CYCLOATE (RO-NEET)

RISK CHARACTERIZATION DOCUMENT

Medical Toxicology and Worker Health and Safety Branches

Department of Pesticide Regulation

California Environmental Protection Agency

November 28, 1995

EXECUTIVE SUMMARY

Introduction

Cycloate, a thiocarbamate, is the active ingredient in Ro-Neet. It is a pre-plant use only, herbicide, used to control weeds in fields for sugar beets, table beets, and spinach. Loss of cycloate from the soil is primarily via evaporation and bacterial breakdown. Results from field studies showed a half-life of 10-11 days for cycloate applied to sandy loam soil. Leaching potential to the groundwater is considered low. Cycloate is available as a liquid concentrate and in granular form. However, only the liquid is registered in California.

The current Risk Characterization Document addresses potential human exposures from the California use of cycloate as an active ingredient in herbicides. The potential dietary risk to the general public from the possible consumption of foods containing the highest legal residues of cycloate is also assessed.

The Risk Assessment Process

The risk assessment process consists of four aspects: hazard identification, dose response assessment, exposure evaluation, and risk characterization.

Hazard identification entails an evaluation of the toxicological properties of each pesticide. The dose response assessment then considers the chemicals toxicological properties and estimates the amount which could potentially cause an adverse effect. The basic principle of toxicology is that at a high enough dose, virtually all substances will cause some type of toxic manifestation. Although chemicals are often referred to as "dangerous" or "safe", as though these concepts were absolutes, in reality, these terms describe chemicals that require low or high doses, respectively, to cause toxic effects. Toxicological activity is determined in a battery of experimental studies which define the kinds of toxic effects that can be caused, and the exposure levels (doses) at which the toxic effect is first seen. State and federal testing requirements, including California's Birth Defect Prevention Act of 1984 (SB 950, Petris), mandate that chemicals be tested at doses high enough to produce toxic effects, even if that testing requires dose 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 amount of the pesticide likely to be delivered though occupational, or dietary routes on an acute or chronic basis. The risk characterization then relates the toxic effects observed in the laboratory studies, conducted with the high doses of pesticides, to potential human exposures to low doses of pesticides in the diet, or work place. The potential for possible adverse health effects in human populations is expressed as the margin of safety (MOS), which is the ratio of the dose which produces no effect in laboratory studies to the theoretical human dose.

Toxic Effects in Animal Studies

Damage to the nervous system is the major concern in the risk assessment of cycloate. Toxic effects were present in both rats and dogs, after even a single treatment, and when cycloate was swallowed, inhaled, or came in contact with the skin.

Animals given a single dose by mouth had brain damage (nerve cell death). Other toxic effects identified in animals exposed to cycloate included decreased brain weight, an

undesirable increase in the number or size of cells of the lining of the nose, and irritation of the skin. Also detected was damage to nerves, muscle, liver, kidney, and heart. The doses at which no toxic effects were observed were determined from the animal studies, or were estimated when animal tests did not use doses low enough to determine no-effect levels.

Potential Human Exposure

A margin of safety of 100 is generally considered sufficient to be protective of human health when the no-effect level is based on results from animal studies. The potential exposure for consumers via dietary sources was ten thousand times lower than the dose which did not cause toxic effects in animals. Therefore, toxic effects are not expected in people from any cycloate in food. For farm workers, there was not always a margin of safety of 100 for potential single or repeated-daily exposures during pre-plant use of the liquid form of cycloate. As continuous "chronic" worker exposure to cycloate is not expected to occur, margins of safety for potential chronic occupational exposure were not determined.

A proposed exposure mitigation involving the use of an effective closed system for mixing and loading emulsible cycloate concentrate, label-required Personal Protective Equipment, and an enclosed cab would lower potential exposures considerably, producing MOSs of greater than 100. In lieu of using an enclosed cab, applicators could wear coveralls and a half-face respirator.

An earlier review of the toxicity of cycloate was conducted for the United States Environmental Protection Agency by 2 experts in nervous system toxicity, Drs. Zoltan Annau of Johns Hopkins University and Mohamed Abou-Donia of Duke University. Drs. Annau and Abou-Donia agreed that the nervous system toxicity of cycloate in rat studies indicated that possibly serious effects could happen in people exposed to the chemical.

Animal studies have also shown that cycloate causes brain damage at dose levels below that of its other effects. The brain areas affected have been reported to be involved with learning and memory formation in both animals and humans. Thus, cycloate, unlike some other nervous system toxins (e.g organophosphate insecticides) does not produce clinical signs of exposure, such as tremors or diarrhea, that would give warning of contamination and subsequent brain damage.

The severity of the toxicity (i.e. brain cell damage) noted in the animal studies suggests that a higher MOS could be considered. However, the conservative estimation factors used to determine potential no-effect levels from animal studies have already established an additional safety margin.

Tolerance Assessment

Based on the 95th percentile of the theoretical consumption of foods at the highest legal residues (tolerances), the acute margins of safety were all greater than 22,000. Based upon pesticide residue monitoring programs, the long-term consumption of foods containing residues at tolerance levels was considered highly improbable. Therefore, an assessment of the margins of safety from potential chronic exposure to foods with tolerance levels of cycloate was not undertaken.

Conclusions

The risk from potential exposure to liquid cycloate, as present in herbicides, was evaluated for farm workers, and for potential exposure in food to farm workers and the general public. The potential risks to the general public from foods with the highest legal levels of cycloate was also assessed.

Normally, a margin of safety of 100 is considered sufficient for protection of human health. All margins of safety for potential food exposures to the general population were above 100. For farm workers, some margins of safety to protect against toxic effects from potential repeated-day work exposures were less than 100. A proposed exposure mitigation would lower potential exposures considerably, producing MOSs of greater than 100.

The margins of safety from the possible consumption of foods with the highest legal levels (tolerances) of cycloate were greater than 100.

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DPR acknowledges the review of this document by the Office of Environmental Health Hazard Assessment.

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I. SUMMARY

Cycloate, a thiocarbamate, is the active ingredient in Ro-Neet. It is a selective herbicide (pre-plant use only) used to control weeds in fields for sugar beets, table beets, and spinach. Dissipation of cycloate from the soil is primarily via volatilization and microbial degradations. Results from field dissipation studies showed a half-life of 10-11 days for cycloate applied to sandy loam soil. Leaching potential to the groundwater is considered low.

Cycloate is absorbed via oral, dermal, and inhalation routes. The absorption rate via the dermal route for rats is approximately 19.3%, and that via the inhalation route is unknown. N-ethyl-cyclohexylamine is the major urinary metabolite in the rat, mouse, and monkey given cycloate. It is also one of the major degradation products found in the soil treated with cycloate or in plants grown in cycloate treated fields.

Neurotoxicity is the major concern in the risk assessment of cycloate. Toxic effects have been detected in both rats and dogs, after exposure periods as short as one dose, and by all exposure routes tested.

Brain damage (neuronal necrosis) was present in rats receiving a single oral dose of cycloate. An estimated No-Observed-Effect-Level (NOEL) of 20 mg/kg for neuronal necrosis was calculated based on the default assumption that a NOEL is 1/10th of the Lowest-Observed-Effect-Level (LOEL). This estimated NOEL was selected for evaluating potential acute single-day human exposures. Other effects identified in animals exposed to cycloate included decreased brain weight, increases in the incidences of epithelial hyperplasia/ hypertrophy of the nasal cavity, irritation of the skin, degeneration of nerve, muscle, testicular, heart, and kidney tissues; and adrenal hyperplasia/hypertrophy. For evaluation of the potential repeated-daily exposures during pre-plant application of cycloate, potential brain damage following dermal and inhalation exposure was examined using a Combined MOS (Hazard Index) approach. A NOEL of 0.02 mg/kg/day was estimated for subchronic inhalation exposure based on the default assumption that a NOEL is 1/10th of the Lowest-Observed-Effect-Level (LOEL) for neurotoxicity (decreased brain weight) in a 15 exposure-day rat nose-only inhalation toxicity study. Potential subchronic dermal neurotoxicity (decreased brain weight) was assessed based on an estimated NOEL of 0.193 mg/kg/day in a 21 exposure-day rat dermal toxicity study. For the effects on the nasal epithelium, a NOEL of 0.01 mg/kg/day was calculated for the subchronic exposure also based on the default assumption that a NOEL is 1/10th of the Lowest-Observed-Effect-Level (LOEL). For potential chronic dietary risks to humans, a NOEL of 0.5 mg/kg/day was selected, and was based on nerve, liver, kidney, adrenal, and heart effects present in chronic dog and rat toxicity studies.

Potential Human Exposure

An earlier review of the toxicity of cycloate was conducted for the US EPA by 2 recognized experts in neurotoxicity, Drs. Zoltan Annau of Johns Hopkins University, and Mohamed Abou-Donia of Duke University. Drs. Annau and Abou-Donia agreed that the neurotoxicity of cycloate observed in rat studies was indicative of possibly serious effects on humans exposed to the compound.

Animal studies have also shown that cycloate causes brain damage at dose levels below that of its other effects. The brain areas affected have been reported to be involved with learning and memory formation in both animals and humans. Thus, cycloate, unlike

some other nervous system toxins (e.g organophosphate insecticides) does not produce clinical signs of exposure, such as tremors or diarrhea, that would give warning of contamination and subsequent neural effects.

A margin of safety (MOS) was calculated to assess the potential exposure to cycloate for consumers via dietary sources or for workers via occupational exposure. A MOS is the ratio of a NOEL identified in an appropriate study to the estimated potential exposure dosage for humans. An MOS of at least 100 is generally considered sufficient to be protective of human health when the NOEL is based on results from animal studies with a comparable duration of exposure for humans. The severity of the toxicity (i.e. brain cell damage) noted in the animal studies suggests that a higher MOS could be considered. However, the conservative estimation factors used to determine potential no-effect levels from animal studies have already established an additional safety margin.

The potential exposure for consumers via dietary sources all had MOSs of greater than 49,000. For agricultural workers, the calculated single-day acute occupational brain damage MOSs were all greater than 230. The margins of safety for brain damage calculated for potential repeated-daily exposure during the planting season ranged from 1-275. The arithmetic mean of the MOSs was 8, while the geometric mean was 21. The margins of safety for damage to the nasal epithelium ranged from 1-234. The arithmetic mean of the MOSs was 8, while the geometric mean was 25. (geometric means presented for comparison purposes only). As continuous "chronic" worker exposure to cycloate is not expected to occur, margins of safety for potential chronic occupational exposure were not determined.

Additional dietary exposure for workers was insignificant compared to the potential occupational exposure, and would not significantly affect the MOS estimates.

Proposed Mitigation

A proposed mitigation involving an effective closed system for mixing and loading cycloate emulsifiable concentrate, label-required Personal Protective Equipment (PPE), and use of an enclosed cab would lower exposures considerably producing MOSs of greater than 100. In lieu of using an enclosed cab, applicators could wear coveralls and a half-face respirator

Tolerance Assessment

Based on the 95th percentile of the potential consumption of foods with an established legal residues for cycloate, the acute margins of safety at the highest legal residue levels (tolerances) were all greater than 22,000. Based upon pesticide residue monitoring programs, the long-term consumption of foods containing residues at tolerance levels was considered highly improbable. Therefore, an assessment of the margins of safety from theoretical chronic exposure to foods with tolerance levels of cycloate was not undertaken.

Conclusions

The toxicological risk from potential exposure to technical cycloate, as present in liquid herbicide formulations, was evaluated for job-related and dietary exposure for agricultural workers, and for the general population from anticipated dietary exposure. The potential risks from consumption of foods containing the highest legal residues (tolerance) of cycloate were also assessed.

The margins of safety for potential dietary exposures to the general population were all greater than 100. For potential occupational exposures, the neurotoxicity margins of safety varied from 1-275, with arithmetic and geometric means of 8 and 21 respectively. The margins of safety for damage to the nasal epithelium ranged from 1-234, with arithmetic and geometric means of 8 and 25 respectively. A proposed exposure mitigation would lower potential exposure considerably, producing MOSs of greater than 100.

The additional dietary exposure for workers was insignificant compared to the occupational exposure, and would not affect the MOS estimates. A risk assessment of the cycloate granular formulation was not conducted, as it is not registered for use in California.

