald R. Taves, M.D., M.P.H., Ph.D. to comments in the "Toxicology Summary Report Worksheet" evaluating the University of Rochester Study of the fertility of workers exposed to Ordram", (D. Taves, 2/11/90). Rebuttal comments to the review in Record No. 019849-019852 (G. Chernoff, 3/23/90).

077 086075-086077, "Molinate: Industrial Summaries - Exposure Data", (Stauffer Chemical Co., 6/4/82). This volume contains summaries of exposure information from three molinate production facilities which were included in the epidemiologic study conducted by the University of Rochester. The data are helpful in clarifying the respiratory exposure levels associated with various jobs in the three plants. However, there is no indication of the nature of the jobs and which jobs may have included some dermal as well as respiratory exposure. Most importantly there is no indication of how many people of various job categories were included in the study population, or what the criteria for inclusion may have been (this review is contained in a memorandum from Dr. Michael O'Malley of Worker Health and Safety to G. Chernoff, dated April 2, 1990).

## TERATOLOGY, RAT

003 035757. Not a true teratogenicity study, but is a spin-off from 3-gen. reproduction study (003 945351). Delivered pups were examined instead of obtaining by C-section. Dosing of dams was ongoing, rather than optimized during organogenesis, as required for a teratogenicity study. **NO ADVERSE EFFECTS** reported by investigators. **NOT ACCEPTABLE** nor upgradeable. Review of full study (003 945351) by J. Christopher, 6/4/85.

****081 088187**, "A Teratology Study in CD* Rats with R-4572 Technical", (Minor, J.L., Ciba-Geigy Environmental Health Center, Report # T-13266, March 30, 1990). R-4572 Technical, 97.6%, Lot #EHC-0866-30, was administered by gavage to groups of 26 female Crl:CD (SD) BRVAF/Plus rats on days 6-15 of gestation at dose levels of 0 (corn oil vehicle control), 2.2, 35, or 140 mg/kg/day. At 140 mg/kg/day, maternal food consumption and weight gain were reduced, and salivation was observed with the presence of cholinesterase inhibition. The number of resorptions at 140 mg/kg was dramatically increased, the litter size decreased, and evidence of intrauterine growth retardation present (decreased fetal weight, dilated brain brain ventricles, incomplete ossification of the sternebra). Maternal NOEL = 35 mg/ml/day (decreased food consumption and weight gain; salivation; cholinesterase inhibition); Developmental NOEL = 35 mg/kg/day (increased resorptions and intrauterine growth retardation). The study is **ACCEPTABLE**, and a **POSSIBLE ADVERSE HEALTH EFFECT** (increased resorptions prior to the onset of maternal toxicity) is noted (G. Chernoff, 5/9/90).

## TERATOLOGY, RABBIT

**044 033591, "A Teratology Study in New Zealand White Rabbits with Ordram [T-11866]", (Stauffer, CT, 6/6/85). Ordram tech., 98.8% purity; tested at 0, 2, 20, and 200 mg/kg/day by gavage. Apparent maternal and developmental

toxicity NOEL = 20 mg/kg/day. The following observations in 200 mg/kg/day dams were statistically non-significant, although possibly treatment-related: increased abortions (incidence of 1, 1, 1, and 4 in increasing doses), slight decrease in body weight during days 14-21 of gestation (diminished food intake during same period), slight increase in liver weights and in incidence of dark brown liver (incidence of 1, 2, 2, and 5 in increasing doses). Fetuses in 200 mg/kg/day group had increased incidence of 5th unossified sternebrae and decreased incidence of extra ribs, (short, bilateral). **NO ADVERSE EFFECTS** noted: evidence for both maternal and developmental toxicity initially evaluated as tenuous with study unacceptable but upgradeable with dose justification (J. Christopher, 9/17/85). Rebuttal in 228-050 (no record number) discusses the dose selection based on a range-finding study at doses of 200, 600 and 800 mg/kg/day with 5/group. Significant toxic effects were seen at 600 and 800 mg/kg/day supporting selection of 200 mg/kg as the high dose. The study is upgraded to **ACCEPTABLE** status based on the justification of dose selection. (Aldous and Gee, 6/17/88).

228-135 119910 Wilczynski, S.L., "A range-finding teratology study in pregnant New Zealand White rabbits with ORDRAM*." Report No. T-11754, 12/20/83. This is a more complete report of the study earlier reported in Document No. 228-050 (see above 1-liner). Two of the 800 mg/kg/day dams died after a single dose, and treatment was terminated for that group after the single dose. The 600 mg/kg/day group suffered the first treatment-related death after 6 dose administrations, and treatment was terminated after the sixth dosing for that group. Only one pregnant, surviving doe was present at cesarean section for each of the 600 and 800 mg/kg/day groups: each had total litter resorptions. There was no evident general or reproductive toxicity at 200 mg/kg/day, hence this dose level was chosen for the definitive study. Aldous, 6/15/94 (no worksheet).

## TERATOLOGY, MOUSE

006 028493, "Ordram Safety Evaluation by Teratological Study in the Mouse", (Woodard Research Corp., 4/20/67). Ordram tech (96.5%). 0, 8, and 24 mg/kg/day in diets from day 6 to day 15 or 18. No maternal nor developmental toxicity indicated. **UNACCEPTABLE,** not upgradeable (too few pregnant dams/group, too few treatment groups, dosages not justified and apparently too low, etc.). (J. Christopher, 6/3/85).

## GENE MUTATION

General Comments: Six studies with microbial systems present a consistent picture of no mutagenicity with or without activation. However, an excellent study with mammalian cells demonstrated weak mutagenicity with activation. Thus there is a **POSSIBLE ADVERSE EFFECT** for this category. All studies in this section (except for Record # 071077) have been reviewed again by Davis, 4/2/87, in making these conclusions. (Aldous, 4/7/87).

**063 071077, "Molinate: An Evaluation in the Salmonella Mutation Assay", (ICI Central Toxicology Laboratory, project no. CTL/P/2246, September 28, 1988). Molinate, purity 97.6%, two trials at 0 (DMSO), 1.6, 8.0, 40, 200, 1000, or 5000 μg/plate with *Salmonella typhimurium* strains TA1535, TA1537, TA1538, TA98 and TA100, with and without Aroclor-1254 induced rat liver S9-mix, 64-68 hours incubation; tested a third time at 0.32, 0.8, 1.6, 4.0, 8.0, or 20.0 μg/plate with S9-mix activation on strains TA1535 and TA1537. No consistent increase in revertants. ACCEPTABLE. (Gee, 11/17/89)

006 945358, "Evaluation of Herbicides for Possible Mutagenic Properties", (K. J. Anderson et al., J. Agr. Food Chem. 20:649-656, 1972). *Salmonella* assay (Columbus Laboratories, Battelle Memorial Institute). Molinate stated to be negative for mutagenicity as tested with 8 mutants of *Salmonella typhimurium*. No adverse effect. Incomplete, **UNACCEPTABLE**. (Christopher 6/3/85).

006 945359, "Assay of Ordram for Mutagenicity Using the Ames Salmonella Tester System", (Woodard Research Corporation, 5/9/75). Ordram = molinate (purity unstated) tested with 0.1 ml of 0, 0.005, 0.5, and 50.0 ppm using strains TA 100, TA 1535, TA 1537, and TA 1538 with and without activation. **NO ADVERSE EFFECT** reported. Incomplete, **UNACCEPTABLE**.--no individual plate counts, inadequate positive controls, strain TA 98 not tested, too few dose levels, inadequate S-9 protocol, test material inadequately characterized. (Christopher, 6/3/85).

006 945360, "Mutagenic Evaluation of Compound Ordram Tech RCK 0701", (Litton Bionetics, Inc. 7/7/75). Ordram (purity unstated) tested at 6 dose levels ranging from 0.01 to 500 ul/plate using *Salmonella typhimurium* strains TA 98, TA 100, TA 1535, TA 1537, TA 1538, and yeast strain D4, with and without activation. Toxicity was found at 100 and 500 ul/plate. **NO ADVERSE EFFECT** reported. Incomplete, **UNACCEPTABLE**. Missing page 1, apparently only one plate for each dose level, negative control values with activation are high, test material inadequately characterized. (Christopher, 6/3/85).

006 945361, "Mutagenicity Testing on Molinate in Microbial Systems", (Institute of Environmental Toxicology, Japan 9/9/77). Molinate (99.8% purity) tested at 0, 10, 50, 100, 500, 1000, and 3000 ug/plate with *Salmonella typhimurium* strains TA 98, TA 100, TA 1535, TA 1537, TA 1538, and *Escherichia coli* strain WP2 hcr with and without activation. 3000 ug/plate was toxic. **NO ADVERSE EFFECT REPORTED.** Incomplete, unacceptable, no test material lot number, no QA or sign-off sheet, only duplicate plates, no confirmatory repeat assay. Study previously classified as acceptable by Christopher (6/3/85), now re-classified as **UNACCEPTABLE** by Davis (4/2/87) for reasons stated above.

228-006 035755 This appears to be a re-numbering of 006:945361 (two different record numbers were used for the two <u>in</u> <u>vitro</u> portion of the report. Aldous, 4/22/91.

006 035756, "Mutagenicity Testing on Molinate in Microbial Systems", (Institute of Environmental Toxicology, Japan 9/9/77). Molinate (99.8% purity) tested at 0, 30, or 100 mg/kg/dose in two doses by gastric intubation to 6 male mice per group, followed immediately by intraperitoneal inoculation with *S. typhimurium* G46 (his-). Three hours later peritoneal fluid was plated in triplicate for each animal and incubated for 2 days. A concurrent <u>in</u> <u>vitro</u> reverse mutation assay was done with the same strain using 0, 10, 50, 100, 500, 1000, and 3000 ug/plate. **NO ADVERSE EFFECT REPORTED**. Incomplete, **UNACCEPTABLE**. No test material lot number, no QA or sign-off sheet, no evidence that the bacteria are exposed to molinate in the host-mediated assay. (Christopher, 3/85).

****043 026459**, "Mutagenicity Evaluation in Mouse Lymphoma Multiple Endpoint Test, Forward Mutation Assay", (Stauffer Chemical Company, Report No. T-11840, 9/25/84). Ordram (= molinate) Technical (Lot No. WRC 4921-8-9, 98.8% purity) in a series of assays in L5178Y TK +/- cells with doses ranging from 0.0125 to 0.28 ul/ml without activation and from 0.01 to 0.10 ul/ml with activation, using 48 or 96 hour expression time. No mutagenicity was observed in the absence of activation, but activation with either mouse or rat liver S-9 extract induced marginal (2 to 5x) increases in mutation frequency. This

was reproducible and not due to artifacts of selection or toxicity. **POSSIBLE ADVERSE EFFECT,** complete, **ACCEPTABLE**. (Remsen (Gee), 9/11/85).