The margins of safety from the potential consumption of foods with the highest legal residues (tolerance) of cycloate were greater than 100.

II. INTRODUCTION

A. CHEMICAL IDENTIFICATION

Cycloate (S-ethyl cyclohexylethylthiocarbamate) is a selective herbicide for pre-plant use only. It is used to control many broadleaf weeds, annual grasses, and nutsedge in fields for sugar beets, table beets, and spinach. Cycloate controls weeds by interfering with normal seed germination and seedling development. It does not control established weeds. Thiocarbamates interfere with cuticle formation by inhibiting the biosynthesis of very long-chain fatty acids, and thereby, aldehydes, alcohols, and wax esters (Corbett, et al., 1984). The possible main site of action is the inhibition of the incorporation of acetate into fatty acid during the chain elongation process. The cholinergic signs (salivation, tremors, etc.) observed in animals given high doses of cycloate are assumed to be attributable to the inhibition of cholinesterase.

B. REGULATORY HISTORY

The United States Environmental Protection Agency (US EPA) has not set a Reference Dose (Acceptable Daily Intake) for cycloate, stating that neurotoxicity considerations precluded establishment of a reference level. Reviews of the toxicity of cycloate by recognized experts in neurotoxicity yielded concerns about possibly serious effects on humans exposed to the pesticide. Cycloate was nominated for Special Review process of the US EPA, based on neurotoxicity observed in laboratory studies of rats and dogs (US EPA, 1993c).

As part of the ongoing reregistration process for pesticides, the US EPA has published a list of outstanding data requirements for products containing cycloate as the active ingredient (EPA, 1991a). Specifically, additional information was requested concerning the effects of cycloate in the following areas: avian acute dietary and reproductive toxicity, mammalian 21-day dermal toxicity, oncogenicity in mice, teratogenicity in rabbits, general metabolism, photodegradation in water, anaerobic soil metabolism, anaerobic aquatic metabolism, leaching and adsorption/desorption, laboratory volatility, a confined rotational crop study, accumulation in fish, nature of residue in plants, nature of residue in livestock, residue analytical method (animals), magnitude of residue in meat/milk/poultry/eggs (feeding/dermal treatment), crop field trials, and the magnitude of residue in processed food/feed.

C. TECHNICAL AND PRODUCT FORMULATIONS

Technical cycloate is a clear, amber liquid. All pesticides containing cycloate as the active ingredient (a.i.) are manufactured by Zeneca Ag Products (ICI Americas) under the trade name Ro-Neet. Commercial production of Ro-Neet began in 1966 (Palshaw, 1985). Ro-Neet is available in two formulations, 10G and 6E. Ro-Neet 10G is the granular product containing 10% a.i., while Ro-Neet 6E is the liquid formulation containing six pounds a.i. per gallon. Ro-Neet 6E is the only formulation registered for use in California.

D. USAGE

Ro-Neet is not a restricted pesticide in California. In 1990, 60,330 pounds of cycloate liquid were used in the State of California. Approximately 95% of the herbicide was applied to beets, and approximately 5% was applied to spinach (DPR, 1991). The current product label for Ro-Neet 6E has a signal word of "CAUTION". "Rubber gloves and protective

clothing" are required to be worn during the application. The label does not, however, specify a field re-entry interval after chemigation.

Ro-Neet 6E may be used for pre-plant application to table and sugar beet fields at the rate of 1/2 to 2/3 gallon of product (3 to 4 pounds AI) per acre. One application per growing season is allowed on sugar beets. The product is not recommended for use on spinach in California except under a Special Local Need (SLN) registration. Another SLN registration in Northern California allowed an increase in application rate of up to 1 gallon of product (6 pounds AI) per acre to sugar beet fields.

Ro-Neet is applied by soil injection (shank), or as a ground spray (broadcast or band spray), and mixed into the soil to a depth of 3 inches. Chemigation has been allowed under a SLN registration. Ro-Neet 6E may be impregnated into dry bulk fertilizers and incorporated into the soil either in the fall before the ground freezes, or before planting. Application must immediately follow impregnation to prevent loss of a.i. through volatilization. Ro-Neet may also be applied in combination with liquid fertilizers. In several midwestern states, the federal label allows Ro-Neet to be applied in combination with another thiocarbamate herbicide, EPTC.

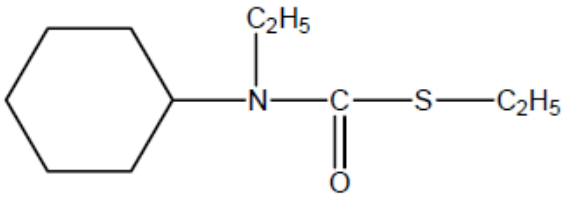
E. ILLNESS REPORTS

The only occupational illness related to Ro-Neet exposure was reported to the State of California in 1983 (Appendix A). The patient (an irrigator) came in contact with the spray during a spray-rig application on spinach. Symptoms included an upset stomach and burning eyes.

F. PHYSICAL/CHEMICAL PROPERTIES (Lee, 1987: DPR, 1992)

Chemical Name:	S-ethyl cyclohexylethylthiocarbamate
Trade Name:	Ro-Neet (ICI Americas)
CAS Registry Number:	1134-23-2

Structural Formula:



Empirical Formula:	$C_{11}H_{21}NOS$
Molecular Weight:	215.4
Physical State:	Amber liquid (Technical)

Boiling Point:	145-146^{o}C at 13 mbar
Specific Gravity:	1.0239 at 20^{o}C
Solubility:	In water: 95 mg/L at 25^{o}C. Miscible with most common organic solvents (e.g. acetone, benzene, kerosene)
Vapor Pressure:	1.6 x 10^{-3}mm Hg (25^{o}C)
Henry's Law Constant:	4.8 x 10^{-6} m^3-atm/g-mole
Octanol/water Partition Coefficient:	1.3 x 10^{4}
Koc (Soil adsorption coefficient):	12,941

G. ENVIRONMENTAL FATE

Hydrolysis

No measurable hydrolysis of cycloate occurred in buffered solutions at pH 5.0, 7.0, or 9.0 at either 25 C or 40^{o}C over the 30-day test period (Myers and Bartell, 1983).

Photolysis

A photolysis study was conducted using aqueous solutions of cycloate buffered at pH of 7.0 at 25^{o}C (Myers and Bartell, 1985). Less than 10% photolytic degradation was observed during the 30-day test period. The half-life under the test conditions was estimated to be 219 days.

Photodegradation of cycloate on soil was studied by applying [ring (U)-^{14}C] cycloate to 0.7-mm layers of soil at surface rates near 6.7 kg/ha (Tarr, 1986). Treated soil layers were exposed to sunlight for 12, 60, 120, and 192 hours. At 192 hours, 85% of the total radioactivity was recovered from the soil and 48% remained as parent compound. The unrecovered radioactivity (15%) was believed to be lost through volatilization. Cycloate sulfoxide and N-ethylcyclohexylamine were the two major degradation products. Only N-ethylcyclohexylamine was produced at greater than 10%. Photolysis appeared to be a relatively unimportant route of dissipation, contributing approximately 9% of the degradation. N-Cyclohexyl-N-ethylformamide and cyclohexylamine, which might be uniquely derived from photolytic processes, accounted for less than 3% of the radioactivity applied.

Persistence in Soil

Studies have shown that cycloate is lost from the soil predominately by vaporization and microbial degradation (Gray and Weierich, 1965). Twenty-four hours after application as a spray at 3 lb/acre to the surface of moist soil, 58% was lost as vapor. No loss was detected 24 hours after application to the dry soil. Increasing the temperature from 40^{o}F to 60^{o}F caused an increase in the rate of loss from moist soil surfaces, but further increases in temperature caused no increase in the loss. The results indicated that cycloate has a relatively short persistence in soil under simulated summer conditions, and microbial decomposition plays an important role in the disappearance of cycloate from moist soil.

Aerobic Metabolism

In a study on soil metabolism, cycloate was incorporated into silt loam soil at a concentration of 6.4 ppm (approximating a field application rate of 6 lbs/acre) and incubated at 30°C for 130 days using a biometer flask apparatus (Spillner, 1986a). After 89 days of incubation, 6% of the applied cycloate had evaporated, 38% was extractable, and 52% was bound to the soil. The major metabolite was cycloate sulfoxide which accounted for 16% of the applied [^{14}C] cycloate 31 days after treatment. After 120 days, ethylcyclo-hexylamine was found at 3%. The half-life of cycloate under the aerobic condition was approximately 41 days. No significant degradation occurred in sterilized soils, although some of the applied cycloate dissipated through volatilization.

Degradation products identified from cycloate incorporated into the soil under the aerobic condition included cycloate sulfoxide, cycloate sulfone, ring-hydroxycycloates, ring-3-hydroxycycloate, ring-ketocycloates, ethylcyclohexylamine, and CO_2 (Spillner, 1989). S-(2-Hydroxylethyl)- cycloate, a metabolite observed in plants, was not found in soil.

Anaerobic Metabolism

An anaerobic soil metabolism study was conducted in silt loam soil treated with [ring-^{14}C] cycloate (Spillner, 1986b). After treatment, the soil was incubated for a total of eleven weeks. For the first three weeks, the study was conducted aerobically. For the remaining eight weeks, it was conducted under anaerobic, flooded conditions. The bound residues increased from 0.4% at day 1 to 32% after eleven weeks of incubation. The organo-soluble fraction decreased inversely in relation to the bound fraction over time. Flooding of the soil at three weeks had no effect on the binding. The organo-soluble fraction of the three-week sample contained 7.2% cycloate sulfoxide, 1.4% cycloate sulfone, and 89.7% parent compound. Volatilization of the compound was minimal, 5-8%, and remained at this level from three to eleven weeks. Mineralization of cycloate to $^{14}CO_2$ did not take place in significant amounts throughout most of the study. However, at eleven weeks, the $^{14}CO_2$ level increased to 4-9% of the applied radioactivity. The calculated half-life of cycloate under the aerobic and anaerobic condition used in this study was 44 days and 77 days, respectively.

The volatilization rate of 5-8% found in the study by Spillner (1986b) was significantly lower than that of 58% found in an earlier study conducted by Gray and Weierich (1965). The discrepancy might be partially attributed to the differences in the experimental apparatus and methodology employed in these two studies: enclosed biometer flasks with minimum air flow (Spillner, 1986b) vs. open aluminum flats with vacuum pump (Gray and Weierich, 1965).

Leaching Studies

Earlier leaching tests showed that cycloate remained predominately in the top six inches of soils and that less leaching occurred in soils higher in clay content (Gray and Weierich, 1965). In another study, cycloate was evaluated for leaching and mobility potential in four types of soil (Miaullis, 1986). The soil types were sand, loam, silt-loam, and sandy-loam. The organic matter ranged from 0.5% to 3.4% with a pH of 5.4 to 7.1. Both parent [^{14}C] cycloate and its degradation products were found to be predominantly of the immobile and low mobility classes. The results suggested that cycloate and its degradation products have a relative low potential for movement in soil. The potential for groundwater contamination was considered to be low. In another evaluation, that only considered the physical-chemical characteristics of cycloate, some theoretical leaching potential was identified (DPR, 1992).

Field Dissipation

In two field dissipation studies, Ro-Neet 6E was applied by broadcasting at a rate of 4 pounds AI per acre to field sites with sandy loam soil (Curry, et al., 1989; Curry, 1989). One site had a pH of 7.6 and an organic content of 0.1 to 0.6%. The other site had a pH of 8.4 and an organic content of 0.4 to 0.9%. The herbicide was incorporated to a depth of 2 inches, and the treated area was planted with sugar beets. Samples of soil were analyzed for residues of cycloate, cycloate sulfoxide, and N-ethylcyclo- hexylamine. The calculated half-life for cycloate residues in the top 6-7 inches of soil was 10-11 days. At the end of the studies (187-198 days after application), cycloate had dissipated to near the detection limit of 0.01 ppm.

N-Ethylcyclohexylamine reached similar concentrations in the field and in the laboratory metabolism study, but dissipated more rapidly in the field. In the field, cycloate sulfoxide did not build up to as high a level, reached the maximum concentration sooner, and dissipated more rapidly than in the study conducted in the laboratory.

The field dissipation studies showed that none of the cycloate, cycloate sulfoxide, or N-ethylcyclohexylamine was detected below 6 inches in any of the samples. The results indicated that significant leaching did not occur under the conditions of the studies. The potential for ground water contamination was negligible.

Ground Water

Cycloate was not detected in 44 California wells analyzed for cycloate during the years 1983 to 1992 (DPR, 1993a).

Plant Metabolism

The fate and metabolism of cycloate labeled with ^{14}C in the cyclohexyl ring was studied in sugar beet seedlings (Gray and Tomlinson, 1967). The radioactivity was rapidly taken up by the roots and translocated throughout all parts of the plant. Seven days after treatment, 1.13% of the applied radioactivity was eliminated as $^{14}CO_2$. No unchanged cycloate could be detected in the
roots or shoots harvested at 3, 7, or 14 days after application to the roots, thereby indicating that it was rapidly metabolized.

In roots, shoots, and leaves, the cell sap accounted for 78-91% of the radioactivity. Ethylcyclohexylamine was identified as one of the main metabolites, and it accounted for 9% of the radioactive metabolites found in the cell sap of leaves 7 days after treatment. No other volatile amines were detected. Extract of leaf cell sap at 14 days revealed eleven minor metabolites. Five of the metabolites were identified as amino acids with Rf values corresponded closely to proline, glycine, asparagine, alanine, and valine. Hydrolysis studies on the cell sap indicated that some ethylcyclohexylamine and other unidentified amines were present as conjugates having characteristic of N-glycosides.

Summary

Cycloate is not persistent in the soil. The major mechanisms for dissipation of cycloate in soils are vaporization and microbial degradation. Environmental fate data indicated that cycloate and its degradation products have a relatively low potential for movement in soils, and the potential for groundwater contamination is low. A limited survey of California wells did not detect cycloate in ground water. Metabolism studies have indicated that cycloate is rapidly metabolized by plants.

III. TOXICOLOGY PROFILE

A. PHARMACOKINETICS/METABOLISM

Oral - Rat

The fate of ^{14}C-cycloate, uniformly labeled in the cyclohexyl ring, was determined following administration of a single dose of approximately 90 mg/kg to rats via oral intubation (Ford, et al., 1966). An average of 97.6 % of the radioactivity was excreted within 96 hours after treatment. Eighty-two percent of the administered radioactivity was excreted in the urine with 15% in the feces and 0.4% in exhaled air. Approximately 2% was found in the tissues with no evidence of selective storage. The un-metabolized ^{14}C-cycloate accounted for about 0.04% of the urinary radioactivity.

Elimination of the radioactivity via the urine and feces was very rapid, averaging 77% in one day (64% in urine and 13% in feces), and more than 93% in two days (78% in urine and 15% in feces). The overall residue levels in the tissues ranged from 0.1 to 10.6 ppm of ^{14}C-cycloate equivalents. The higher levels were generally found in those regions actively associated with transport, metabolism, and elimination (e.g. blood, liver, kidney, and skin, etc.). The lower levels were associated with the storage depots (e.g. fat). The data suggested that storage of radioactivity in the tissues was very transient.

Oral - Rat

In another single-dose oral pharmacokinetics study, the fate of ^{14}C-cycloate labeled in the cyclohexyl ring was determined following doses of 10 or 160 mg/kg to female rats via oral intubation (Lappin et al., 1991). Within 48 hours following administration of either dose, radioactivity was rapidly eliminated primarily in urine (80%) and feces (11%). High levels of residual activity were found in liver, kidneys, lung, spleen, and heart. The pattern of elimination and tissue distribution were reported to be similar to the previous report in male rats.

Oral - Rat

In a 10 mg/kg repeat-dose oral pharmacokinetics study, the fate of ^{14}C-cycloate labeled in the cyclohexyl ring was determined following 14 daily oral doses of unlabeled cycloate prior to receiving a single oral dose of radiolabeled cycloate (Bratt et al., 1991). Within 48 hours after the final dose, 74.1% to 87.1% of the radioactivity was eliminated in the urine, whereas 10.5% to 22.3% of the radioactivity was detected in the feces. Residual concentrations of radioactivity in the tissue were similar to those obtained from rats administered a single oral dose of radiolabeled cycloate. Pre-dosing rats with unlabeled cycloate had little effect on the route and rates of elimination of a single oral dose of radiolabeled cycloate. There was no evidence for bioaccumulation.

I.V./Gavage - Rat

In a metabolism study of ^{14}C-cyclohexyl ring labeled cycloate following 10, 40, or 160 mg/kg oral, or 10 mg/kg I.V. doses to male rats, cycloate was extensively metabolized prior to excretion in the urine (Chin et al., 1990). N-ethylcyclohexylamine was identified as the major metabolite, and was probably formed by hydrolytic cleavage of the amide bond of the

parent compound. Cyclohexylamine was also identified, but only accounted for about 1% of the total urinary radioactivity. Fecal excretion of radioactivity was independent of the route of administration, and averaged 6.9 and 9.7% of the dose following iv and oral dosing, respectively, indicating 97% oral absorption. One percent of the dose was found in expired CO_2. Residual radioactivity remaining in tissues at the end of 8 days after dosing was less than 1% of the administered dose, indicating that there was little bioaccumulation of cycloate and/or metabolites.

I. V./Gavage - Rodent

A comparative pharmacokinetics study was conducted in male Sprague-Dawley rats and male CD-1 mice administered a single dose of radiolabeled cycloate (purity 99.90%) via I.V. injection at 10 mg/kg or via oral gavage at 10, 40, or 160 mg/kg (Chin, 1983). The study showed significant differences, qualitatively as well as quantitatively, in the pharmacokinetics of cycloate in rats and mice. The half-life for elimination of plasma radioactivity was approximately 60 hours and 30 hours for rats and mice, respectively. The disappearance of radioactivity from the plasma of both species was rapid, biphasic, and followed first order kinetics.

Cycloate was extensively metabolized by both rats and mice. Nine of thirteen metabolites isolated from rat urine (93% of the urinary radioactivity) were identified, while six of twelve metabolites (72% of urinary radioactivity) isolated from mouse urine were identified. No unchanged cycloate was found in the urine of rats following a single dose of cycloate at 160 mg/kg. In mice, however, unchanged cycloate accounted for approximately 3.7% of the total urinary radioactivity.

Urinary excretion was the primary route for the elimination of cycloate in both rats (95% of the administered dose) and mice (65%). In mice, fecal excretion accounted for 25% of the administered dose, while in rats it represented only 8% of the dose. Biliary excretion appeared to play a role in the fecal excretion of cycloate and/or its metabolites. Radioactivity remaining in the tissues was less than one percent of the administered dose at the end of eight days for both rats and mice. These findings indicated that the bioaccumulation of cycloate and/or its metabolites is minimal.

N-ethylcyclohexylamine was identified as a major metabolite constituting approximately 28% and 51% of the urinary radioactivity from mice and rats, respectively. The desethyl derivative, cyclohexylamine, accounted for approximately one percent of the urinary radioactivity in both species. Hydroxylation of the cyclohexane moiety of cycloate resulted in the formation of both structural and conformational isomers of the hydroxylated derivatives of N-ethylcyclohexylamine and cyclohexylamine.

In rat urine, 3-(N-ethylamino)cyclohexanol (19%) was more prevalent than 4-(N-ethylamino)-cyclohexanol (9%). In the mouse, 4-(N-ethylamino)-cyclohexanol accounted for 35% of the urinary metabolites and 3-(N-ethylamino)-cyclohexanol was not found. Other major metabolites identified in rat urine included N-cyclohexyl-ethyl-carbamic acid (8%) and its glucuronide conjugate (5%). Approximately 28% of the mouse urinary metabolites remained unidentified. A smaller fraction (<9%) of the rat urinary metabolites were unknown.

Oral - Rat

In an abstract of an article published in the Russian literature, it was reported that following single gavage dose of cycloate (1000 mg/kg), the highest concentration were found

in the lungs, kidneys, liver, heart, and spleen at one hour. At two hours the highest concentration was in the brain (Aleksandrovna, 1978) Additional details were not provided.

I. V./Gavage - Monkey

A study on the pharmacokinetics and metabolism of cycloate was also conducted in male cynomolgus monkeys (Chin, 1984). Monkeys were administered a single dose of radiolabeled cycloate via I.V. at 10 mg/kg or by oral gavage at 10, 40, or 160 mg/kg. The study showed that monkeys are qualitatively more similar to rats than to mice in their ability to absorb, metabolize, and excrete cycloate. The oral absorption rate was calculated to be approximately 65% and not appreciably affected over the dosing range. On the other hand, the recovery studies indicated a greater than 80% absorption from the gastrointestinal tract. The disappearance of radioactivity was biphasic and followed first-order kinetics. The plasma half-life was approximately 55-65 hours.

Urinary excretion was found to be the primary route of elimination for cycloate and its metabolites. Approximately 88% of the administered radioactivity had been accounted for in the urine by the end of the eight-day study. The majority (90%) of the excretion occurred within the first 24 hours after dosing. Radioactivity remaining in the tissues at the end of the study was less than 1% of the dose administered. Liver and kidney had the highest concentrations of radioactivity, regardless of the dose. Fecal excretion played only a minor role in elimination, irrespective of route of administration or dose level. Only two percent of the administered radioactivity was found in the feces at the end of the eight-day study.

Cycloate was extensively metabolized by monkeys. No unchanged cycloate was found in the urine after a single oral dose of 160 mg/kg. Ten of 27 metabolites isolated were identified, representing approximately 75% of the urinary radioactivity. N-ethylcyclo-hexylamine was a major metabolite accounting for approximately 28% of the urinary radioactivity. The desethyl derivative, cyclohexylamine, was also found. Hydroxylation of the cyclohexane moiety resulted in the formation of structural isomers of hydroxylated forms of N-ethylcyclohexylamine and cyclohexylamine. Glucuronide conjugates of ring-hydroxylated cycloate have also been identified and accounted for approximately 27% of the urinary radioactivity.

Oral - Human

Cycloate (99.8%) in corn oil was administered orally to six human volunteers at a single dose of 60-74 ug/kg (Marsh, 1993). Cycloate was not detectable in any blood samples (limit of detection ~10ng/ml). Peak urinary excretion of the metabolite 4-hydroxy cycloate (as a conjugate) occurred within the first 2 hours and was almost complete by 24 hours. However, small amounts were detectable up to 60 hours in two of the volunteers. The amount of 4-hydroxy cycloate excreted in the urine was equivalent to a mean of 50% of the administered cycloate (range 31-65%, s.d. 11).

Peak excretion of N-ethyl cyclohexylamine occurred within the first 24 hours, and this metabolite was detectable in urine for up to 120 hours post-administration. The amount excreted in the urine was equivalent to a mean of 9.4% of the administered cycloate (range 8.2-11%, s.d. 1.1).

No adverse clinical, biochemical, or hematological effects were reported. Although, many details were not provided. Administration of the cycloate did not cause a reduction in plasma or erythrocyte cholinesterase activity in any of the volunteers.

The study demonstrated that 4-hydroxy cycloate is a major metabolite of cycloate, and that this metabolite could be useful in estimation of cycloate absorption during occupational exposures.

The above studies indicate that cycloate is well absorbed from the gastrointestinal tract. The recent study by Chin (1990) which indicated ~97% oral absorption, was considered the most reliable for risk assessment purposes. With absorption essentially complete, adjustment factors for the percent absorbed in oral toxicity studies were not necessary.

B. ACUTE TOXICITY (Miller, 1981; Meyding, 1965; Beliles, 1965, NACA, 1992; US EPA, 1993a)

A summary of the general acute toxicity studies in animals exposed to cycloate is presented in Table 1. Information on acute neurotoxicity testing is presented in Section **H. NEUROTOXICITY**.