CHROMOSOME EFFECTS

General Comment: It is noted that there were some elevated frequencies of both chromosome aberrations and sister chromatid exchanges with activation in Record 26460. This could support the weak mutagenicity with activation found in the same cell line in Record 26459. However, the cytogenetic effects were not consistent in repeat assays and were not dose-related, since none occurred in the high dose. Furthermore, the bone marrow micronucleus assay was negative. On balance the test material appears to be negative for this category of genotoxicity. We conclude that there is **NO ADVERSE EFFECT**. The studies in this section (except for Record # 072638) have been reviewed again by Davis, 4/2/87, in making these conclusions.
The publication in Mutation Research, 1990, by Pinter et al. reports an increase in the incidence of micronucleated polychromatic erythrocytes. The report is unacceptable based primarily on the lack of individual data. There is already an acceptable study of this type spanning the same dose range without an effect. Therefore, there is no demonstrable adverse effect. Added comment by Gee, 8/11/94.

**065 072638, "Molinate: An Evaluation in the In Vitro Cytogenetic Assay in Human Lymphocytes", (ICI Central Toxicology Laboratory, Report no. CTL/P/2402, Dec. 15, 1988). Molinate, purity 97.6%, at levels of 0 (DMSO), 190, 95 and 24 mg/ml with lymphocytes of 2 donors (1 male and 1 female), in vitro - 3.25 to 3.75 hours exposure, in the presence and absence of Aroclor-1254 induced male rat liver activation. No adverse effect. Molinate showed no clastogenic effect with human lymphocytes in vitro. ACCEPTABLE. (Kishiyama, 7/21/89 and Gee, 11/17/89).

**043 026461, "Mutagenicity Evaluation in Bone Marrow Micronucleus", (Stauffer Chemical Company, Report No. T-11820, 11/22/83). Ordram ( molinate) Technical (Lot No. WRC 4921-8-9, 98.8% purity) at 0, 200, 400, or 600 mg/kg for 15 male mice/group and 0, 100, 200, or 400 mg/kg for 15 female mice/group by single oral gavage. Sampled 5000 bone marrow cells of 5 mice/sex/time point at 24, 48, and 72 hours (except for 24 hour female group where sampled 10,000 cells). **NO ADVERSE EFFECT**, Complete, **ACCEPTABLE**. [Remsen (Gee), 9/11/85].

**043 026460, "Mutagenicity Evaluation in Mouse Lymphoma Multiple Endpoint Test. Cytogenetic Assay." (Stauffer Chemical Company, Report No. T-11856, 12/2/83). Ordram (molinate) Technical (Lot No. WRC 4921-8-9, 98.8% purity) in a series of assays in L5178Y mouse lymphoma cells with doses ranging from 0.0125 to 0.20 ul/ml without activation and from 0.0025 to 0.0400 ul/ml with activation. Neither chromosome aberration frequency nor sister chromatid exchange (SCE) frequency was increased in the absence of activation. Activation with rat liver S-9 extract resulted in some statistically significant elevations in both chromosome aberration and SCE frequencies but these were not dose-related and not repeatable. **NO ADVERSE EFFECT**, complete, **ACCEPTABLE**. (Remsen, 9/11/85).

No record number. "Cytogenetic effect of the thiocarbamate herbicides butylate, molinate and vernolate in the mouse bone marrow micronucleus test." Pinter, A. et al. in: Mutation Research 242: 279-283 (1990) Molinate, 97.4%, was given to CFLP mice in a single oral dose at 0 (sunflower oil), 175, 350 or 525 mg/kg. Groups of 5/sex (usually) were sacrificed at 24, 48 and 72 hours

for control and high dose and at 48 hours at the low and mid doses. Micronuclei in polychromatic erythrocytes were scored, 1000 per animal. AT 48 hours at the mid and high doses, the mean incidence of micronucleated polychromatic erythrocytes was statistically significantly increased in both males and females compared with controls. Possible adverse effect. UNACCEPTABLE (no individual data, missing information on study conduct). (Gee, 8/11/94)

DNA DAMAGE

General Comment: The evidence shows **NO ADVERSE EFFECT** in this category. See also the general comments for the Chromosome Mutation category and the one-liner for Record 26460. The studies in this section (except Record # 073902) have been reviewed again by Davis, 4/2/87 in making these conclusions.

**068 073902, "Molinate: Assessment for the Induction of Unscheduled Synthesis in Primary Rat Hepatocyte Cultures", (ICI Central Toxicology Laboratory, Study no. SV0332, Report No. CTL/P/2484, March 22, 1989). Rat hepatocytes were exposed to molinate, purity 97.6%, at concentrations of $10^{-2}$, $10^{-3}$, $10^{-4}$, $10^{-5}$, $10^{-6}$, $10^{-7}$, $10^{-8}$, or $10^{-9}$ molar for 17 to 20 hours in 2 independent tests. Doses of $10^{-4}$ and $10^{-5}$ were selected as the high concentration for experiments 1 and 2, respectively, and the three subsequent lower concentrations from each experiment were also were selected for UDS evaluation. Net nuclear grain counts were less than zero for all molinate treatments examined. No adverse effects indicated. ACCEPTABLE. (Kishiyama and Gee, 11/17/89).

**006 945361, "Mutagenicity Testing on Molinate in Microbial Systems", (Institute of Environmental Toxicology, Japan 9/9/77). Molinate (99.8% purity) tested at 0, 1, 5, 10, 25, 50, and 100% v/v on the paired B. subtilis strains H17 (repair competent) and M45 (repair deficient) in a disc diffusion assay using the streak technique. **NO ADVERSE EFFECT**. Complete, **ACCEPTABLE**. (Christopher, 6/3/85).

NEUROTOXICITY, HEN

**046 041548** Study T-10510, "Acute Delayed Neurotoxicity Study with Ordram Technical in adult hens", (Stauffer, 6/16/83). [T-10510]. Molinate, tech. Dosages (see section V.A. of this review for details) 0.02, 0.063, 0.20, 0.63, and 2.0 g/kg + pos. and neg. controls. Additional hens for recovery study. NOEL for neurotoxicity (apparently not identical to classical TOCP-like acute delayed toxicity) = 0.2 g/kg, based on microscopic lesions (esp. axonal degeneration in upper spinal cord and cerebellar peduncle areas), walking behavior deficits, and other observations of neuromuscular weakness in 2.0 and 0.63 g/kg hens. Mortality, weight deficits, and other general toxicity findings were dose-related and essentially limited to the same upper two dosage groups. **NOT ACCEPTABLE**, but fully upgradable on receipt of additional clinical observation data. (C. Aldous, Aug. 7, 1986).

**228-147 129725** Horner, J.M., "Molinate: Acute neurotoxicity study in rats", Zeneca Central Toxicology Laboratory, Alderley Park, Report No. CTL/P/4180, 3/22/94. Twelve Alpk:APfSD rats/sex were dosed once with 0, 25, 100, or 350 mg/kg by gavage. Rats were evaluated for limited Functional Observational Battery (FOB) parameters, motor activity, cholinesterase effects, and histopathology of nervous system. No NOEL was found for the following acute effects: food consumption and body weight decrements (M), sensory response (tail flick) (M & F), and motor activity (M & F). All of the above had

dose-response relationships. Brain cholinesterase NOEL at day 15 = 25 mg/kg (dose-related changes in M & F). There were no effects which persisted for more than a few days after a single dose of 25 mg/kg, which is the NOEL for non-transient effects (based on slight brain weight decrements in males). A **""possible adverse effect"** is indicated, based on neuronal cell necrosis in the pyriform cortex of 350 mg/kg females. The resulting NOAEL = 100 mg/kg. The study is **unacceptable, possibly upgradeable.** Deficiencies which do not invalidate the study are (1) some groups were too small for meaningful evaluation, such as N = 3 for glial fibrillary acidic protein (GFAP) assays, and (2) there were no concurrent positive controls (however these were supplied by Zeneca in support of another active ingredient). A deficiency which would need to be addressed to upgrade the study is that the Functional Observational Battery (FOB) was not performed in the systematic manner recommended in guidelines, and findings were typically not graded as to severity. An upgrade of the study acceptability status would require an adequate resolution of the FOB parameters in both the positive controls and the test groups. Aldous; June 9, 1994.

## METABOLISM STUDIES

NOTE: Metabolism studies are not routinely evaluated under SB-950, hence the 1-liners below do not represent an exhaustive listing of such studies which may be available. In particular, there are rat metabolism studies cited in a record dated 11/30/92 entitled "Molinate: NOELs being used in CDPR risk assessment" (DPR Record No. 119319) which suggest a metabolite pattern in rats which is markedly different from man. Humans excrete molinate metabolites almost entirely in the urine, and 4-hydroxymolinate is by far the major metabolite. Rats apparently excrete more molinate mercapturate conjugate than 4-hydroxymolinate, and an appreciable amount of rat metabolites are found in feces. Aldous, 6/16/94.

074 090135, ICI America Inc., 12/20/89. This report consists of 4 tables from a draft report on a dermal absorption study conducted as part of a research project that did not use the "Zendzian" protocol. An additional in vitro dermal penetration study, followed by an in vivo (Zendzian protocol) study are scheduled with tentative reporting dates of 4/1/90 and 12/31/90, respectively. No worksheet provided at this time (G. Chernoff, 1/25/90).

228-127 118234 Batten, P.L. et al., "Molinate: Metabolism in man following a single oral dose", Report No. CTL/R/1099, March 6, 1992. Report was submitted in support of DPR risk assessment process. Oral administration of molinate in corn oil led to rapid urinary excretion. Principal metabolites were conjugates of 4-hydroxy molinate (about 39% of administered dose). It was suggested that this metabolite could be used to estimate absorbed dose. No DPR worksheet by DPR SB-950 Data Review Group (not a required study type). Aldous, 6/15/94.

228-132 118003 Lythgoe, R.E. et al., "Molinate: Excretion and blood kinetics in the monkey". Ring-labeled molinate was administered iv or orally to male cynomolgus monkeys. Excretion was rapid, and almost entirely via urine. Animals were not sacrificed for general tissue analyses, however levels in RBCs and plasma dropped quickly. Major urinary metabolites following a 60 mg/kg oral dose were: glucuronide conjugate of 4-hydroxymolinate (33%), cysteine conjugate (12%), and molinate mercapturate (10%). [Contrast with rat metabolism in Record No. 118234, above]. Useful information, but not requiring SB-950 worksheet at this time. Aldous, 6/15/94.

RISK ASSESSMENT/PROPOSITION 65 GENERAL SUBMISSIONS

228-136 120196 "Submission to January 1993 SAP: Molinate reproductive toxicology executive summary", ICI Agrochemicals, 12/21/92. A discussion referring primarily to SB-950-related studies, presenting several lines of evidence that reproductive effects in rat reproduction studies are species-specific phenomena. Information is potentially useful, but not relevant for an SB-950 review worksheet. Aldous, 6/15/94.

228-136 120199 Paddle, G.M., "Epidemiological assessment of fertility in male workers exposed to 'Ordram' at the Stauffer Chemical Company", from a larger report with the same title by Taves et al., dated 4/20/84. Some information to partially characterize chemical plant worker exposure ranges is provided. Data include seasonal sperm counts, % normal sperm by season, and fertility analyses, with these data categorized in some cases by exposure groups. Seasonal data are provided because the primary exposure is seasonal (especially winter and spring). Data do not implicate molinate as a male reproductive toxicant. Information is potentially useful, but not relevant for an SB-950 review worksheet. Aldous, 6/15/94.