Table 1. Summary of Acute Toxicity Observed in Animals Exposed to Cycloate Liquid

Routes/Formulation	Species	(Sex)	LD_{50} (mg/kg)
Oral	Rat	(F) (M)	3,690 - 4,175 3,160 - 3,250
Dermal	Rabbit	(M/F)	>5,000
Inhalation (R-2063 6E)	Rat	(M/F)	LC_{80} = 15.7 mg/l (8 hr.) LC_{10} = 14.7 mg/l (4 hr.)
Eye Irritation (Ro-Neet 6E)	Rabbit		Moderate Irritant
Skin Irritation (Ro-Neet 6E)	Rabbit		Moderate irritant
Skin Sensitization	G. Pig		Negative

Clinical Signs

Signs of acute toxicity following oral administration of cycloate liquid to rats included depression, tremors, blood-like facial stains, hyperactivity, salivation, ruffled fur, and alopecia on the back. Depression and labored breathing were noted in rats during exposure to cycloate via inhalation. On days one through seven following inhalation exposure, depression, redness around eyes and nose, and wheezing were noted in rats that had been exposed to 15.7 mg/L for 8 hours. The reported 15.7 mg/L was the nominal concentration. The actual concentration in the chamber was not measured.

Necropsy

Common necropsy findings in rats exposed to acutely toxic doses of cycloate via the oral routes included bloated stomachs, red lungs and gastrointestinal tract, and dark discoloration of the liver and spleen. Rats acutely exposed to cycloate via inhalation of 15.7

mg/l for 8 hours, 14.7 mg/l for 4 hours, or 9.1 mg/l for 1 hour and necropsied 14 days later had hemorrhagic lungs. Increased lung weight was also noted at 15.7 and 14.7 mg/l.

C. SUBCHRONIC TOXICITY

Summaries of the subchronic toxicity studies are presented in this section. Additional discussion of subchronic neurotoxicity testing is also presented in Section **H. NEUROTOXICITY**.

Gavage - Rat

Cycloate was administered at a dose level of 10 mg/kg/day to both male and female rats by gavage for 14 days (Boberg, 1986). No significant differences in terminal body weight, absolute liver weight, and relative liver weight were observed between control and cycloate treated animals. Cycloate at 10 mg/kg/day did not induce hepatic microsomal enzymes in rats.

Dietary - Dog

Technical cycloate (97.8% pure) was fed to 16 purebred beagle dogs at dose levels of 2,700, 5,400, or 10,800 ppm (corresponding to 60, 120, or 240 mg/kg/day) for 13 weeks (Johnston, 1965; Johnston, 1966a). Preliminary experiments showed food refusal at dietary level equivalent to 240 and 120 mg/kg. All feedings began at 60 mg/kg for one week and increased to 120 mg/kg and 240 mg/kg at the end of the 7th and 14th day, respectively. No adverse effects were observed as determined by the survival, body weight gain, general condition, hemograms, and clinical chemistry. Some early instances of diarrhea, emesis, or slight to moderate focal microscopic calcification of the kidney tissues were observed in a few dogs in the high- and middle-dose groups. The No-Observed-Effect-Level (NOEL) was 60 mg/kg/day.

Dietary - Rat

Technical cycloate (97.8% pure) was administered to rats (20/sex/group) in the diet at 0, 55, 167, or 500 mg/kg/day for 13 weeks (Johnston, 1965; Johnston, 1966a). Interim evaluation at 5 weeks showed significant differences in the absolute and relative organ weights of the animals at the high- and middle-doses compared to those of the controls (Johnston, 1965). In the males, the absolute and relative weights of the liver of the high-level were significantly increased, and those of the pituitary of all three compound-fed groups were significantly decreased. In the females, the absolute and relative liver weights were increased in both the high- and middle-dose groups, and the increase in the kidney weight was observed at the high-dose.

Increases in liver weights and some degenerative histological changes in the liver and kidneys were observed in rats fed cycloate at 167 and 500 mg/kg/day at the end of the 13-week exposure (Johnston, 1966a). Slight or moderate periportal hepatic cell hypertrophy and cytoplasmic degenerative appearances were observed. The amount of albuminous material in the upper nephrons was moderately greater than that of the controls. Decreases in food consumption and body weight gain were seen. The absolute and relative liver weights were also significantly increased in males at the 55 mg/kg/day level. Significant decreases in the absolute and relative ovarian weights were seen in females of all three compound-treated groups. A NOEL was not established for the 13-week exposure. The Lowest-Observed-Effect-Level (LOEL) based on the changes in the liver and ovarian weights was 55 mg/kg/day.

Dermal - Rabbit

Ten albino rabbits per group were treated with water, solvent (kerosene) control, or one of the pre-determined daily doses of R-2063 6E (an old designation for a cycloate formulation) undiluted at 1.0 ml/kg or diluted with water (1:5) at 2.5 ml/kg once daily, 5 days a week for 3 weeks (Johnston, 1966b). Severe skin irritation was noted in groups receiving the kerosene solvent control or undiluted formulation. Little difference was seen between groups with abraded skin and those with intact skin. Groups treated with diluted formulation showed moderate irritation at the second or third week. Mortality was observed in animals treated with the kerosene solvent (1/10), undiluted formulation (3/20), and diluted formulation (1/10).

Gross autopsy findings included consistent skin thickening and drying (except in water controls), and scattered instances of pitted kidneys, discolored liver or thyroid, or enlarged adrenals. Hyperplasia of the epithelial structures with marked hyperkeratosis and some chronic inflammatory changes in the skin were noted in all groups except the water controls. Systemic effects, consisting of microscopic changes in the liver and kidneys, were also observed in animals treated with the undiluted formulation or kerosene solvent. These findings indicate that the kerosene vehicle may have played a role in the production of skin and systemic toxic effects.

Dermal - Rat

Five Alpk:APfSD rats/sex/dose were treated with 0 (sham control), 10, 50, or 200 mg/kg (0, 0.065, 0.31, or 1.23 mg/cm^2) technical cycloate (97.8% purity) 6 hours/day for 5 days/week amounting to 21 dermal applications over a 30 day period (Kinsey et al., 1991). Based on a dermal absorption factor of 19.3% (Appendix A), the absorbed dosages were 0, 1.93, 9.65, or 38.6 mg/kg/day. Hematology, clinical chemistry, necropsy, and microscopic examinations of the skin, liver, and kidney were conducted. Perfusion fixation of tissues was not employed, and neural tissue was not histologically examined. Two 200 mg/kg/day rats were sacrificed prior to study termination due to poor condition or self-mutilation due to bandaging or skin irritation. One of these animals also had necrosis and atrophy of the stomach. Clinically, there was a dose-related incidence and severity of skin irritation in the 50 and 200 mg/kg/day rats consisting of desquamation, erythema, edema, and thickening of the skin. Males at 200 mg/kg/day had a slight decrease in body weight and food consumption. There were slight, though not statistically significant, reductions in plasma glucose and urea levels in 200 mg/kg/day males. There also was a 7 to 9% decrease in red blood cell counts in males at 50 and 200 mg/kg. A dose-related, 2%, 3%, and 8% decrease in absolute brain weight was present in all cycloate-treated male groups, reaching statistical significance at 200 mg/kg/day. Microscopically, several animals at 50 and 200 mg/kg/day had slight acanthosis (increased thickness of the stratum spinosum of the skin). Based on skin irritation, the topical NOEL was 10 mg/kg/day (0.065 mg/cm^2). Based on decreased brain weight, the systemic LOEL was 1.93 mg/kg/day. Using an uncertainty factor of 10 as a default procedure for the determination of a NOEL from a LOEL (Beck et al., 1989), the estimated no-effect level was 0.193 mg/kg/day (193 ug/ kg/day). The US EPA did not consider the study acceptable for FIFRA guideline requirements due to the lack of histological examination of perfusion-fixed neural tissue (US EPA, 1993a). However, that did not prevent DPR use of the decreased brain weight and skin irritation present in the study to evaluate the risk of neurotoxicity and skin irritation from potential human exposure.

Inhalation - Rat

Two subchronic whole-body inhalation studies in Sprague-Dawley CD rats were submitted by the Stauffer Chemical Company (Knapp and Thomassen, 1984; Knapp and

Thomassen, 1986). In the first study cycloate was present predominantly as a vapor at the lower exposure levels and predominantly as an aerosol at the high exposure level. In the first study, technical cycloate (96.8%) was administered to 18 rats/sex/group at 0, 2.5, 17, or 120 mg/m^3 (~0, 0.2, 2.04, or 14.4 mg/kg/day) for 6 hours/day, 5 days/week for a total of 65 exposure days (Knapp and Thomassen, 1984). Decreases in body weights and food consumption were noted in the mid- and high exposure groups of both sexes. At the high exposure of 120 mg/m^3, there were increased incidences of chromorhinorrhea, chromodacryorrhea and rough hair coat in both sexes, and decreased absolute ovary weight in females. Six to 12% decreases in absolute brain weight, and increases in relative weight in various organs were noted in both sexes at mid- and high exposure levels. Relative brain weights did not increase, as would typically be expected in the presence of the decreased body weight (Hayes, 1989; Scharer, 1977), confirming the deleterious effect on the brain. Treatment-related histopathologic changes included a significantly increased incidence and severity of hypertrophy and/or hyperplasia of nasal respiratory epithelium in females at the high dose and in treated males at all doses (Table 2). Neither necrosis or inflammation of the nasal epithelium was present. A slightly increased severity of gynecomastia was observed in males at the high exposure level. The LOEL based on the hyperplasia/hypertrophy of the nasal respiratory epithelium, was 2.5 mg/m^3 (0.2 mg/kg/day) A NOEL was not established.

Table 2. Incidence of Epithelial Hyperplasia/Hypertrophy in Rats Exposed to Cycloate for 13 Weeks[a,b]

	Cycloate Treatment Level (mg/kg/day)[c]			
Animals	**0**	**0.2**	**2.04**	**14.4**
Females	0/18	3/18	4/18	10/18***
Males	0/18	5/18*	6/18**	13/18***
Combined	0/36	8/36**	10/36***	23/36***

[a] Data are derived from Knapp and Thomassen, 1984. Incidence of the nasal epithelial hyperplasia/hypertrophy shown as number of animals affected/number of animals treated.

[b] Significant at * $p<0.05$, ** $p<0.01$, *** $p<0.001$ based on the Fisher Exact Test. Using the same test, the registrant reported the statistical significance at $p<0.05$ for males at the mid and high doses, and for females at the high dose.

[c] The calculation used for conversion of mg/m^3 to mg/kg/day was: (exposure level of mg/m^3) x (rat respiratory rate of 0.96 m^3/kg/day) x (50% respiratory absorption/retention) x (duration of exposure hrs/24 hrs) x (number of exposure days per week/7 days).

Perfusion-fixation of tissue was not employed in the first study (Knapp and Thomassen, 1984), so the true incidence of changes in the nervous system may not have been determined, since such procedures are necessary in order to reliably detect cycloate-induced neurotoxicity. (Stauffer Chemical Co., 1986). Hypertrophy/hyperplasia of the mammary glands were found in the high exposure groups. The average grade of the lesion, but not the number of animals affected, was significantly different between the control and high dose group. Histopathological evaluation of that tissue at lower doses was incomplete due to loss in a fire at an independent histology laboratory. The study was considered acceptable for risk assessment purposes.

In a 2-week pilot inhalation toxicity study involving exposure to 0, 4.3, 27, or 210 mg/m^3, it was reported that cycloate technical had an apparent irritating effect on the eyes and nasal passages at concentrations of 27 and 210 mg/m^3 as evidenced by an increased number of animals showing chromorhinorrhea, facial staining, and chromodacryorrhea (Knapp, 1983). Female body weight means were significantly decreased in the 210 mg/m^3 exposure level at study day 14. There were no apparent treatment-related gross changes seen at necropsy, and no histopathological examinations were conducted. Relative liver weights of the 210 mg/m^3 males and females were increased as were the relative heart weights of the 27 and 210 mg/m^3 females. A trend towards increased relative kidney weights with increased concentration of cycloate technical was observed in the males, and the 210 mg/m3 weights were significantly increased above the control value. Brain weights were not reported. A complete study report was not submitted to DPR for evaluation.

Inhalation - Rat

A repeat whole-body subchronic toxicity study using the inhalation route was conducted in young adult Sprague-Dawley CD rats (18/sex/group) exposed to technical cycloate (97.6% purity) at 0, 1.2, 12, or 120 mg/m^3 for 6 hours/day, 5 days/week for 68 to 71 exposure days (Knapp and Thomassen, 1986). Special neuropathology procedures, including perfusion fixation and teased nerve preparations, were included in the study design. Decreases in mean body weight and food consumption were observed at the high exposure level of 120 mg/m^3. Other significant observations in the high exposure groups included increases in the incidence of chromodacryorrhea, chromorhinorrhea, salivation, tremors, and degeneration of axons of the spinal cord, spinal nerve rootlets and sciatic nerve. Additional changes present in the high exposure groups included significant increases in relative weights of the liver, kidneys, and heart, a 12% decrease in the absolute brain weight, and dilatation of the lateral ventricles of the brain. The degeneration of the spinal cord was detected only in animals which had received perfusion tissue fixation at necropsy, and degeneration of spinal nerve rootlets and sciatic nerve was more frequently detected in perfused animals (Stauffer Chemical Co., 1986). Decreases in serum cholinesterase were present in females at 120 mg/m^3. Based on the observation of cholinergic signs, degeneration of nerves and spinal cord, and changes in organ weights, the NOEL was 12 mg/m^3 (~1 mg/kg/day).

Rhinitis was observed in both sexes at all exposure levels including controls. The incidence within each severity grade was increased for all exposure levels when compared to controls. The rhinitis involved a spectrum of changes of the nasal mucosa including inflammation, hypertrophy/hyperplasia and squamous metaplasia, and deposits of purulent exudate in the nasal meatuses. The anterior portion of the nose was distinctly more frequently and severely involved than the posterior region. The inflammatory and proliferative responses to cycloate at all exposure levels exceeded the controls and were considered treatment-related. However, the presence of similar lesions in non-treated rats suggested the presence of a concurrent infection such as murine respiratory mycoplasmosis. The authors hypothesized that exposure to vaporized and aerosolized cycloate additively or synergistically increased the response of the nasal mucosa to injury. A NOEL based on rhinitis was not established. The LOEL for rhinitis was 1.2 mg/m^3 (~0.1 mg/kg/day). The presence of the concurrent infection precludes use of respiratory tract data for risk assessment purposes.

Inhalation-Rat

An additional inhalation toxicity study was conducted to determine the no-effect level for nasal passage toxicity and/or neurotoxicity after shorter-term inhalation of 97.8% technical

cycloate (Lewis et al., 1992). In the main part of that study, 5 Hsd-Ola rats/sex/dose were exposed nose-only to concentrations of 0, 2.05, 21.23, or 207 mg/m^3 (~0, 0.175, 1.81, or 17.64 mg/kg/day) 6 hours/day, 5 days/week, giving a total of 15 exposure-days out of a 21-day period. The animals were sacrificed after the last exposure. A satellite group of 5 Hsd-Ola rats/sex/dose were similarly exposed to 0, 2.13, 22.79, or 209.6 mg/m^3 (~0, 0.18, 1.94, or 17.86 mg/kg/day), they were then maintained without exposure for an additional 30-day "recovery" period before sacrifice. Salivation and lacrimation were noted at the high-exposure level during the exposure period, but were not present during the recovery period. No significant changes in mean body weight or food consumption were noted during the study. Slight, though statistically significant, increases in absolute and relative kidney weights were present in 17.64 mg/kg/day main-study males. Absolute and relative heart weight and absolute kidney weight elevations were present on main-study females at that dose level. Organ weights were not altered in the satellite group sacrificed after 30 days recovery. Necropsy changes present at sacrifice of the main-study groups included mottled lungs in two male and two female rats at 17.64 mg/kg/day, and in one female at 1.81 mg/kg/day. No treatment-related changes were noted in animals sacrificed at the end of the recovery period. No treatment-related microscopic changes were recorded for any treatment group at either time point, although only brain, spinal cord and sciatic nerve were examined in the recovery group. Perfusion fixation of tissues at necropsy was not undertaken, which limited the ability to detect cycloate-induced lesions of the nervous system (Stauffer Chemical Co., 1986). It is also of note that marked cerebellar hypoplasia of the brain was present in one female in every group, including control, in the main study. The investigators concluded that the lesion was due to a viral infection at a young age. The brain damage was severe enough to hinder the animals walking ability. Lung inflammation consisting of minimal to slight alveolitis, alveolar macrophage infiltration, pneumonitis, and/or perivascular cuffing with mixed inflammatory cells were present in the lungs of both control and test groups in the main-study (recovery animal lungs were not microscopically examined). The lesions were considered by the investigators to be part of "background" pathology. Based on mottled lungs at necropsy, the NOEL for the main-study group was 0.175 mg/kg/day. The NOEL for the nasal cavity and nervous system was apparently $\geq$ 17.86 mg/kg/day, although, as previously stated, adequate examination of the nervous system was not accomplished. It is of note that it has been reported that different methods of tissue fixation (immersion vs. perfusion) may produce different histological appearances of rat nasal epithelium (Hurtt et al., 1987). Due to the inadequate tissue fixation at necropsy, and other deficiencies, the study was not acceptable for risk assessment purposes.

Inhalation - Rat

In a repeat 15 exposure-day inhalation study designed to evaluate neurotoxicity, groups of 10 male and 10 female Sprague-Dawley rats were exposed nose-only to air concentrations of 0, 2.3, 20.9, or 208.6 mg/m^3 (~0, 0.2, 1.8, or 17.8 mg/kg/day) for 6 hours/day 5 days/week for 3 consecutive weeks (Coombs, 1993). Five males and 5 females from each group were sacrificed following 3 weeks of exposure; the remaining animals were sacrificed following a 4-week withdrawal period. Whole body perfusion was used for all test animals. A functional observation battery was performed on half the animals at termination of exposure, and on the remaining rats 4 weeks later. At exposure termination, there was a slight increase in activity and hindlimb grip strength in 17.8 mg/kg/day males. A slight decrease in activity was present in that group after 4 weeks withdrawal from treatment. One high-dose female lacked a startle response at the withdrawal observation.

There were no mortalities during the study. Reduced body weight gain was present in 1.8 and 17.8 mg/kg/day females. A 7-12% decrease in brain weight was present in females at all cycloate dose levels at 3 weeks, and a 4-6% decrease in 1.8 and 17.8 mg/kg/day females after 4 weeks withdrawal. Histologically, slight axonal degeneration in the spinal cord

was present in 4 of 5 high-dose males sacrificed at 3 weeks. No degeneration was present in control animals. Axonal degeneration was also present in 4 of 5 high-dose females sacrificed after 4 weeks withdrawal. The 0.2 and 1.8 mg/kg/day groups were not examined histologically at either time point. The inadequate histological examination precluded determination of a NOEL/LOEL for axonal degeneration of the spinal cord. Based on decreased absolute brain weight, the study LOEL was 0.2 mg/kg/day. A NOEL was not established. Relative brain weights did not increase, as would typically be expected in the presence of the decreased body weight (Hayes, 1989; Scharer, 1977), confirming the deleterious effect on the brain. Using a default uncertainty factor of 10 for determination of a NOEL from a LOEL (Beck et al., 1989), the estimated no-effect level was 0.02 mg/kg/day. The decreased brain weight NOEL of 0.02 mg/kg/day was one of the definitive NOEL's used to evaluate the risk of potential human repeated daily exposure.

Inhalation - Rat

In a more recent 15 exposure-day rat inhalation toxicity study designed to evaluate nasal cavity toxicity and neurotoxicity, groups of 18 Crl:CD(SD)BR rats/sex/dose were exposed to cycloate (98.4%) 6 hr/day, 5 days/week for 3 weeks (Parr-Dobrzanski, 1994). The chamber concentrations were 0, 1.2, 12, or 120 ug/l (~0, 0.1, 1.0, or 10 mg/kg/day). In addition, a recovery study was performed, in which the same group sizes were exposed to the same concentrations, but the latter groups were taken off treatment 70 days before sacrifice. Rats were evaluated for clinical observations, body weight gain, food consumption, and for histopathological changes in the respiratory tract, central nervous system, and peripheral nervous system. Perfusion tissue fixation was employed. No NOEL was observed in the study, however dose-response relationships were typically well defined. Absolute brain weight was decreased 2-7% at the two highest dose levels. Histologically, at the 3-week sacrifice several lesions in the nasal cavity (rhinitis, goblet cell hyperplasia, respiratory epithelial cell hyperplasia, and respiratory transitional cell hyperplasia), and in the larynx (squamous epithelial hyperplasia) were observed at all dose levels in females, and in most cases also in males (Table 3). Neuronal necrosis in the pyriform cortex of the brain was present at 1 and 10 mg/kg/day. After 70 days without treatment, nasal cavity goblet cell hyperplasia and respiratory epithelial cell hyperplasia were still present at all dose levels in both sexes. Based on the nasal cavity changes, the study LOEL was 1.2 ug/l (~0.1 mg/kg/day). Using a default uncertainty factor (Beck et al., 1989) of 10 for determination of a NOEL from a LOEL, the estimated no-effect level was 0.12 ug/l (~ 0.01 mg/kg/day). The estimated nasal epithelial NOEL of 0.12 ug/l (~0.01 mg/kg/day) was one of the NOELs used to evaluate the risk of potential human seasonal exposure.

D. CHRONIC TOXICITY/ONCOGENICITY

Dietary - Rat

Two chronic toxicity studies were conducted in rats administered technical cycloate in the diet. In the earlier study, Sprague-Dawley albino rats were fed cycloate (95.3% purity) at measured dosages of 0, 8, 24, or 72 mg/kg/day for 104 weeks (Kundzins, 1979). A dose-related histopathologically-confirmed peripheral neuromyopathy of the sciatic nerve and muscle was present in both sexes at 8, 24 and 72 mg/kg/day. Females were affected more frequently than males. The peripheral neuropathy consisted of one or more of the following: mineralization in the adventitia of small thick-walled vessels, subacute neuritis with multinucleate cells, and cholesterol clefts. Perfusion tissue fixation was not employed, so the true incidence of any spinal cord changes may not have been determined (Stauffer Chemical

Table 3. Incidence of Nasal Cavity Lesions in Rats Exposed to Cycloate for 3 Weeks, or Exposed to Cycloate for 3 Weeks Followed by a 70-Day Recovery Period

	Air Concentration (mg/m^3)[a,b]			
Observation	**0**	**1.2**	**12.0**	**120**
3-Weeks Exposure				
		MALES		
Nasal Cavity	(18)[c]	(18)	(18)	(18)
Rhinitis	0^{+++}	1	2	9***
Goblet Cell Hyperplasia	0^{+++}	1	9***	16***
Respiratory Epithelial Cell Hyperplasia	0^{+++}	1	9***	16***
Transitional Epithelial Cell Hyperplasia	0^{+++}	3	15***	17***
Larynx	(18)	(17)	(18)	(18)
Squamous Epithelial Hyperplasia	0^{+++}	0	4	15***
		FEMALES		
Nasal Cavity	(18)	(18)	(18)	(18)
Rhinitis	0	1	1	3
Goblet Cell Hyperplasia	0^{+++}	5*	10***	15***
Respiratory Epithelial Cell Hyperplasia	0^{+++}	5*	9***	15***
Transitional Epithelial Cell Hyperplasia	0^{+++}	5*	10***	16***
Larynx	(18)	(18)	(18)	(17)
Squamous Epithelial Hyperplasia	0^{+++}	3	8**	14***
3 Weeks Exposure + 70 Days Recovery				
		MALES		
Nasal Cavity	(17)	(18)	(18)	(17)
Goblet Cell Hyperplasia	0^{++}	1	1	5*
Respiratory Epithelial Cell Hyperplasia	0^{++}	1	1	4
		FEMALES		
Nasal Cavity	(18)	(18)	(18)	(18)
Goblet Cell Hyperplasia	0^{++}	4	9***	12***
Respiratory Epithelial Cell Hyperplasia	0^{+}	4	7**	8**

[a] Approximately 0, 0.1, 1,0, or 10 mg/kg/day, whole body exposure. [b] The calculation for conversion of mg/m^3 to mg/kg/day for the study was: (exposure level mg/m^3) x (rat respiration rate of 0.96 m^3/kg/day) x (duration of exposure hrs/24 hrs) x (duration of exposure days/7 days) x (50% respiratory absorption/retention). [c] Number examined in parenthesis. [+] Significant trend at $p \leq 0.05$ based on dose-weighted chi-square trend test (Peto et al., 1980). [++] Significant trend at $p \leq 0.01$ based on dose-weighted chi-square trend test. [+++] Significant trend at $p \leq 0.001$ based in dose-weighted chi-square trend test. * Significantly different from the control group at $p \leq 0.05$ by the Fisher Exact Test. ** Significantly different from the control groups at $p \leq 0.01$ by the Fisher Exact Test. *** Significantly different from the control group at $p \leq 0.001$ by the Fisher Exact Test.