228-129 118021 "Molinate: A review of reprotoxicity" (Prepared by ICI Agrochemicals for submission to the California Proposition 65 Scientific Advisory Panel), 9/30/92. General discussion of reasons why the writers feel that molinate should not be classified by California Proposition 65 SAP as a reproductive toxin. Primary thrust of arguments is that high quality human epidemiological studies have been carried out, and no evidence of human reproductive toxicity has been found. No formal review or worksheet is relevant by this group at this time. Aldous, 6/16/94.

OTHER

066 072779, "21-Day Dermal Toxicity to the Rat", (ICI Toxicology Laboratory, Report No. CTL/p/2321, 1/27/89). Molinate, purity 97.6%, was administered undiluted to the dorso-lumbar region of 5 Wistar-derived albino rats/sex/group at concentrations of 0 (occlusive bandages only), 10, 25 or 50 mg/kg for 6 hours per day for 21 days. An increased incidence and severity of skin irritation (desquamation, thickening of skin, erythema, and oedema), and slight to moderate hydronephrosis was observed at doses greater than 10 mg/kg/day. NOEL = 10 mg/kg/day based on skin irritation and hydronephrosis. This study is ACCEPTABLE as a supplemental report (J. Kishiyama and G. Chernoff, 1/25/90).

# APPENDIX B

## Occupational Exposure Assessment

# ESTIMATION OF EXPOSURE OF PERSONS IN CALIFORNIA TO PESTICIDE PRODUCTS THAT CONTAIN MOLINATE

BY

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HS-1543, June 21, 1991
Revised January 24, 1995

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## ABSTRACT

Molinate is a selective herbicide that is used exclusively on rice in California. It is applied predominantly by air as a granular formulation. There have been 12 reported illness/injury cases that were associated with exposure to molinate alone or in combination with other pesticides from 1982 to 1992 in California. Following ingestion, molinate is readily absorbed, metabolized, and excreted in animals and humans. Workers involved in loading molinate were identified as having the greatest potential for exposure. Worker exposure studies show that the exposure to workers loading molinate has consistently been in decline for the last 15 years as additional personal protective equipment, engineering controls, improved formulation, worker training, and use restrictions have systematically been implemented. This report was prepared as part of the Department's risk characterization document for molinate because molinate reproductive toxicity and neurotoxicity studies have shown possible adverse effects in exposed laboratory animals.

HUMAN PESTICIDE EXPOSURE ASSESSMENT

California Environmental Protection Agency
Department of Pesticide Regulation
Worker Health and Safety Branch

MOLINATE

June 21, 1991
Revised January 24, 1995

## Introduction

Molinate is on the list of the first 200 products to be reviewed under the California Birth Defect Prevention Act of 1984 (SB-950). Studies with molinate have shown possible adverse reproductive and neurotoxicity effects in laboratory animals. A human exposure assessment was prepared for molinate on December 5, 1989 and was revised prior to each growing season since that time. Since the initial studies in 1980-81, several additional molinate worker exposure monitoring studies have been conducted. This revision of molinate human exposure assessment includes exposure studies conducted following the previous revision. Human exposure assessment provides essential information for the risk assessment of pesticides. This human exposure assessment document will be an integral part of the Risk Characterization Document of the Department of Pesticide Regulation (DPR) for molinate. It will also serve as a basis for developing mitigation strategies if exposure to molinate is found to cause excessive risk.

## Chemical and Physical Properties

Molinate (CAS # 2212-67-1) is the common name for S-ethyl hexahydro-1H-azepine-1-carbothioate. Molinate has a molecular formula of $C_9H_{17}NOS$ and a molecular weight of 187.3 g/Mol. It is a liquid at normal temperature and pressure and slightly soluble in water but miscible in common organic solvents. Molinate has a boiling point of 202$^{o}$C and a vapor pressure of 5.0 x $10^{-3}$ mm Hg at 25$^{o}$C (Myers, 1987; 1988; Stauffer, 1971). Its half-life in soil is 28 days (Lay, 1990). Molinate is a selective herbicide that is used for control of weeds in rice fields.

## Regulatory Status

The Department of Pesticide Regulation has issued an interim risk characterization document each year from 1990 to 1994, recommending consecutive exposure mitigation measures to minimize the exposure of workers handling molinate in California. The Department also required additional worker exposure monitoring studies to better define the potential exposure of workers to molinate. The mitigation measures included personal protective equipment (PPE), engineering controls, worker training, use restrictions, and restriction on the amount handled by workers. The additional mitigation measures were implemented through Worker Safety Permit Conditions issued prior to each rice growing season.

## Formulations

Ordram® 8E and Ordram® 10G are the only two molinate-containing products registered in California. Ordram® 8E is an emulsifiable concentrate formulation with 90.9 percent active ingredient (a.i.) and contains 8 pounds (lb) a.i. per gallon. Ordram® 10G is a granular formulation that contains ten percent a.i.

## Usage

Molinate is a selective herbicide that is used exclusively on rice in California for control of barnyardgrass. The application rate for Ordram® 10G is 3 to 5 lb a.i. per acre and for Ordram® 8E is 3 lb a.i. per acre. Ordram® 10G is applied either by ground or air equipment. Ordram® 8E application is allowed only by ground equipment in California (see attachment I). The majority of molinate used in California is applied by air, using the granular formulation. A total of 1,387,600 lb of molinate was applied to 350,990 acres of rice fields in California in 1992 (DPR, 1994a).

## Label Precautions

Both Ordram® 8E and Ordram® 10G are toxicity category II pesticides carrying "Warning" as a signal word. Aerial application must be made only when the wind velocity is ten miles per hour or less. The latest molinate worker safety permit conditions (1994) require personal protective equipment (PPE), engineering controls, and training for workers handling molinate. Attachment I is a copy of the 1994 permit conditions. The application of Ordram® 8E by air is not allowed in California according to the permit conditions.

## Human Illnesses and Injuries

There have been 12 reported illness/injury cases that were associated with exposure to molinate alone or in combination with other pesticides from 1982 to 1992 in California (DPR, 1994b). From 1982 to 1985, no molinate-associated illnesses or injuries were reported. The 12 reported cases occurred from 1986 to 1992. Of the 12 cases, six were systemic, three were eye injuries, and three were skin injuries.

## Dermal Irritation/Sensitization

Technical molinate is a mild to moderate skin and eye irritant in the rabbits. Dermal irritation studies with formulated molinate in rabbits at 0.5 g/animal showed no effect or slight erythema at 24 hours. The symptom appeared to be transient and subsided by 72 hours (Stauffer, 1968). Molinate formulations did not cause sensitization in guinea pigs (Mutter, 1986).

## Metabolism

Simonsen albino rats were administered a single (72 mg/kg) gavage dose with $^{14}C$-labeled molinate in two studies (DeBaun, *et al*., 1987a; DeBaun, *et al*., 1987b). The first study consisted of two parts: a mass balance and tissue residue. Urine, feces, air, tissues and cage washes were collected from two rats of each sex. By 72 hours post-exposure, 82.1, 10.6, 5.8, and 0.9 percent of the dose were recovered in

urine, feces, cage wash, and exhaled air, respectively. Tissue distribution of the $^{14}C$-labeled molinate was observed in six rats of each sex. Blood and liver had the highest residues. In the second study, thin-layer chromatography, NMR, and mass spectral analysis were used to identify urinary metabolic products. Urine samples were the composite of the first 48 hours of output. Molinate mercapturate (35.4 percent of urinary radioactivity), 3- and 4-hydroxy molinate glucosiduronic acid (26.1 percent), hexamethyleneimine (14.6 percent), and 3- and 4-hydroxy hexamethyleneimine (10.3 percent) were the major metabolites and accounted for 86.4 percent of $^{14}C$ found in urine. The primary metabolic pathways of molinate were sulfoxidation, glutathione conjugation, and ring hydroxylation.

In a series of studies (Ritter *et al*., 1991; Ritter, 1991a; Peffer, 1991; Ritter, 1991b), Sprague-Dawley rats were given a single low dose gavage, 14 days gavage, a single high dose gavage, or a single intravenous dose of $^{14}C$-labeled molinate (94.2 - 96.7 percent purity, 17.5 mCi/mmol). Most of the radiolabel (64-85 percent) was excreted in the urine in these studies within 36 hours. Radiolabel in feces ranged from 2.5 to 14 percent of the dose. Molinate mercapturic acid was the major metabolite, accounting for 21.2 to 51.3 percent of the administered dose in these studies. Other metabolites were 3- and 4-hydroxy molinate glucoronide, hexamethyleneimine, 3- and 4-hydroxy hexamethyleneimine, 4-keto hexamethyleneimine, hydroxy molinate mercapturic acid, and 3- and 4-hydroxy molinate, each accounting for 0.5 to 15 percent of the dose.

Male Cynomolgus monkeys (4/dose) were administered $^{14}C$-molinate (99.9 percent purity) either by gavage at 6 mg/kg or 60 mg/kg, or intravenously at 6 mg/kg (Lythgoe *et al*., 1992). Monkeys receiving an i.v. injection excreted in the urine 87.4 percent of the administered dose during the first 24 hours and 95.8 percent of the administered dose within 192 hours. Total recovery was 97.5 percent of the administered dose. The major urinary metabolite was the glucuronide conjugate of 4-hydroxy molinate (33.3 percent). Other metabolites were cysteine conjugate of molinate (11.7 percent), molinate mercapturate (10.2 percent), the glucuronide conjugate of 3-hydroxy molinate (4.7 percent), the methyl ester of glucuronide conjugate of 4-hydroxy molinate (2.4 percent), the acetic acid conjugate of molinate (3.2 percent), the glucuronide conjugate of ring hydroxylated molinate (1.9 percent), hexamethyleneimine (0.7 percent), and 4-hydroxy molinate (0.3 percent). The identified metabolites accounted for 68 percent of the urinary radioactivity. Monkeys administered a single oral dose at 6 mg/kg excreted 48 percent of the administered dose in urine in 24 hours. During the following seven days an additional 2.3 percent was excreted in urine. Total recovery was 51.1 percent of the administered dose despite the analysis of feces. Monkeys administered a single oral dose at 60 mg/kg excreted 79 percent of the administered dose in urine in 24 hours. In the following seven days an additional 1.2 percent was excreted in urine. Excretion in feces accounted for 1.8 percent of the total dose in eight days.

Human volunteers (six) were given a single oral dose (5 mg) of molinate (99.7 percent purity) in corn oil (Batten *et al*., 1992). The major metabolite was 4-hydroxy molinate. Peak excretion of 4-hydroxy molinate occurred within the first four hours and was almost complete by 24 hours. An average of 39 $\pm$ 9.7 percent of the administered dose was excreted in the form of 4-hydroxy molinate in the urine by 24 hours. Molinate mercapturate urinary excretion accounted for 0.9 $\pm$ 0.35 percent of the administered dose in 24 hours. Human metabolism of molinate appears much more similar to monkeys than to rats. Molinate was detected in some blood samples collected after 0.5 to 1.0 hour of dosing but the concentrations were close to the limit of detection of 1 ng/mL.

Following ingestion, molinate is readily absorbed, metabolized, and excreted in animals and humans. The primary urinary metabolite in humans is 4-hydroxy molinate. The primary metabolite, 4-hydroxy molinate, is a viable exposure indicator that can be used in monitoring human exposure to molinate.

## Dermal Absorption

The dermal absorption of molinate has been studied in rats and *in vitro* in humans. Dermal absorption (i*n vivo*) of $^{14}$C-molinate was determined in dorsally shaved Charles River Sprague-Dawley rats (Holmes, 1990). Five to six rats per dose level (0.61, 1.39, and 10.6 mg/kg) were administered $^{14}$C-molinate dissolved in methanol and placed into individual metabolism chambers. The majority of rats had the treatment site covered with an occlusive wrap which was kept on for 24 hours until the site was washed. One group of rats dosed at 1.39 mg/kg did not have the treatment site occluded and were exposed for seven hours prior to washing. All rats remained in metabolism chambers for 72 hours. At sacrifice, radiocarbon was quantified in carcass, urine, feces, exhaled air, and treatment site.

Absorption in the occluded rats was uniform across dosages (56.0 $\pm$ 3.0 percent) and recovery of radiocarbon was complete (97.3 $\pm$4.5 percent). Radiocarbon recovery was 80.6 $\pm$ 7.0 percent in the unoccluded rats and the absorption, when corrected for recovery, was 47.1 percent.

In a separate *in vivo* dermal absorption study, rats were administered 0.1, 1.0, or 10.0 mg of molinate (as molinate in solution) or 1.0 mg of molinate (as an Ordram 15G surrogate using kaolin clay carrier) (Little, 1991). All molinate formulations used $^{14}$C-molinate. The application site was protected with an activated charcoal filter patch to trap volatilizing molinate. Material was left on the skin for the duration of the study (up to 120 hours) for all except the Ordram surrogate which was washed after 10 hours. The surrogate was also applied dry to the skin. Urine, feces, cage wash, filter patch, and carcass were analyzed for radiocarbon. A dermal absorption of 53 percent in 24 hours was estimated based on this study (Thongsinthusak, 1991).

The Department (Thongsinthusak, 1991) reviewed an *in vivo*/*in vitro* rat and *in vitro* human dermal absorption study (Scott, 1991) and an *in vitro* rat and human dermal absorption study (Scott and Clowes, 1991) to make an estimate of *in vivo* human dermal absorption. The Department derived a ratio of *in vitro* rat to *in vitro* human (average ratio = 5.2) dermal absorption and divided the *in vivo* rat (53 percent absorption) by that ratio to estimate an interim *in vivo* human dermal absorption. The estimated human *in vivo* dermal absorption of 10.2 percent was conditionally accepted for regulatory purposes pending a confirmatory human *in vivo* dermal absorption study to be submitted in 1992. No confirmatory human *in vivo* dermal absorption study has been submitted to the Department, therefore, a dermal absorption of 53 percent will be used in this document based on rat *in vivo* dermal absorption rate.

## Dislodgeable Foliar Residue

Because of the nature of rice growing and mechanized harvesting, dislodgeable foliar residue data to determine the exposure to field workers are unnecessary.

## Worker Exposure

Several molinate worker exposure studies have been conducted over the years. The earlier studies were conducted using passive dosimetry (pad or t-shirt, hand wash, air sampling). The later and most recent studies were conducted using biological monitoring.

Maddy et al. (1982) monitored dermal and inhalation exposure of one pilot, two loaders, and two flaggers handling Ordram® 10G (packaged in 50-lb bags) during aerial application to rice fields. The workers were monitored during two workdays. The application rate was 4 lb a.i. per acre. Air samples were taken from the breathing zone of workers, using XAD-4 filters, assuming all airborne residues

were in vapor phase. Hand exposure was measured using distilled water hand washes. Durham and Wolfe-type patch dosimeters were attached to the exterior of coveralls or clothing at various body parts to measure potential dermal exposure. No spiked samples were taken to determine field recoveries.

The loading procedure during this study was substantially different from that currently in practice. One loader would toss a 50-lb bag onto the sawtooth blade welded along the hopper cover screen. And the other loader would pull the bag along the blade, hemisecting the bag and emptying the contents into the hopper. Once the hopper was full, it was positioned over the plane and the contents were transferred into the plane's holding tank. Each hopper contained 1,600 lb of Ordarm® 10G (32 bags) and required ten minutes per loading cycle. The pilot was not involved in loading. Flaggers were sometimes subject to accidental application. Workers wore a long or short-sleeved shirt, long pants, shoes, and coveralls. The coveralls were provided by the investigators for monitoring purposes.

Knarr (1980) monitored the exposure of workers applying either Ordram® 10G at 3 to 5 lb a.i. per acre or Ordram® 8E at 1 lb a.i. per acre by air in Arkansas. The exposure monitoring techniques and the work tasks were similar to the study conducted by Maddy *et al*., 1982. Two additional pads were placed under the coveralls to establish a clothing penetration factor. Penetration factors of 53 and 30 percent were estimated for the 10G and 8E formulations, respectively. Analytical recoveries for laboratory and field spikes were 95 percent or more for the 10G formulation and 80 to 117 percent for the 8E formulation. Results were adjusted for laboratory recoveries.

The estimates of exposure for pilots, flaggers, and loaders handling the 10G formulation in Table 1 are based on both Maddy *et al*., 1982 and Knarr, 1980 studies. In Maddy *et al*. (1982), more than 85 percent of dermal dosimeter samples for pilots and flaggers contained no detectable residues. The minimum detection limit (MDL) was not reported and was assumed to be the lowest value reported (0.01 ug/cm$^2$). Samples with no detectable residues were assumed to contain residues one-half the MDL.

Table 1
The Estimate of Exposure of Pilots, Flaggers, and Loaders
Handling Ordram® 10G

| Work Task (n) | Head +Neck | Potential Body* | Hand | Inhalation | Absorbed Daily Dosage (ADD) |
|---|---|---|---|---|---|
| | ug/4-hours | ug/4-hours | ug/4-hours | ug/4-hours | ug/kg/4-hours |
| **10G:** | | | | | |
| Pilot** (5) | 37.3 | 629.6 | 15.8 | 121.4 | 3.5 |
| Flagger (8) | 66.8 | 1597.0 | 20.5 | 55.8 | 10.5 |
| Loader (12) | 1752.3 | 35154.9 | 662.2 | 3323.6 | 201.1 |
| **8E:** | | | | | |
| Pilot** (4) | 29.8 | 763.3 | 5.4 | 32.5 | 2.4 |
| Flagger (4) | 2627.5 | 56777.8 | 60.1 | 290.5 | 174.4 |
| Loader (8) | 249.5 | 25071.6 | 163.4 | 135.3 | 87.3 |

All data are geometric means (log-normally distributed).
n - Number of replicates.
* - Total exposure to the body.
** - The pilot who routinely assisted loaders filling the plane in Knarr, 1980 study was excluded.
Based on: Male pilots and loaders, female flaggers, clothing of long-sleeved shirt, long pants, and shoes. Body weight, body surface area, and inhalation rates from Thongsinthusak *et al*., 1993. Normal clothing penetration of 53 and 30 percent for 10G and 8E formulations, respectively (Knarr, 1980), dermal absorption of 53 percent (see Dermal Absorption section), and inhalation uptake of 50 percent (Raabe, 1987).

The estimates of exposure for pilots, flaggers, and loaders handling the 8E formulation in Table 1 are based on the Knarr, 1980 study. The workday in Maddy *et al*., 1982 study in California was two to four hours of Ordram application and the remainder of the day fertilizer and seed applications. This is a common practice for most rice herbicide applications in California. The workdays in Knarr, 1980 study in Arkansas were 1.5 to four hours during the 10G formulation and 1.5 to nine hours during the 8E formulation. The exposure estimates in Table 1 were normalized for a four-hour workday.

In the 1990 worker exposure study conducted by Chester (1991) in Arkansas, Ordram® 15G was applied by air. Both 50-lb and 1500-lb bulk bags were used. The exposure was monitored both by dosimetery and biomonitoring. In the biomonitoring portion, workers' absorbed dosages were estimated based on the amount of molinate mercapturate found in the urine samples. Later, further investigation of the pharmacokinetics of molinate in humans following a single oral dose determined that 4-hydroxy molinate was the major metabolite of molinate in urine. The urine samples of loaders were then analyzed for 4-hydroxy molinate and the results were reported (Chester *et al.*, 1992). The averages (arithmetic mean $\pm$ standard deviation) estimated ADD based on 4-hydroxy molinate for loaders of 50-lb bags and loaders of 1500-lb bags were 788 $\pm$ 428 (geometric mean = 711) and 491 $\pm$ 210 (geometric mean = 450) ug/kg/day, respectively. There were 12 replicates for 50-lb bags and nine replicates for 1500-lb bags. A t-test showed that there is a significant difference ($\alpha$=0.05) between the two means, demonstrating a modest advantage in using bulk bags in exposure reduction.

In 1991, a study was conducted in Arkansas in which the loading of Ordram® 10G into airplane hoppers was simulated, using two loading techniques (ICI, 1991). Either 1500-lb or 350-lb bags were loaded. The first technique (trans-loading) involved loading the bulk bags into a hopper truck and then into the airplane simulator (bin). A fan was positioned at the loading site to provide an airflow simulating an airplane. Bags were lifted using a forklift. One loader opened the bag and guided the material into the truck hopper and then emptied the truck hopper into the airplane simulator. The other loader rode with the hopper truck. The second technique (direct-loading) involved direct loading into the airplane simulator. One loader remained inside the cab of the truck which was positioned as the lifting device while the other one emptied the bag into the plane simulator. The two loaders traded places so that each drove and loaded the same number of bags.

The exposure of workers to molinate was estimated by analyzing workers' full 24-hour urine samples for 4-hydroxy molinate. Urine samples were collected for 24 hours prior to loading, for the day of loading, and for two days after loading. No 4-hydroxy molinate was detected in samples collected prior to the day of loading. Results were corrected for molecular weight difference, the metabolite (4-hydroxy molinate) representing 39 percent of the dose in urine (Batten *et al*., 1992 in the metabolism section), and actual body weight. Workers wore goggles, rubber gloves, hat, long-sleeved shirt, long pants, and shoes. The exposure for each worker was expressed as a three-day average. Estimates of ADD for loaders (n=10) of the trans-loading technique and loaders (n=10) of the direct-loading technique were 40.5 $\pm$ 28.9 (geometric mean = 30.9) and 15.1 $\pm$ 7.4 (geometric mean = 13.2) ug/kg/day, respectively.