Co., 1986). The myopathy consisted of muscle fiber atrophy, muscle and/or sarcolemmal nuclei hyperplasia, and subacute myositis secondary to the peripheral neuropathy. Brain cholinesterase reductions (85-89% of control) reached statistical significance in the high-dose male group at week 53 and in the high-dose female group at week 53 and termination. A dose-related 4-15% decrease in absolute brain weight was present at all dose levels at week 53, and a 10-15% decrease was present at 24 and 72 mg/kg/day by termination. Increased absolute weight of some organs (e.g., liver and kidneys) was observed although there was no histopathological correlation. The LOEL was 8 mg/kg/day based on the peripheral neuromyopathy and decreased brain weight. A NOEL was not established.

Dietary - Rat

In a second two-year rat study Sprague-Dawley-derived rats (70 rats/sex/group) were fed technical cycloate (98% purity) at 0, 2, 10, 60, or 300 ppm in the diet (~ 0, 0.1, 0.5, 3.0, or 15.0 mg/kg/day; assuming food consumption at 5% of body weight per day (Sprague, 1984). Interim sacrifices were performed at 12 and 18 months, and the study was terminated at 24 months. Tissues from the central and peripheral nervous systems as well as selected skeletal muscles were examined for microscopic changes; however the lumbar spinal cord was not examined. Perfusion tissue fixation was not employed, so the true incidence of any spinal cord changes may not have been determined, as such procedures are necessary in order to reliably detect cycloate-induced neurotoxicity (Stauffer Chemical Co., 1986). In the spinal nerves, statistically significant increases in axonal degeneration were found in the lumbosacral regions at 0-15 months and the cervical-thoracic regions at 16-21 months only in female rats treated at 300 ppm (Table 3). Incidences of lumbosacral axonal atrophy were significantly increased in the 300 ppm group at 16-21 and 22-24 months, at 60 ppm at 22-24 months, and at 300 ppm in the cervical-thoracic nerves at 22-24 months. Although not statistically significant, incidents of axonal atrophy in the lumbosacral regions of the spinal nerves were increased in females exposed to cycloate for 22-24 months at the two lower doses of 2 and 10 ppm.

In the peripheral nerves, statistically significant increases in the axonal degeneration in the lumbosacral region at 16-21 and 22-24 months and in the femoral nerve at 22-24 months were found only in females at 300 ppm (Table 4). Axonal atrophy was found in the 22-24 month females and included significant increases in frequency and severity in both nerves at 300 ppm and in the lumbosacral nerve at 60 ppm. The incidence of axonal atrophy in the lumbosacral region of the peripheral nerves were increased at 22-24 months for females treated at 10 ppm; however, the increase was not statistically significant. Increases in muscular atrophy were statistically significant in both sexes at the 300 ppm level in both the 16-21 and 22-24 month groups. A decrease of 5-10% in absolute brain weight was present at 300 ppm at various timepoints of the study. Increased relative weights for heart, kidney, and occasionally for other organs were also noted at this high dose level. The NOEL for this study was 10 ppm (0.5 mg/kg/day) based on statistical significance (Fisher's Exact test) in the increased incidence of axonal atrophy of the spinal and peripheral nerves in female rats. The higher background incidence of such changes in aging male rats (up to 100%) limited detection of treatment-related change in those tissues. Collectively, the two dietary studies in rats (Kundzins, 1979; Sprague, 1984) fulfilled the requirements under the Federal Insecticide, Fungicide, and Rodenticide Act (FIFRA) Guideline.

Table 4. Incidence of Treatment-Related Axonal Atrophy Observed in Female Rats Fed Cycloate for 2 Years.

	Cycloate Treatment Level (ppm)[a]				
Site of Observation	**0**	**2**	**10**	**60**	**300**
Spinal Nerves	4/29^{+++} (14%)	8/28 (29%)	9/29 (31%)	21/37*** (57%)	33/33*** (100%)
Peripheral Nerves	5/29^{+++} (17%)	4/28 (14%)	9/29 (31%)	18/37*** (49%)	32/33*** (97%)

[a] The treatment level corresponds to ~0, 0.1, 0.5, 3.0, or 15 mg/kg/day, respectively.
*** Significant at p<0.001 based on the Fisher Exact Test performed by DPR.
$^{+++}$ Significant at p<0.001 based on the trend analysis performed by DPR.

Dietary - Mouse

A two-year chronic dietary study was conducted in mice administered technical cycloate (97.2% purity) at 0, 20, 60, or 180 mg/kg/day (Goldenthal, 1979a). The interim examination was performed at 52 weeks for 10/sex/group, and the remainder were examined after 104 weeks of study. Histopathological examination included brain, spinal cord, nerve, and muscle tissues in addition to various other organs and tissues. No compound-related gross pathological observations were seen at 12 months or at the terminal sacrifice. Statistically significant mean organ weight variations were noted in a few organs at 12 months and at the terminal examination. No compound-related gross or microscopic changes were observed. However, perfusion tissue fixation was not employed. Inflammatory, degenerative, proliferative or neoplastic lesions were reported to be of spontaneous nature and unrelated to compound administration. The study was considered unacceptable under the FIFRA Guideline requirements due to the lack of dose justification and diet analyses during the early parts of the study.

Dietary - Mouse

In a repeat carcinogenicity study, cycloate (purity 98.1%) was administered to mice at 0, 300, 1000, or 3000 ppm (corresponding to 0, 38, 127, or 395 mg/kg/day for males and 49, 167, or 515 mg/kg/day for females) in the diet for 18 months (Stonard, 1991). There was a statistically significant increase in the number of inactive ovaries in animals at 3000 ppm and a statistically significant increase in the number of blood filled ovarian cysts in animals at 1000 and 3000 ppm. Reductions in mean cell volume and mean cell hemoglobin levels were seen in both sexes. There was no evidence of oncogenic effects or neuromyopathy, however, perfusion tissue fixation was not employed. The NOEL was 300 ppm (38 mg/kg/day for males and 49 mg/kg/day for females) based on the effects on the hematological changes in red blood cell parameters and ovaries. The study met the FIFRA Guideline requirements.

Gavage - Dog

Cycloate (97.6% purity) was administered by oral intubation in corn oil or as neat material to 5-6 months old beagle dogs, 3/sex/group at 0 or 1,200 mg/kg for 3 months (Kurtz, et al., 1985) and 4/sex/group at 0, 0.5, 50, 200, or 850 mg/kg for 1 year (Kurtz, et al., 1987). Perfusion tissue fixation was employed for the 3-month sacrifice only. Brain, spinal cord, and nerve degeneration, as well as dilation of cerebral ventricles was observed at the end of 3 months at 1,200 mg/kg. Neuromyopathy, dilatation of cerebral ventricles, Wallerian degeneration, and degeneration of spermatogenic cells of the testis were elicited at dosages of 200 and 850 mg/kg/day for one year. A 13-16% decrease in absolute brain weight was present at 200 and 850 mg/kg/day. Toxicity of the liver (hypertrophy, fibrosis, inflammation), kidney (papillopathy, pyelopathy, pyelonephropathy), adrenals (cortical hyperplasia/ hypertrophy), heart (focal myocardial degeneration/atrophy), and inhibition of growth was evident at dosages of 50 mg/kg and higher. The NOEL was 0.5 mg/kg based on the hepatic, renal, adrenal, and myocardial effects. This one-year study in dogs met the FIFRA Guideline requirements.

E. GENOTOXICITY

In tests for genotoxicity, cycloate produced increased mutation frequency, sister chromatid exchange, and chromosome aberrations in mouse lymphoma cytogenetic assays. Studies involving Salmonella typhimurium, bone marrow micronuclei, or human lymphocyte cultures were negative.

Mutagenicity

Cycloate at six concentrations from 0 to 5 ul/plate was assayed (with and without activation) in Salmonella typhimurium, strains TA1535, TA1537, TA1538, TA98, and TA100 for revertants (Litton Bionetics, 1977). No increase in the reversion rate was observed. The study was not acceptable for federal guideline requirements. There was no repeat trial, and only one plate per concentration was used.

An acceptable Ames assay was conducted in Salmonella typhimurium strains TA1535, TA1537, TA98 and TA100 incubated with technical cycloate (purity 97.6%) at 0, 0.012, 0.037, 0.111, 0.333, or 1.0 ul/plate, with or without activation (Majeska, 1985). No increase in the reversion rate was observed.

Technical cycloate (unknown purity) was evaluated for mutagenicity using Saccharomyces, strain D4 (Litton Bionetics, 1977). No increase in the revertants was observed at a concentration of 0 to 5 ul/plate, with or without activation. The study was not acceptable because there was no repeat trial, the test material was not described, and there was only one plate per concentration.

The mutagenicity potential of cycloate was evaluated in a L5178Y mouse lymphoma multiple endpoint test forward mutation assay (Majeska, 1988). Mouse lymphoma L5178Y cells were exposed for 4 hours in duplicate cultures to technical cycloate (97.6% purity) at concentrations between 0.0050 and 0.0600 ul/ml in a single assay without activation and at levels between 0.010 and 0.080 ul/ml in six repeat assays with activation. The expression time was 48 hours or longer. Dose-related increases in mutation frequency were observed with activation. The assay was not acceptable for the federal guideline requirements, only because there was no confirmatory assay in the absence of activation. Since there were repeat assays with activation, the positive findings were considered valid.

Chromosome Aberration

A bone marrow micronucleus assay was used to evaluate the mutagenic potential of cycloate (Majeska, 1985a). Technical cycloate (97.6% purity) was given to CD1 mice by gavage, 5/sex/group, at 0, 1,000, 1,500, or 2,000 mg/kg. Examinations of bone marrow cells were performed at 24, 48, and 72 hours. There was no reported increase in micronuclei. The study was considered acceptable to meet the federal guideline requirements.

A cytogenetic assay using mouse lymphoma multiple endpoint test was used to evaluate the mutagenicity of cycloate (Majeska, 1985b). Mouse lymphoma cells were exposed to technical cycloate (97.6% purity) at 0 to 0.10 ul/ml for four hours, with and without rat liver activation. Without activation, no increase in structural aberrations was observed at any dose. In the presence of an Aroclor 1254 induced rat liver activation system, two trials were done. In the first trial, cycloate was evaluated for 100 cells/dose level over a range of 0.005 to 0.08 ul/ml. There was an apparent dose related increase in structural aberrations at 0.06 and 0.08 ul/ml. In the second trial, cycloate was evaluated over a dose range of 0.03 to 0.10 ul/ml for 50 cells/culture. The results showed a significant increase in both the number and complexity of structural aberrations at 0.06 and 0.10 ul/ml. Numerical aberrations in the form of polyploid cells were also increased. The study was acceptable for meeting the federal guideline requirements.

In an evaluation using an in vitro cytogenetic assay in human lymphocytes, cycloate technical (98.1% purity) was assayed at concentrations of 10, 50, 100 ug/ml in the presence and absence of metabolic activation (Mackay, 1990). Blood was from 1 male and 1 female donor. Treatment time was for 3 hours after cells were incubated for 44 hours from initiation. Cycloate treatments did not induce chromosomal aberrations in human lymphocytes. The study was acceptable to DPR.

DNA Alteration

Mouse lymphoma cells were exposed to 0, 0.005, 0.01, 0.02, 0.04, or 0.06 ul/ml technical cycloate (97.6% purity) for four hours with and without activation (Majeska, 1985b). Forty cells per concentration were evaluated. Increases in sister chromatid exchange were observed at 0.005, 0.01, 0.04, and 0.06 ul/ml in the presence of the Aroclor 1254 induced rat liver activation. The study met the federal guideline requirements.