Workers' exposure to molinate was monitored in Sacramento Valley during actual loading of Ordram® 10G for aerial application (ICI, 1992a). Two loading techniques as described in the previous the previous ICI (1991) study were used. The direct loading involved bulk containers (1500 lb bags) and the trans-loading involved both the bulk containers and 50-lb bags. A total of 20 workers were monitored. Each worker was monitored for three days of exposure and one day of pre-exposure as a baseline. Attempts were made to assure the workers were not exposed to molinate the day before the exposure day started (baseline day), but the study report indicates that workers did handle molinate on "baseline day". Workers were divided into four job classifications as follows: 1) Direct-loading drivers (n = 2) who remained in a closed cab during loading; 2) Trans-loading drivers (n =5), who would leave the closed cab to assist the loader, after hoisting the bag; 3) Direct-loading loaders (n = 3), who were involved only in emptying the bag into the airplane hopper; and 4) Trans-loading loaders (n = 10), who were involved in emptying the bags into a bucket and then into the airplane hopper.

Urine samples were taken starting on the morning of the pre-exposure day and finishing with the first void of the day after the last monitoring day. Personal air sampling pumps equipped with XAD-2 solid sorbent resin tubes were used to take air samples from workers' breathing zones. Urine samples were analyzed for 4-hydroxy molinate and the results were corrected for the metabolite representing 39 percent of the dose and molecular weight difference between the metabolite and molinate. The majority of workers did not wear clean chemical-resistant suits every day. Some workers wore the same suit during the entire monitoring period. Generally all workers wore the full face respirator during loading but there was a question of whether the filters were changed at all. Use permit deviations such as loading both bulk (1500 lb) and small (50 lb) bags, and not wearing respirator or gloves during loading were considered significant. Table 2 shows molinate ADDs and molinate residues in the breathing zones for different job classifications when work was done in compliance with permit conditions. Air monitoring data suggest that most of the exposure occurred via dermal route.

Table 2
Workers' Estimated ADDs of Molinate
During Actual Loading of Ordram® 10G from 1500-lb Bags

| Job Classification (n) | ADD* ug/kg/day | Air residues* ug/$m^3$ | Ordram Handled lb/person/day |
|---|---|---|---|
| Direct-loading: | | | |
| driver (6) | 1.26 ± 1.82 | 2.09 ± 1.83 | |
| loader (9) | 3.31 ± 2.61 | 6.78 ± 4.63 | 2500 |
| Trans-loading: | | | |
| driver (11) | 2.78 ± 1.60 | 7.19 ± 3.59 | |
| loader (23) | 10.31 ± 2.54 | 27.41 ± 4.41 | 6220 |

n - Number of replicates
* - Geometric mean ± geometric standard deviation (log-normally distributed).
Clothing consisting of work clothing, full-body chemical resistant suit, hat, chemical
resistant gloves, full-face respirator, and chemical resistant foot coverings.

In 1992, ICI Americas conducted a study to measure the exposure of workers to a new formulation (montmorillonite) of molinate during simulated direct-loading and trans-loading of 1250-lb bulk bags (ICI, 1992b). The trans-loading operation was monitored using a hopper truck equipped with and without

Table 3
Workers' ADDs of MolinateDuring Simulated
Loading of Ordram® 10G from 1250-lb Bags

| Loading Method (n) | ADD* (ug/kg/day) |
|---|---|
| Direct-loading (10) | 3.0 ± 2.25 |
| Trans-loading (shroud) (10) | 5.3 ± 2.91 |
| Trans-loading (no shroud) (10) | 6.6 ± 2.35 |

n- Number of replicates
* - Geometric mean ± geometric standard deviation (log-normally distributed).

following collection by the study personnel. Urine samples were analyzed for 4-hydroxy molinate. The results (Table 4) were corrected for 39 percent recovery of molinate in urine as 4-hydroxy molinate and the molecular weight difference. The exposure for each worker was expressed as a three-day average.

The estimate of exposures for loaders wearing Tyvek or carbon suits during direct loading or direct plus trans-loading are shown in Table 4. For two out of three work tasks monitored (direct loading and drivers), the estimated exposure values for workers wearing carbon suits are lower than those wearing Tyvek or no protective clothing. It is not clear why the work task that requires handling the Ordram® twice (trans-loading) resulted in higher exposure for persons wearing carbon suits than Tyvek.

The maximum number of 1,200-lb. bags which could be loaded directly by an individual in a given day would be 14 (16,800 lb. of the 10G formulation), based on physical limitations (Ross, 1994). An individual doing direct loading for the season would be limited to 226,800 lb. (189 bags) based on the seasonal margin of safety (Cochran, 1994). The weight limit for an individual doing a combination of direct and trans-loading for the season would be 135,000 lb., also based on the seasonal margin of safety (Cochran, 1994). The seasonal average daily dosages (SADDs) in Table 5 were calculated based on the estimated absorbed doses/1000 lb. in Table 4 and the above bag limits.

Table 5
Workers' Estimated ADD, SADD, and AADD During Loading
of a New Formulation (montmorillonite) of Ordram® 10G from Bulk Bags

| Work Task | Type of Suit Worn | Absorbed Dose (ug/kg/1000 lb.) | ADD* (ug/kg/day) | SADD** (ug/kg/day) | AADD*** (ug/kg/day) |
|---|---|---|---|---|---|
| direct loader | Tyvek | 0.63 | 10.58 | 4.10 | 0.39 |
| direct loader | Carbon | 0.41 | 6.89 | 2.66 | 0.25 |
| direct + trans | Tyvek | 0.74 | 3.7 | 2.85 | 0.27 |
| direct + trans | Carbon | 0.97 | 4.85 | 3.74 | 0.36 |
| driver | None | | 0.76 | | |
| driver | Carbon | | 0.56 | | |

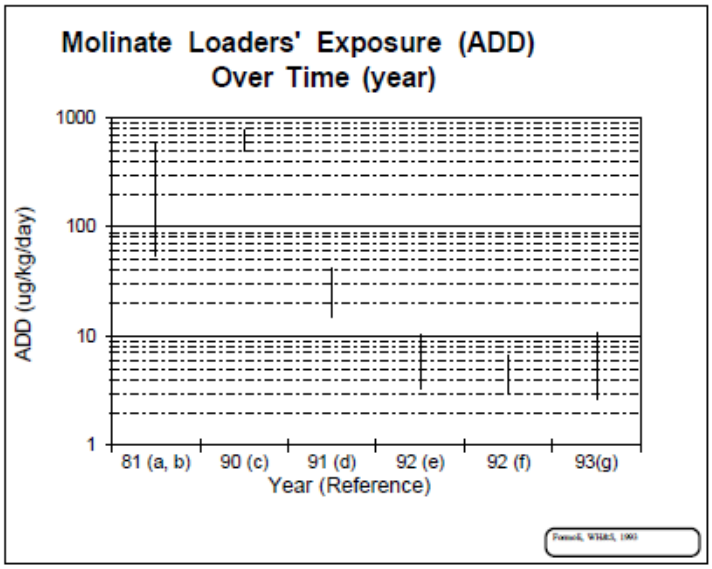
* - ADD based on daily direct loading of 16,800 lb. or direct plus trans-loading of 5,000 lb. of Ordram 10G.
** - SADD based on direct loading of 226,800 lb. or direct plus trans-loading of 135,000 lb. during approximately 27 days of handling period in a 35-day season (Zeneca, 1993b).
*** - Annual average daily dosage (AADD) based on 27 days of handling molinate in a 365-day year.

While inhalation exposure is significant, dermal exposure appears to be the major route of exposure for workers handling molinate 10G formulation (see Tables 1 & 2). The earlier worker exposure studies showed that loaders of Ordram® 10G have the greatest potential for exposure to molinate compared to the pilots and flaggers. Based on the earlier studies (Maddy *et al*., 1982; Knarr, 1980), the ADD for pilots was conservatively estimated at 3.5 ug/kg/workday (Table 1). In Maddy et al., 1982 study, most of the dermal exposure samples for pilots had no detectable residues and these samples were assumed to contain residues at half the MDL. In addition, the work practices, engineering controls, and personal PPE have improved dramatically since these studies were conducted. The use of bulk bags, direct loading, and improved formulation (montmorillonite) may have reduced the exposure to pilots from the levels estimated based on earlier studies. The ADD of 10.5 ug/kg/day for flaggers was also estimated based on Maddy *et al*., 1982 and Knarr, 1980 studies. The current (1994) molinate use permit requires flaggers to stay in an enclosed vehicle during application or wear coveralls (in addition to work clothing), goggles, head covering, chemical resistant gloves, and a half-face respirator. These exposure mitigation alternatives should provide 90 percent dermal and inhalation exposure protection

(Thongsinthusak *et al*., 1991) to flaggers and reduce the estimated exposure in Table 1 to 1.1 ug/kg/workday.

The more recent studies focused mainly on monitoring the exposure of workers involved in loading Ordram® 10G for aerial applications. These studies show a gradual decline in molinate exposure to loaders as more reliable monitoring techniques, improved formulation, and additional exposure mitigation measures were systematically implemented over the years. Figure 1 shows the estimated range of exposure to loaders.

Figure 1




a) Knarr, (1980)
b) Maddy, et al., 1982
c) Chester,et al., 1992.
d) ICI, 1991
e) ICI, 1992a
f) ICI, 1992b
g) Zeneca, 1993a

**Protective Clothing and Engineering Controls:**

a, b) Long- sleeved shirts, long pants, chemical resistant gloves, loading of 50-lb bags of Ordram 10G.
c) Long- sleeved shirts, long pants, chemical resistant gloves, goggles, loading 50-lb and 1500-lb bags of Ordram 15G.
d) Long- sleeved shirts, long pants, chemical resistant gloves, goggles, hat, leather shoes, direct loading and trans-loading of 350-lb and 1500-lb bags of Ordram 10G.
e) Work clothing, chemical resistant suit, chemical resistant gloves, full-face respirator, hat, shoe coverings, direct-loading and trans-loading of 1500-lb bags of Ordram 10G.
f) Work clothing, chemical resistant suit, chemical resistant gloves, full-face respirator, hat, shoe coverings, direct-loading and trans-loading (with and without shroud) of 1250-lb bags of a new formulation (montmorillonite-based) of Ordram 10G.
g) Work clothing, chemical resistant suit (Tyvek or carbon-impregnated), chemical resistant gloves, full-face respirator, hat, shoe coverings, direct-loading or direct plus trans-loading of 1280-lb bags of Ordram 10G (montmorillonite formulation).

The quantity of the emulsifiable concentrate formulation of molinate (Ordram® 8E) used in California is limited compared to that of the granular formulation. The use of Ordram 8E by air is prohibited in California. It can be used by ground equipment as a preplant soil incorporation. There are no studies that monitored molinate exposure to workers involved in ground application of Ordram 8E. The pesticide handlers exposure database (PHED), a software system that was developed by Versar Inc., was used as surrogate to obtain an estimate of exposure for mixer/loader/applicators (M/L/A) of Ordram 8E. There were 20 records for the M/L/A file with emulsifiable formulation and ground boom application subsets. There were no (zero) records for the soil incorporation method of application. The

estimate of dermal exposure for a M/L/A wearing long-sleeved shirt, long pants, and gloves was 27.1 ug/lb a.i. sprayed (see attachment II). Molinate use permit conditions require workers handling the liquid formulation of molinate to wear chemical resistant coveralls or chemical resistant full-body protective clothing, a full-face respirator, chemical resistant foot coverings, and a tightly woven head covering in addition to a long-sleeved shirt, long pants, and chemical resistant gloves. The additional PPE should provide 95 percent dermal protection to the covered areas (Thongsinthusak *et al*., 1991) and reduce the PHED estimated dermal exposure to 2.3 ug/lb a.i. sprayed. Based on an application rate of 3 lb a.i./acre, treatment of 40 acres in a workday, and dermal absorption of 53 percent, the ADD for a ground M/L/A weighing 75.9 kg will be 1.9 ug/kg/day. Because of the fairly high vapor pressure of molinate, it is not appropriate to use the estimate of inhalation exposure from the PHED. Air samples collected from the breathing zone of the ground crews during aerial applications of Ordram 8E showed an average molinate residues of 54 ± 46 ug/$m^3$ (Knarr, 1980). With a full-face respirator providing 98 percent protection for inhalation exposure (Thongsinthusak, *et al.*, 1991), respiratory exposure of a ground M/L/A of Ordram 8E to molinate will be insignificant, assuming that the air in their breathing zone is in the same range as observed in the Knarr, 1980 study.

Potential exposure to workers entering treated fields following molinate application is mainly respiratory since no dermal contact is expected. Air samples taken from four feet above the water surface of rice fields showed 48 ug/$m^3$ molinate immediately after application (Ross and Sava, 1986). Molinate concentration declined to 8.3 ug/$m^3$ three days after the application. The three-day average concentration was 21.9 ug/$m^3$. The estimate of ADD for a worker spending one hour a day in a treated rice field is 0.12 ug/kg/day, using the average molinate concentration, male inhalation rate of 0.84 $m^3$/hour, a body weight of 75.9 kg (Thongsinthusak, 1993), and inhalation uptake of 50 percent (Raabe, 1988).

In the 1992 worker exposure study (ICI, 1992b), the exposure to loaders using transloading equipment with and without a cover were monitored. The mean exposure to workers using a hopper truck equipped with a metal cover was lower than the mean exposure to those using a hopper truck without a cover (see Table 3). However, a t-test at $\alpha = 0.05$ indicated that the exposure difference between the two types of equipment was not significant. In addition, site air monitoring data were collected by the Department during transloading operation using hoppers equipped with and without a cover (Schneider, 1994). Air samples were taken from near the middle of the bucket and also from near the edge of the bucket. A sampling train consisting of a glass fiber filter cassette followed by a solid sorbent resin tube was used. Arthimetic mean molinate levels were 0.967 (n=4, SD=0.74), 0.439 (n=5, SD=0.663), and 0.316 (n=6, SD=0.232) ug/L for the hopper with pvc funnel and cover, the hopper with flat cover, and for the hopper without a cover, respectively. Results of ANOVA (p=0.21) indicated no significant difference between the three groups.

The worker safety permit conditions for 1994 (section II-A) require the use of a transloader cover when loading granular molinate to the transfer equipment. However, some aerial applicators of molinate were experiencing problems when loading two bags into a transloader equipped with a cover. The cover would impede the process and would create a barrier to the material, creating the potential for greater exposure. Therefore, the Department allowed the use of trans-loading equipment without a transloader cover (Andrews, 1994).

## Non-Occupational Exposure

Members of the general population with potential exposure to molinate include those who regularly eat rice and those served by the water utilities using the Sacramento River as the source of drinking water. The Sacramento River receives water drained from the Sacramento Valley rice fields treated with molinate. Potential routes of exposure to molinate in the drinking water supply include ingestion as well as inhalation and dermal contact from general household uses. Exposure to molinate in the

ambient air is also a concern for the general public residing in communities in the proximate vicinity where molinate is applied by aerial spraying.

**1. Water Supplies (Sacramento/West Sacramento)**

***a. Oral Route***

Table 6 shows the peak concentrations of molinate detected at various monitoring sites from 1986 to 1990 (O'Brian, 1989; CDFA, 1986, 1987, 1989, 1991b). The peak concentrations at all sites were found around mid-May through mid-June of the year. Concentrations detected at the Colusa Basin Drains ranged from 16 to 59 ppb during the peak period of 1990 with an average concentration of about 35 ppb. The Sacramento River water had concentrations ranging from 2 to 9 ppb and an average concentration of 4 ppb during the same period.

Table 6
Water Concentrations of Molinate Detected at Various Monitoring Locations in 1986 to 1990*

| Locations | Concentration (ppb) 1986 | 1987 | 1988 | 1989 | 1990 |
|---|---|---|---|---|---|
| CBD1 Maximum | 77 | 43 | 67 | 51 | 51 |
| Average** | 52 | 35 | 47 | 37 | 35 |
| | (7.4) | (2.8) | (5.1) | (3.1) | (5.0) |
| CDB5 Maximum | 88 | 53 | 89 | 60 | 59 |
| Average** | 67 | 37 | 55 | 40 | 34 |
| | (9.4) | (3.3) | (7.5) | (3.6) | (6.3) |
| SR1 Maximum | 11 | 8 | 8 | 6 | 9 |
| Average** | 6 | 5 | 6 | 4 | 4 |
| | (0.7) | (0.8) | (0.5) | (0.5) | (0.9) |
| SRR Maximum | 14 | 6 | 5 | 5 | 7 |
| Average** | 5 | 3 | 3 | 2 | 4 |
| | (0.6) | (0.4) | (0.2) | (0.3) | (0.3) |

* Samples taken by either the City of Sacramento or the California Department of Fish and Game.

** Data represent the arithmetic mean concentrations detected during the peak period from mid-May to mid-June. Numbers in the parenthesis are standard errors. Only concentrations $\geq$ 20 ppb for CBD1 and CBD5 and $\geq$ 2 ppb for SR1 and SRR are included in the calculations unless they are within the peak period.

CBD1: Colusa Basin Drain at Roads 109 and 99E near Knight's Landing in Yolo County.

CBD5: Colusa Basin Drain at Highway 20 in Colusa County.

SR1 : Sacramento River at Village Marina in Sacramento County considered to be representative of the intake for the West Sacramento.

SRR : Sacramento River at the intake to the City of Sacramento water treatment facility.

Molinate is oxidized to its sulfoxides by the chlorination process employed in the water treatment facilities (Ross, 1983). Analyses performed by the City of Sacramento from 1983 to 1986 indicated that molinate was not detected in the tap water (CDFA, 1984; Myers, 1983-89). On the other hand, molinate sulfoxide was found in the finished tap water at a level comparable to the level of the parent molinate observed in the pretreated Sacramento River raw water. DPR concurred with the opinion of the CDHS that measurements of the molinate concentration in Sacramento River water intake for the water treatment facilities could be used as a surrogate to evaluate potential human exposure to molinate and its by-products in drinking water supplies (Berteau, 1984).

California Department of Health Services (CDHS) set a maximum concentration level (MCL) of 20 *u*g/L for molinate in drinking water (CDHS, 1989). Based on the 1990 data, molinate concentrations of 2 to 9 ppb (*u*g/L) detected in the Sacramento River water were below the MCL of 20 *u*g/L. The potential daily exposure dosages of molinate and its by-products from ingesting water from Sacramento River are presented in Table 7. Young children residing in West Sacramento potentially ingested an average of 0.43 *u*g/kg/day to a maximum of 0.89 *u*g/kg/day during mid-May to mid-June. The potential average daily dosage and maximum daily dosage for adults was 0.12 *u*g/kg and 0.25 *u*g/kg, respectively. The potential daily dosage of molinate and its by-products from drinking water for residents in the City of Sacramento was about 80 percent of that observed for residents in West Sacramento.

Table 7
Potential daily exposure to molinate from drinking water*

| Sources | Exposure Dosage (*u*g/kg/day) | | | |
|---|---|---|---|---|
| | Adult | | Child | |
| | Average | Maximum | Average | Maximum |
| SR1 | 0.12 | 0.25 | 0.43 | 0.89 |
| SRR | 0.10 | 0.19 | 0.35 | 0.65 |

* Assumes 70 kg body weight anddaily water consumption of 2 liters for adults. Assumes 10 kg body weight and daily water consumption of 1 liter for the child.
SR1 Sacramento River at Village Marina in Sacramento County considered to be representative of the intake for West Sacramento.
SRR Sacramento River at the intake to the City of Sacramento water

Molinate concentrations in the Sacramento River peaked from mid-May to mid-June, coinciding with the seasonal application of the herbicide in rice fields and the subsequent drainage of the contaminated water into the Sacramento River after a holding period of up to 19 days. The duration of exposure to detectable concentrations of molinate in drinking water is approximately one month per year. The arithmetic average water concentration during the peak period is used to estimate the potential exposure level.

Even though the use-season for molinate is limited to a 6-week period, data suggest the neurotoxic effects of molinate may not be reversible (Cochran, 1994). Consequently, the potential annual exposure to molinate was also calculated. As detectable levels of molinate have been measured in the river water for approximately 30 days each year, the annual exposure would equal the seasonal exposure multiplied by 30 days/365 days per year. Thus, the annual exposure from drinking water would range from 0.01 *u*g/kg/day for adults to 0.04 *u*g/kg/day for children in West Sacramento.

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| SR1 | 0.12 | 0.25 | 0.43 | 0.89 |
| SRR | 0.10 | 0.19 | 0.35 | 0.65 |

* Assumes 70 kg body weight anddaily water consumption of 2 liters for adults. Assumes 10 kg body weight and daily water consumption of 1 liter for the child.
SR1 Sacramento River at Village Marina in Sacramento County considered to be representative of the intake for West Sacramento.
SRR Sacramento River at the intake to the City of Sacramento water

Molinate concentrations in the Sacramento River peaked from mid-May to mid-June, coinciding with the seasonal application of the herbicide in rice fields and the subsequent drainage of the contaminated water into the Sacramento River after a holding period of up to 19 days. The duration of exposure to detectable concentrations of molinate in drinking water is approximately one month per year. The arithmetic average water concentration during the peak period is used to estimate the potential exposure level.

Even though the use-season for molinate is limited to a 6-week period, data suggest the neurotoxic effects of molinate may not be reversible (Cochran, 1994). Consequently, the potential annual exposure to molinate was also calculated. As detectable levels of molinate have been measured in the river water for approximately 30 days each year, the annual exposure would equal the seasonal exposure multiplied by 30 days/365 days per year. Thus, the annual exposure from drinking water would range from 0.01 *u*g/kg/day for adults to 0.04 *u*g/kg/day for children in West Sacramento.

The combined potential exposure dosage from daily ingestion of rice and water from the Sacramento River ranges from 0.13 *u*g/kg/day for the adult population to 0.46 *u*g/kg/day for non-nursing infants less than one year old, with major contribution being from the water (Table 8). The combined potential exposure for ingestion of rice and water ranges from 0.04 *u*g/kg/day for adults to 0.07 *u*g/kg/day for children.