F. REPRODUCTIVE TOXICITY

Dietary - Rat

A three generation, two litters per generation, reproduction study was conducted using Charles River CD rats (Goldenthal, 1979b). Three lots of technical cycloate (purity of 97.2% and 95.3%) were administered in the diet to 15 male and 30 female rats per test group at measured dosages of 0, 8, 24, or 72 mg/kg/day. Food consumption for females at the 72 mg/kg/day level was consistently lower than the control females throughout the F_2 generation. Body weights of the rats at all generations receiving technical cycloate at 24 and 72 mg/kg/day were significantly lower than the controls. Body weights of pups were significantly lower than the controls at the 72 and 24 mg/kg/day levels. Survival of the pups at the 72 mg/kg/day was significantly lower in at least one measurement period in each litter. Lower pup survival at day 4 of lactation was the most consistently observed variation at this dosage. No compound related gross or microscopic pathologic lesions were seen in any F_2 parental

rats sacrificed at the study termination, although perfusion fixation was not employed. The reproductive NOEL based on the decreased pup survivability was 24 mg/kg/day. The systemic NOEL based on the decrease in the maternal and pup body weight gain was 8 mg/kg/day. The study was considered acceptable according to the FIFRA Guideline requirements.

Dietary - Rat

A two generation, two litters per generation, reproduction study was conducted using Crl:CD(SD)BR VAF/plus rats (Minor, et al., 1990). Cycloate technical (98.1% purity) was administered in the diet to 25 rats/sex/group at 0, 50, 400, or 1000 ppm (~0, 2.5, 20, or 50 mg/kg/day). P0 and P1 adults were examined for microscopic effects on the reproductive organs, nervous system, liver, and kidneys. Body weights of parental males and females were reduced 10-23% at 400 ppm and at 1000 ppm. Perfusion tissue fixation was not employed. Microscopically, mineralization of the thalamic region of the brain was present in P1 males at 400 and 1000 ppm, reaching statistical significance at 1000 ppm. P1 females at 1000 ppm were also affected. The investigators stated that such changes were not typically seen in toxicity studies conducted in their laboratory; however, there were literature reports of similar lesions in aged rats. Dilatation of the ventricles of the brain (generally slight) occurred in male and female P1 animals at 400 ppm and above. Dose-related, statistically significant 2-15% decreases in absolute brain weight were present in P0 females at 50 ppm and above. Degeneration of the white matter of the thoracic spinal cord and/or sacral spinal cord nerve roots was also present in females of both generations at 400 and 1000 ppm. Bile duct hyperplasia in the liver was increased in incidence in both generations of males and females, reaching statistical significance in 1000 ppm P1 males and 400 ppm P1 females. Reproductive effects included smaller litters, 30-40% decreased pup survival, and a 42% decreased pup weight at 1000 ppm. A 30% decrease in pup weight was present at 400 ppm. Based on the decreased body weights, brain mineralization, brain ventricle dilatation, spinal cord degeneration, and bile duct hyperplasia, the parental NOEL was 50 ppm (2.5 mg/kg/day). Based on decreased pup weights, the reproductive NOEL was also 2.5 mg/kg/day. The study was considered acceptable according to the FIFRA Guideline requirements.

G. DEVELOPMENTAL TOXICITY

Gavage - Rat

Technical cycloate (96.8% purity) was administered by gavage to Charles River COBS CD female rats (25/group) at 0 (corn oil), 10, 75, 175, or 400 mg/kg/day on days 6-15 of gestation (WIL Research Lab., 1985). Maternal toxicity was indicated in the 400 mg/kg/day group by increased alopecia, salivation, dried red material around the eyes, nares and mouth, dried brown material around the ventral neck, yellow urogenital matting and significantly reduced body weight, body weight gains, net body weights and food consumption. Increased alopecia in the lateral abdominal and hip area, salivation, dried red material around the eyes and nares, and a 60% decrease in body weight gain during the first 3 days of treatment were also observed at 175 mg/kg/day. Developmental effects were not detected. The maternal NOEL based on the clinical signs and the decrease in body weight gain was 75 mg/kg/day. Based on lack of effects, the developmental NOEL was $\geq$400 mg/kg/day. The study was acceptable for meeting the FIFRA Guideline requirements.

Gavage - Rabbit

Groups of 15 to 18 New Zealand white rabbits were gavaged with technical cycloate (97.6%) at 0 (corn oil), 10, 37.5, or 150 mg/kg/day on days 7-19 of gestation (Wilczynski, 1986). Decreased food consumption and an increased incidence of fetuses with reduced gall bladder size were observed at the highest dosage of 150 mg/kg/day. The registrant indicated that the developmental NOEL was 37.5 mg/kg/day based on the increased incidence of reduced gallbladder size, which was outside of the historical range. The DPR review set the developmental NOEL at $\geq$150 mg/kg/day, since the biological significance of the reduced bladder size was questionable, and it is not an infrequent finding in control rabbits. This study was acceptable in meeting the FIFRA Guideline requirements.

Gavage - Rabbit

Groups of 20 mated New Zealand white rabbits were gavaged with technical cycloate (95%) at 0 (corn oil), 30, 100, or 300 mg/kg/day on days 8-20 of gestation (Horner, 1992). Maternal food consumption and body weight were reduced in the 300 mg/kg/day group. Based on the food consumption and body weight decrease, the maternal NOEL was 100 mg/kg/day. There were no adverse developmental effects. This study was acceptable to DPR under FIFRA Guideline requirements.

Dietary - Mouse

Technical cycloate (97.7% pure) was fed in the diet to mice (20 per group) at 0, 8, or 24 mg/kg, on days 6-18 of gestation (Scott and Benson, 1967). No clinical signs of maternal or teratological changes were observed at any dosage. The NOEL for maternal and developmental toxicity was $\geq$ 24 mg/kg/day. The study was considered unacceptable because the dose selection was not justified. There was no evidence of achieving the maximum tolerated dose. The data on skeletal and visceral findings were inadequate.

H. NEUROTOXICITY

Neurotoxicity is the major concern in the risk assessment of cycloate. Neurotoxic effects have been detected in both rats and dogs, after dosing periods as short as a single dose, and by all exposure routes tested. A listing of the cycloate studies that detected neurotoxic effects is presented in Table 5. Specific neurotoxicity studies are discussed in this section.

Gavage - Hen

A neurotoxicity study was performed in two groups of hens (12 hens/group) given technical cycloate (98.8% purity) at 10,170 mg/kg (2 doses with 21 days between each treatment) or 3,051 mg/kg (5 daily treatments and repeated 21 days later) via gavage (Sprague, 1979). The LD_{50} for hens was greater than 10,170 mg/kg. Toxic signs in the hens included slight unsteadiness, feather loss, markedly pale combs, laying soft-shelled eggs, reduced egg production, loss of body weight, reduced food consumption, and behavioral signs of cholinergic stimulation. Hens treated ten times with cycloate exhibited a slightly higher incidence of neuronal swelling and chromatolysis in the spinal cord. One hen in this treatment group also showed minimal axonal degeneration in the ventral and lateral funiculi of sacrolumbar spinal cord, but was not accompanied by similar changes in the sciatic nerve or in the rest of the central nervous system. The investigators concluded that cycloate did not produce conclusive evidence of acute delayed neurotoxicity since no behavioral or

histopathological evidence of muscle or nerve degeneration was evident in the hens. The study was acceptable according to the FIFRA Guideline requirements.

Gavage - Rat

Technical cycloate (97.6% purity) in corn oil vehicle was administered via single oral gavage to groups of 10 Alpk:APfSD (Wistar derived) rats/dose/sex at 0, 200, 750 or 2000 mg/kg (Rattray, 1993). The animals were then observed for 14 days. At weekly intervals, quantitative assessment of landing foot splay, sensory perception and muscle weakness, and locomotor activity were performed. There were no mortalities. Clinically, cholinergic signs and increased muscle rigidity were present at 750 and 2000 mg/kg. There was no evidence of any effect on the neurobehavior parameters. At the end of the study, 5 rats/sex/dose were sacrificed by perfusion tissue fixation prior to histologic examination of brain, spinal cord, spinal roots, dorsal root ganglia, sciatic nerve, sural nerve, tibial nerve, and gastrocnemius muscle. Tissues from the remaining 5 rat/dose/sex were fixed in formalin, but were not histologically examined. A slight decrease in mean brain weight (4%) was present in 2000 mg/kg females, and considered, by the investigators, to be cycloate-related. Histologically, dose-related neuronal cell necrosis in the pyriform cortex and dentate gyrus of the brain was present at all cycloate levels. Lesions at 200 mg/kg were detected in only one male animal, and were stated to be minimal. No evidence of spinal or peripheral nerve injury was reported. Based on neuronal necrosis of the brain, the study LOEL was 200 mg/kg. Using an uncertainty factor of 10 as a default procedure for determination of a NOEL from a LOEL (Beck et al., 1989), the estimated no-effect level was 20 mg/kg. The study was not acceptable to DPR due to the lack of submission of concurrent or historical positive control data, as required by US EPA neurotoxicity testing guidelines (US EPA, 1991). However, that did not prevent DPR use of the estimated NOEL of 20 mg/kg to calculate the margin of safety for potential acute single-day human exposure to cycloate. The DPR evaluation which indicated the lack of a NOEL for neuronal necrosis is in agreement the US EPA review of the study (US EPA, 1994).

Gavage - Rat

Technical cycloate (97.6% purity) was administered via gavage to groups of 10 female Sprague-Dawley rats (Sprague and Thomassen, 1988; Turner, 1988). For functional and neuropathologic changes, there were three treatment groups, 400 mg/kg/day for 3 days, 220 mg/kg/day for 9 days, or 120 mg/kg/day for 27 days, and three associated corn oil vehicle control groups. Recovery or delayed changes were evaluated in 10 additional rats/group given the same treatments, but held for 28 additional days. It was stated that the highest (400 mg/kg/day) and the lowest (120 mg/kg/day) dose levels were chosen based on incorporation of a 100-fold safety factor from estimates of the maximum exposure associated with open and closed applications of cycloate in field use. To optimize microscopic examination of the brain, spinal cord, and sciatic nerve, whole-body perfusion fixation and plastic embedding of tissue specimens was employed. Clinical signs of toxicity suggestive of cholinesterase inhibition increased in severity with absolute dose, not with the treatment duration of cycloate. A significant increase in the incidence of salivation was observed in rats treated with cycloate at 400 mg/kg/day for 3 days or at 220 mg/kg/day for 9 days. Chromorhinorrhea and stained coat/skin (likely resulting from diarrhea) were seen in rats with 3 day treatment at both 220 and 400 mg/kg/day. A significant increase in the incidence of chromorhinorrhea was also observed in animals treated at 400 mg/kg/day for 1 day. Measurements of the cholinesterase activity did not correlate with the cholinergic signs. Significant inhibition of cholinesterase in red blood cells ($\geq$ 78% of control) and the brain neurotoxic esterase ($\geq$ 86 control) was

Table 5. Summary of Cycloate Neurotoxicity

Species/Sex	Exposure Regimen	Neurotoxicity	NOEL (mg/kg/day)	LOEL (mg/kg/day)	Reference/Comment
Rats (M/F)	Single-dose gavage Neur otox	Brain necrosis, 4% ↓ brain wt.	20[a,b]	200[c]	(Rattray, 1993) Positive control data not submitted.
Rats (M/F)	3-week inhalation (nose only) Neur otox	Spinal cord & nerve degeneration, 7-12% ↓ brain wt., behavior changes	0.02 [a,d]	0.2[c]	(Coombs, 1993) Incomplete histological examination.
Rats (M/F)	3-week inhalation (whole body)	Brain necrosis, 2-7% ↓ brain wt.	0.1	1.0	(Parr-Dobrzanski, 1994)
Rats (M/F)	4-week dermal	2-8% ↓ brain wt.	0.193[a,d]	1.93[c]	(Kinsey, 1991), Neural tissue not histologically examined.
Rats (F)	3,9,27-day gavage	Nerve degeneration (see text)	-	120[c]	(Sprague, 1988)No brain weight
Rats (M/F)	13-week inhalation (whole body)	6-12% ↓ brain wt.	2.5	17	(Knapp, 1984) No Perfusion
Rats (M/F)	13-week inhalation (whole body)	5-8% ↓ brain wt, spinal cord & nerve degeneration, brain ventricle dilation.	1	10	(Knapp, 1986)
Rats (M/F)	13-week diet Neur otox	Brain necrosis, 7-14% ↓ brain wt, behavior changes.	4	40	(Horner, 1993)
Dogs (M/F)	13-week gavage	Brain, spinal cord, nerve degeneration, brain ventricle dilation.	-	1200	(Kurtz, 1985), No brain weight.
Dogs (M/F)	1-year gavage	Brain, spinal cord, nerve degeneration, 13-15% ↓ brain wt, brain ventricle dilation.	50	200	(Kurtz, 1987), No Perfusion.
Rats (M/F)	2-year diet	Nerve degeneration, 4-16% ↓ brain wt.	-	8[c]	(Kunins, 1979)No Perfusion.
Rats (M/F)	2-year diet	Nerve degeneration, 5-10% ↓ brain wt.	0.5[e]	3	(Sprage, 1984)No Perfusion.
Rats (M/F)	Reproduction diet	Spinal cord & nerve degeneration both generations, 2-15% ↓ brain wt, brain mineralization and ventricle dilation.	-	2.5[c]	(Minor, 1990), No Perfusion.