Table 8
Combined potential exposure of West Sacramento residents to molinate from drinking water and diet (rice).

| | Exposure Dosage (*u*g/kg/day) | | |
|---|---|---|---|
| Population | Rice | Water* | Combined |
| Adults | 0.005 | 0.12 | 0.13 |
| Non-Hispanics Other Than Black and White | 0.029 | 0.12 | 0.15 |
| Nursing Infants (<1 year old) | 0.007 | 0.43 | 0.44 |
| Non-Nursing Infants (<1 year old) | 0.029 | 0.43 | 0.46 |

* Data is taken from Table 7, the Average Daily Dosage from SR1, Sacramento River water intake for West Sacramento.

***b. Inhalation Route***

Molinate is very volatile, and evaporation is expected to be the major route of dissipation from water. In general, volatility also increases with the increase in temperature when water is used for showering, laundry, and washing purposes. It is suggested that the contribution from inhalation of volatile organic compounds, such as tetrachloroethene and trichloroethene, from domestic uses of the water supply could

Table 9
Comparison of Henry's Law Constant at 20°C

| Compounds | Henry's Law Constant |
|---|---|
| Molinate | 0.000065 |
| Tetrachloroethene | 0.847 |
| Trichloroethene | 0.393 |

$$\text{Henry's Law Constant } H = \frac{\text{Concentration in Air } (u\text{g/L})}{\text{Concentration in Water } (u\text{g/L})} = \frac{16.04\ P*M}{T*S}$$

Where: P is the equilibrium vapor pressure in torr.
M is the gram molecular weight per mole.
T is the temperature in °K. °K = 273 + °C.
S is the solubility in water in mg/L.

be as much as that from the ingestion of two liters of water (Andelman, 1985). A comparison of the calculated Henry's Law Constant (Table 9) shows that the volatility of molinate is about 0.007 to 0.015 percent of that for tetrachloroethene and trichloroethene. Therefore, the potential exposure to molinate via inhalation from household uses of the water supply is expected to be negligible.

***c. Dermal Route***

Dermal contact with contaminated water while swimming or bathing is a potential route of exposure to molinate. However, as environmental factors (water temperature and flow volume) limit swimming in the Sacramento River and adjacent waterways during May, and no molinate was measured in domestic waters supplies, it is believed that the dermal route is insignificant compared to the oral and inhalation routes.

**2. <u>Ambient Air</u> (Maxwell and Williams)**

***a. Inhalation***

In 1986, the ambient air concentrations of molinate were measured on the rooftops of the public buildings in four Sacramento Valley towns (Seiber *et al*., 1989). Sampling was carried out for four 24 hour intervals (Monday a.m. through Friday a.m.) for four weeks during the period selected to represent the highest uses of molinate. The measurement of ambient air concentration did not discriminate between fine particulate aerosol and the vapor phase of molinate (Mischke, 1989). The maximum concentration and the highest arithmetic average concentration were detected at Maxwell at 1.7 $u$g/m$^3$ and 0.65 $u$g/m$^3$, respectively. After adjusting for the collection efficiency of 67 percent, the arithmetic average ambient air concentration of molinate encountered by residents of Maxwell was 0.28 $u$g/m$^3$.

The arithmetic average ambient air concentration of molinate in 1992 at Maxwell according to measurements taken by the California Air Resources Board was 0.72 $u$g/m$^3$ (range = 0.4 to 1.17 $u$g/m$^3$) and at Williams it was 0.39 $u$g/m$^3$ (range = 0.16 to 0.50 $u$g/m$^3$) (EMPM, 1992).

Using the standard default value for human inhalation of 0.29 m$^3$/kg/day for adults and 0.6 m$^3$/kg/day for infants (Anderson *et al*., 1983), and assuming a 50 percent retention and 100 percent absorption (Raabe, 1986, 1988), the estimated seasonal dosage for an individual in Maxwell in 1986 was:

Adults- (0.28 $u$g/m$^3$)x(0.29 m$^3$/kg/day)/2 = 0.04 $u$g/kg/day

Infants- (0.28 $u$g/m$^3$)x(0.6 m$^3$/kg/day)/2 = 0.09 $u$g/kg/day

The estimated seasonal dosage for an individual in Maxwell in 1992 was:

Adults- (0.72 $u$g/m$^3$)x(0.29 m$^3$/kg/day)/2 = 0.10 $u$g/kg/day

Infants- (0.72 $u$g/m$^3$)x(0.6 m$^3$/kg/day)/2 = 0.22 $u$g/kg/day

The estimated seasonal dosage for an individual in Williams in 1992 was:

Adults- (0.39 $u$g/m$^3$)x(0.29 m$^3$/kg/day)/2 = 0.06 $u$g/kg/day

Infants- (0.39 $u$g/m$^3$)x(0.6 m$^3$/kg/day)/2 = 0.12 $u$g/kg/day

Potential annual exposures may be estimated by amortizing the seasonal dosage (which occurs during a 35 day period) over the entire year (365 days). Thus, the arithmetic average annual daily dosage would be equivalent to the seasonal dosage multiplied by 35/365:

| Maxwell | 1986 | 1992 |
|---|---|---|
| Adults | 0.004 *u*g/kg/day | 0.01 *u*g/kg/day |
| Infants | 0.009 *u*g/kg/day | 0.021 *u*g/kg/day |
| | | |
| Williams | | |
| Adults | not measured | 0.006 *u*g/kg/day |
| Infants | not measured | 0.012 *u*g/kg/day |

A theoretical worst case scenario can be derived from the supposition that a family could live in a home adjacent to a treated rice field. Rice fields are only treated once during the rice growing season, so the exposure would follow the pattern described by Ross and Sava (1986). They reported that the highest measured air concentration of molinate, 48 cm above the surface of a treated rice paddy, was 48 *u*g/$m^3$ on the day of application. However, the air concentrations dropped precipitously with time, theoretically reaching non-detectable levels in a week. The 5 day arithmetic average was estimated to be 18.7 *u*g/$m^3$. Theoretically, individuals living in a home next to the rice paddy would receive the following short-term daily dosages through the inhalation route:

Adults- (18.7 *u*g/$m^3$)x(0.29 $m^3$/kg/day)/2 = 2.71 *u*g/kg/day
Infants- (18.7 *u*g/$m^3$)x(0.6 $m^3$/kg/day)/2 = 5.61 *u*g/kg/day

As a rice paddy is treated only once during the year with molinate, there would not be a seasonal, or annual exposure to consider.

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# Attachment I

MOLINATE (ORDRAM) WORKER SAFETY PERMIT CONDITIONS
(1994)

The Worker Safety Permit conditions for Molinate (Ordram 8E and 10G) have changed. Please read these conditions carefully for changes which will affect application of this material.

WORKER PROTECTION

I. Training

A. All persons, prior to handling molinate, must be trained and maintain proof that they attended or were trained by an attendee of a 1994 worker protection training program approved by the Department of Pesticide Regulation (DPR).

B. Employers shall maintain records of the date and name of each person who received training. Records shall be made available for inspection upon request by the county agricultural commissioner or the Director.*

II. Engineering Controls

A. A transloader cover must be used when transferring granular molinate from the bag to transfer equipment. This cover must fit the opening and reduce escaping dust.

B. Liquid molinate (Ordram 8E) shall not be applied by air.

C. If an enclosed cab is used which meets criteria for Type 2 enclosed cabs, ground applicators shall wear cloth coveralls, or long-sleeved shirt and long-legged trousers. However, the applicator shall have available a full-face respirator, gloves, and foot coverings in the cab. This equipment shall be worn when exiting the cab and coming in contact with contaminated soil or equipment.

III.General Requirements

**The employer shall provide and require employees to wear all necessary protective clothing as specified, and provide for cleaning, repair, and replacement when necessary. All protective clothing and equipment are the property of the employer.**

A. Aerial Application Handling Requirements (Granular Formulation)

1. Bag Handling Requirements

a. No person shall load more than the equivalent of 190 bags of Ordram 10G per season (228,000 pounds total).

b. Ordram 10G shall be loaded only in the following manner:

(1) Directly from the bulk bag into the application vehicle hopper (direct loading); or

(2) Directly from the bulk bag into a loading cone and then to the application vehicle hopper (transloading).

c. The employer shall maintain a record of persons loading Ordram 10G and make these records available for inspection by the county agricultural commissioner or the Director upon request. Records shall be kept as follows:

(1) Name of person.

(2) The date and total pounds of Ordram 10G loaded per day.

2. Loaders or any persons having contact with full, or handling partial, or empty Ordram 10G bags shall wear the following personal protective clothing and equipment:

a. A charcoal cloth suit (long-sleeved and long-legged) as the underlayer.

b. Either a cloth coverall or long-sleeved shirt and long-legged trousers as the outer layer [equivalent to work clothing as defined in California Code of Regulations (CCR) Section 6000.4(s)].

c. A tightly woven head covering.

d. Chemical resistant foot coverings.

e. Chemical resistant gloves.

f. NIOSH or MSHA approved full-faced respirators having both dust and organic vapor pesticide cartridges.

3. Pilots shall wear the following personal protective clothing and equipment.

a. Cloth coveralls or long-sleeved shirt and longlegged trousers [equivalent to work clothing as described in CCR Section 6000.4(s)].

b. Pilots involved in loading or equivalent activities (load leveling, handling the bucket sock) where they may come in contact with Ordram 10G shall wear the same protective clothing and equipment as required for loaders in III, A, 2.

4. Flaggers shall wear the following personal protective clothing and equipment:

a. Two layers of any of the following:

(1) Cloth coveralls or long-sleeved shirt and long-legged trousers [equivalent to work clothing described in CCR Section 6000.4(s)].

(2) Disposable coveralls (long-sleeved and long-legged) made of synthetic materials capable of excluding particles 45 microns or larger in diameter. Examples of these are Tyvek Q, Kleen

Guard, Polypropylene, or other brands of coveralls approved by the Worker Health and Safety Branch, DPR.

b. Protective eyewear (safety glasses)

c. NIOSH or MSHA approved half-face respirator having organic vapor pesticide cartridges.

d. Tightly woven head covering.

e. Chemical resistant gloves.

f. If an enclosed vehicle is used while flagging, flaggers shall wear cloth coveralls or long-sleeved shirt and long-legged trousers. However, the flagger shall have available a respirator, protective eye wear, gloves, and head covering in the vehicle. This equipment shall be worn when exiting the vehicle during the application to perform flagging activities.

B. Ground Application Requirements

1. Granular Formulation Requirements

a. Ground applicators shall wear the following personal protective clothing and equipment:

(1) Two layers of any of the following:

(a) Cloth coveralls or long-sleeved shirt and long-legged trousers [equivalent to work clothing defined in CCR Section 6000.4(s)].

(b) Disposable coveralls (long-sleeved and long-legged) made of synthetic materials capable of excluding particles 45 microns or larger in diameter. Examples of these are Tyvek Q**, Kleen Guard**, Polypropylene**, or other brands of coveralls approved by the Worker Health and Safety Branch, DPR.

(2) NIOSH or MSHA approved full-faced respirator having both dust and organic vapor pesticide cartridges. A respirator is not required for applicators using ground equipment that injects or incorporates molinate into soil.

(3) Chemical resistant gloves.

(4) Chemical resistant foot coverings.

(5) If an enclosed cab is used which meets criteria for Type 2 enclosed cabs, ground applicators shall wear cloth coveralls or long-sleeved shirt and long-legged trousers. However, the applicator shall have available a full-face respirator, gloves, and foot coverings in the cab. This equipment shall be worn when existing the cab and coming in contact with contaminated soil or equipment.

2. Liquid Formulation Requirements

a. All handlers (mixers, loaders, and applicators) shall wear the following protective clothing and equipment:

(1) Two layers of the following:

(a) Cloth coveralls or long-sleeved shirt and long-legged trousers [equivalent to work clothing described in CCR Section 6000.4(s)], and

(b) Chemical resistant coveralls (long-sleeved and long-legged) or equivalent to 6738(d)(1): ...chemical resistant full body protective clothing that covers the torso, head, arms, hands, legs, feet. Examples of these are Tyvek QC**, Tyvek laminated with Saranex**, Polypropylene laminated with polyethylene**, Encase II** or other brands of coveralls approved by the Worker Health and Safety Branch, DPR.

(2) NIOSH or MSHA approved full-faced respirator having organic vapor pesticide cartridges. Respirators are not required for applicators using ground equipment that injects or incorporates molinate into the soil or when using vehicle-mounted spray nozzles which are both located below the applicator and are directed downward.

(3) Chemical resistant gloves.

(4) Chemical resistant foot coverings.

(5) A tightly woven head covering.

b. Persons using a closed system that meets the Director's criteria are not required to wear chemical resistant coveralls and full-faced respirator. However, a chemical resistant apron shall be worn.

c. If an enclosed cab is used which meets criteria for Type 2 enclosed cabs, ground applicators shall wear cloth coveralls or long-sleeved shirt and long-legged trousers. However, the applicator shall have available a full-face respirator, gloves, and foot coverings in the cab. This equipment shall be worn when exiting the cab and coming in contact with contaminated soil or equipment.

*CCR Section 6000.4(s)*

*"Work Clothing" means a long-sleeved shirt and long-legged trousers or a coverall-type garment, all of closely woven fabric or equivalent covering the body, including arms and legs. The clothing need not cover the head, hands or feet.*

*Type 2 Enclosed Cab Criteria*

*These cabs must provide a nonporous physical barrier totally surrounding the worker in the cab that prevents contact with dust or spray mist. A type 2 enclosed cab may have air intakes, heating and air conditioning.*

* The term Director means employees of DPR or agents of the Director.
** Use of brand names does not imply endorsement by DPR.

## Attachment II

Summary Statistics Developed in PEHD
for a Ground Mixer/loader/applicator of Ordram 8E

SUMMARY STATISTICS FOR CALCULATED DERMAL EXPOSURES

SCENARIO: Long pants, long sleeves, gloves

| PATCH LOCATION | DISTRIB. TYPE | MICROGRAMS PER LB AI SPRAYED Median | Mean | Coef of Var | Geo. Mean | Obs. |
|---|---|---|---|---|---|---|
| HEAD (ALL) | Lognormal | 24.96 | 43.394 | 124.5027 | 19.2875 | 20 |
| NECK.FRONT | Lognormal | 1.155 | 8.4143 | 270.133 | 1.4842 | 20 |
| NECK.BACK | Lognormal | 3.344 | 2.7456 | 102.7462 | .8417 | 15 |
| UPPER ARMS | Lognormal | .582 | 1.552 | 108.2539 | 1.0576 | 3 |
| CHEST | Lognormal | .71 | 1.42 | 86.6056 | 1.127 | 3 |
| BACK | Lognormal | .71 | 1.42 | 86.6056 | 1.127 | 3 |
| FOREARMS | | | | | | 0 |
| THIGHS | Other | .764 | .764 | 0 | .764 | 3 |
| LOWER LEGS | Other | .476 | .476 | 0 | .476 | 3 |
| FEET | | | | | | 0 |
| HANDS | Lognormal | .276 | 16.9617 | 190.2144 | .9801 | 12 |
| TOTAL DERM: | | 27.1451 | 32.977 | 77.1476 | 27.1451 | |
| INHALATION: | Lognormal | 5.0678 | 5.1332 | 87.649 | 1.8802 | 20 |
| COMBINED: | | 29.0253 | 38.0448 | 82.2808 | 29.0253 | |

95% C.I. on Mean: Dermal: (-923.6871, 1077.9823)

95% C.I. on Geo. Mean: Inhalation: (.033, 106.9864)

Inhalation Rate : 14 Liters/Minute
Number of Records: 20
Data File: MIXER/LOADER/APPLICATOR Subset Name: MOLINATE.MLAP

# APPENDIX C

## Calculation of Oncogenic Potency

DATE: 08/10/1994 TIME: 09:59:06

# GLOBAL 86 (MAY 1996)

BY RICHARD B. HOWE AND CYNTHIA VAN LANDINGHAM

CLEMENT ASSOCIATES
1201 GAINES STREET
RUSTON, LA 71270
(318) 255-4800

molinate, SD rats diet, kidney/cort adenoma-carcinoma

POLYNOMIAL DEGREE SELECTED BY PROGRAM, (POLY-DEGREE=0)
MONTE CARLO TEST USED IN SELECTION
THE BACKGROUND Q(0) HAS BEEN SET TO ZERO

| GROUP | DOSE | #RESPONSES OBSERVED/#ANIMALS | #RESPONSES PREDICTED |
|---|---|---|---|
| 1 | .000000 | 0/ 47 | .00 |
| 2 | .300000 | 0/ 46 | .00 |
| 3 | 1.80000 | 0/ 49 | .00 |
| 4 | 13.0000 | 5/ 48 | 5.00 |

CHI-SQUARE GOODNESS OF FIT STATISTIC IS 3.79819E-05

P-VALUE FOR THE MONTE CARLO TEST IS .5800000000

FORM OF PROBABILITY FUNCTION:
P(DOSE) = 1 - exp( -Q0 - Q1 * D - Q2 * D^2 - . . . - Q 6 * D^ 6 )

MAXIMUM LIKELIHOOD ESTI .ATES OF DOSE COEFFICIENTS

---

Q( 0) = .000000000000
Q( 1) = .000000000000
Q( 2) = .000000000000
Q( 3) = .000000000000
Q( 4) = .000000000000
Q( 5) = .000000000000
Q( 6) = 2.278939538950E-08

MAXIMUM VALUE OF THE LOG-LIKELIHOOD IS -16.0388919683

CALCULATIONS ARE BASED UPON EXTRA RISK

LINEARIZED MULTISTAGE CONFIDENCE LIMITS

**********

| RISK | MLE DOSE | LOWER BOUND ON DOSE | UPPER BOUND ON RISK | CONFIDENCE LIMIT SIZE |
|---|---|---|---|---|
| .10000 | 12.907 | 13.086 | 9.86994E-02 | 90.0 |
| | | 9.4263 | .13434 | 95.0 |
| | | 7.9845 | .15660 | 97.5 |
| | | 6.8043 | .18115 | 99.0 |
| l.OOOOOE-02 | 8.7245 | 1.2483 | 6.78325E-02 | 90.0 |
| | | .89917 | 9.29126E-02 | 95.0 |
| | | .76164 | .10875 | 97.5 |
| | | .64906 | .12637 | 99.0 |
| l.OOOOOE-03 | 5.9395 | .12427 | 4.66944E-02 | 90.0 |
| | | 8.95119E-02 | 6.42315E-02 | 95.0 |
| | | 7.58209E-02 | 7.53820E-02 | 97.5 |
| | | 6.46136E-02 | 8.78664E-02 | 99.0 |
| l.OOOOOE-04 | 4.0462 | 1.24211E-02 | 3.20519E-02 | 90.0 |
| | | 8.94716E-03 | 4.42182E-02 | 95.0 |
| | | 7.57868E-03 | 5.19917E-02 | 97.5 |
| | | 6.45845E-03 | 6.07306E-02 | 99.0 |
| l.OOOOOE-05 | 2.7566 | l.24206E-03 | 2.19497E-02 | 90.0 |
| | | 8.94676E-04 | 3.03418E-02 | 95.0 |
| | | 7.57834E-04 | 3.57216E-02 | 97.S |
| | | 6.45816E-04 | 4.17865E-02 | 99.0 |
| l.OOOOOE-06 | 1.8781 | 1.24205E-04 | l.50069E-02 | 90.0 |
| | | 8.94672E-05 | 2.07729E-02 | 95.0 |
| | | 7.57831E-05 | 2.44776E-02 | 97.5 |
| | | 6.45813E-05 | 2.86619E-02 | 99.0 |
| l.OOOOOE-07 | 1.2795 | l.24205E-05 | l.02487E-02 | 90.0 |
| | | 8.94671E-06 | l.41997E-02 | 95.0 |
| | | 7.57830E-06 | l.67422E-02 | 97.5 |
| | | 6.45813E-06 | 1.96175E-02 | 99.0 |
| l.OOOOOE-08 | .87172 | l.24205E-06 | 6.99382E-03 | 90.0 |
| | | 8.94671E-07 | 9.69617E-03 | 95.0 |
| | | 7.57831E-07 | l.14370E-02 | 97.5 |
| | | 6.45812E-07 | l.34074E 02 | 99.0 |

END OF LINEARIZED MULTISTAGE CONFIDENCE LIMITS

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GLOBAL 86 LOWER CONFIDENCE IMITS ON DOSE FOR FIXED RISK

**********

| RISK | MLE DOSE | LOWER BOUND ON DOSE | CONFIDENCE LIMIT SIZE | COEFFICIENTS FOR CONFIDENCE LIMIT |
|---|---|---|---|---|
| 1.00000E 05 | 2.7566 | 8.94676E 04 | 95.0% | Q( 0) = .00000<br>Q( 1) = 1.11773E-02<br>Q( 2) = .00000<br>Q( 3) = .00000<br>Q( 4) = .00000<br>Q( 5) = .00000<br>Q( 6) = .00000 |
| 1.00000E-06 | 1.8781 | 8.94672E-05 | 95.0% | Q( 0)= .00000<br>Q( 1) = 1.11773E-02<br>Q( 2) = .00000<br>Q( 3) = .00000<br>Q( 4) = .00000<br>Q( 5) = .00000<br>Q( 6) = .00000 |

GLOBAL 86 UPPER CONFIDENCE IMITS ON RISK FOR FIXED DOSE

**********

| DOSE | MLE RISK | UPPER BOUND ON RISK | CONFIDENCE LIMIT SIZE | COEFFICIENTS FOR CONFIDENCE LIMIT |
|---|---|---|---|---|
| 3.0000 | 1.66133E 05 | 3.29759E-02 | 95.0% | Q( 0) = .00000<br>Q( 1) = 1.11773E-02<br>Q( 2) = .00000<br>Q ( 3) = .00000<br>Q( 4) = .00000<br>Q( 5) = .00000<br>Q( 6 ) = .00000 |

Thus, the rat $Q_1^*$ = rat $Q_1^*$ = $1.1 \times 10^{-2}$

Human $Q_1^*$ = rat $Q_1^* \times (BW_h/BW_r)^{1/4}$

= $1.1 \times 10^{-2} \times (70/0.35)^{1/4}$

= $4.1 \times 10^{-2}$