[a] NOEL estimated from LOEL using a default uncertainty factor of 10. [b] Used as the definitive NOEL to estimate potential acute single-day human health hazard. [c] Lowest dose tested, NOEL not established. [d] One of the NOELs used to estimate potential human health hazard from repeated daily (seasonal) exposures. [e] Used as the definitive NOEL to estimate potential chronic dietary human health hazard.

observed in rats treated with cycloate at 220 mg/kg/day for 9 days or at 120 mg/kg/day for 27 days. No significant inhibition in cholinesterase (serum, red blood cells, or brain) or brain neurotoxic esterase activity was shown in rats treated at 400 mg/kg/day for 3 days. Other signs of toxicity in rats treated at 120 mg/kg/day for 27 days included stained coat and reduced grip strength. Hindlimb, but not forelimb, grip strength was stated to be significantly reduced (90% of control), but no affect was apparent 4 weeks after treatment was discontinued. Brain weights were not reported. Microscopically, no significant histologic change was reported for any treated, recovery or control animal. However, in the 120 mg/kg/day group sacrificed at the end of treatment, there was a statistically significant increase in the number of sciatic nerves examined which contained degenerative fibers. The increase was not present in animals examined 4 weeks later. The total percentage of degenerative nerve fibers (~<1%) was so small that the change may not have been biologically significant. Any possible relationship between the reduced hindlimb grip strength and the increased number of sciatic nerves with degenerative fibers is unknown. Based on the clinical observation of cholinergic signs, the NOEL was 120 mg/kg/day for an exposure of 2 to 3 days and 220 mg/kg/day for a one day exposure. The study was considered a supplemental study.

Inhalation - Rat

In a 15 exposure-day inhalation neurotoxicity study, groups of 10 male and 10 female Sprague-Dawley rats were exposed nose-only to air concentrations of 0, 2.3, 20.9, or 208.6 mg/m^3 (~0, 0.2, 1.8, or 17.8 mg/kg/day) for 6 hours/day 5 days/week for 3 consecutive weeks (Coombs, 1993). Five males and 5 females from each group were sacrificed following 3 weeks of exposure; the remaining animals were sacrificed following a 4-week withdrawal period. Whole body tissue perfusion was used for all test animals. A functional observation battery was performed on half the animals at termination of exposure, and on the remaining rats 4 weeks later. At exposure termination, and after withdrawal, there was a slight increase in hindlimb grip strength in all cycloate-dose female groups. One high-dose female lacked a startle response at the withdrawal observation.

There were no mortalities during the study. Reduced body weight gain was present in 1.8 and 17.8 mg/kg/day females. A 6-12% decrease in brain weight was present in females at all cycloate dose levels at 3 weeks, and a 3-6% decrease in 1.8 and 17.8 mg/kg/day females after 4 weeks withdrawal. Relative brain weights did not increase, as would be typically expected in the presence of the decreased body weight (Hayes, 1989; Scharer, 1977), confirming the deleterious effect on the brain. Histologically, slight axonal degeneration in the spinal cord was present in 4 of 5 high-dose males sacrificed at 3 weeks. No degeneration was present in control animals. The axonal degeneration was also present in 4 of 5 high-dose females sacrificed after 4 weeks withdrawal. Degeneration was also present in the sciatic nerve at both time points. The 0.2 and 1.8 mg/kg/day groups were not examined histologically at either time point. The inadequate histological examination precluded determination of a NOEL/LOEL for axonal degeneration of the spinal cord. Based on decreased brain weight the study LOEL was 0.2 mg/kg/day. A NOEL was not established. Using an uncertainty factor of 10 as a default procedure for the determination of a NOEL from a LOEL (Beck et al., 1989), the estimated no-effect level was 0.02 mg/kg/day (20 ug/kg/day). The study was not acceptable to DPR due to the lack of submission of concurrent or historical positive control data, lack of dose justification, and inadequate histological examination of neural tissue. However, that did not prevent DPR use of the estimated NOEL of 0.02 mg/kg to calculate the margin of safety for potential human repeated daily exposure to cycloate during the planting season. The DPR evaluation which indicated the lack of a NOEL for decreased brain weight is in agreement the US EPA review of the study (US EPA, 1994).

Inhalation - Rat

In a more recent 15 exposure-day rat inhalation toxicity study designed to evaluate nasal cavity toxicity and neurotoxicity, groups of 18 Crl:CD(SD)BR rats/sex/dose were exposed to cycloate (98.4%) 6 hr/day, 5 days/week for 3 weeks (Parr-Dobrzanski, 1994). (A review of non-neurotoxic effects present in the study was presented in Section C. Subchronic Toxicity) The chamber concentrations were 0, 1.2, 12, or 120 ug/l (~0, 0.1, 1.0, or 10 mg/kg/day). In addition, a recovery study was performed, in which the same group sizes were exposed to the same concentrations, but the latter groups were taken off treatment 70 days before sacrifice. Rats were evaluated for clinical observations, body weight gain, food consumption, and for histopathological changes in the respiratory tract, central nervous system, and peripheral nervous system. Perfusion tissue fixation was employed. Absolute brain weight was decreased 2-7% at the two highest dose levels. At the 3-week sacrifice, neuronal necrosis in the pyriform cortex of the brain was present at 1 and 10 mg/kg/day. After 70 days without treatment, neuronal necrosis was no longer detected, suggesting that damaged neural tissue had been removed by CNS phagocytic mechanisms. Based on the decreased brain weight and neuronal necrosis, the neurotoxicity NOEL was 1.2 ug/l (~0.1 mg/kg/day).

Diet - Rat

Technical cycloate (97.5% purity) was administered in the diet to groups of 12 Alpk:APfSD rats/sex/dose at levels of 0, 40, 400, or 4000 ppm (~0, 4, 40, or 400 mg/kg/day) for 13 weeks (Horner, 1993). Rats at 40 and 400 mg/kg/day had reduced food consumption and weight gain. Females at 400 mg/kg day also had urinary incontinence, increased response to touch, and upward curvature of the spine. Males at 400 mg/kg/day also had lower landing foot splay values. Histologically, necrosis of neurons in the dentate gyrus of the brain was present in both sexes at 400 mg/kg/day and in females only at 40 mg/kg/day. Females at both 40 and 400 mg/kg day had decreased brain weight and reduced locomotor activity. Based on brain damage, and locomotor activity, the NOEL was 4 mg/kg/day. The study was not acceptable to DPR due to lack of submission on positive control data (historical), and no justification of dose levels.

I. EPIDEMIOLOGICAL SURVEYS/HUMAN STUDIES

An epidemiological survey was conducted to evaluate the prevalence of neurological symptoms and/or illnesses among workers at plants which manufactured (one plant) or formulated (three plants) cycloate (Palshaw, 1985). The survey was based on health insurance claims of 1070 employees from the year cycloate was first manufactured at each location through 1983 and reports of physical examinations of 276 workers examined in 1983. The Time-Weighed-Average (TWA) geometric mean of the air samplings conducted between 1978 and 1983 for two plants ranged from 0.13 mg/m^3 to 0.56 mg/m^3. Among the 276 employees who received physical examinations in 1983, there was one worker diagnosed with a neurological condition, an epilepsy case receiving treatment. Ten of the employees in two plants (one manufacturing and one formulating plant) reported neurological symptoms, such as "persistent numbness, tingling, weakness or paralysis of any part of the body" and "unsteadiness in walking or balance". The study noted that "the occurrences of the symptoms affected a very small percentage of employees, and the examining physicians did not consider them of clinical importance".

Ten of the 1,070 employees included in the survey filed health insurance claims for neurological disorders. Only one employee had a neurological condition, peripheral neuropathy. The employee was reported as known "to have never been assigned to work

with cycloate". It was concluded that "the findings did not suggest that exposure to cycloate had resulted in the development of clinical neurological disorders at the levels existing at the plants".

There are no American Conference of Government Industrial Hygienists or Occupational Safety and Health Administration standards for cycloate. It has been reported that registrant currently maintains its facilities such that employee exposure to active ingredient is below 0.14 mg/m^3 (CPP, 1993).

No adverse effects were reported in a very brief summary of a Russian article on physiological and hematological investigations of workers exposed to cycloate aerosols and vapors at concentrations as high as 6.2 mg/m^3 for 5 consecutive days (Rebrin et al., 1971).

In a study of the pharmacokinetics of cycloate in humans, a single oral dose of ~60-74 ug/kg cycloate (99.8%) in corn oil was given to 6 volunteers (Marsh, 1993). No adverse clinical effects were reported. However, neurological examinations were not performed after dosing. The report stated that no significant changes in biochemical or hematological parameters were present, but details were not provided. Administration of the cycloate did not cause a reduction in plasma or erythrocyte cholinesterase activity in any of the volunteers. (A more detailed review of the study was presented in Section A. Pharmacokinetics/Metabolism.)

IV. RISK ASSESSMENT

The Birth Defect Prevention Act of 1984 (SB 950, Petris) requires DPR to review the toxicological data for all active ingredients currently registered in California. Cycloate was placed on the list for risk assessment by the DPR based on the possible adverse effects identified in the following studies: mutagenicity, reproduction, and chronic toxicity.

A. HAZARD IDENTIFICATION

Acute Toxicity

In the acute neurotoxicity study using rats, a single oral administration of cycloate at 200 mg/kg or greater produced brain damage (neuronal necrosis). As a NOEL was not established in the study, an estimated NOEL of 20 mg/kg was calculated from the LOEL of 200 mg/kg using a default uncertainty factor of 10 (presented in: III. TOXICOLOGY PROFILE, H. NEUROTOXICITY). This estimated NOEL was selected for evaluating potential acute, single-day human exposures. The DPR evaluation which indicated the lack of a NOEL for neuronal necrosis is in agreement the US EPA review of the study (US EPA, 1994).

Subchronic Toxicity

Subchronic toxicity observed in animals exposed to cycloate via the dermal route included decreased brain weight, skin epithelial hyperplasia, acanthosis, hyperkeratosis, desquamation, erythema, or edema. A slight decrease in red blood cell numbers was also present. (Johnston, 1966b; and Kinsey et al., 1991). A topical NOEL for skin lesions from subchronic exposure via the dermal route was calculated to be 0.065 mg/cm^2 based on information from the 21 exposure-day dermal toxicity study in rats (Kinsey et al., 1991).

Subchronic toxicity of cycloate via the inhalation route included brain, spinal cord and nerve degeneration or necrosis, decreased brain weight, inflammation and epithelial hyperplasia/ hypertrophy of the nasal cavity, and alterations in organ weights (Knapp and Thomassen, 1984,1986; Lewis, 1992; Coombs, 1993; and Parr-Dobranski, 1994).

A NOEL of 0.12 mg/m^3 (~0.01 mg/kg/day) was calculated from a LOEL of 1.2 mg/m^3 cycloate air concentration, based on hyperplasia of nasal epithelium in a 15 exposure-day whole-body rat inhalation toxicity study using a default uncertainty factor of 10 (Parr-Dobranski, 1994 presented in: III. TOXICOLOGY PROFILE, C. SUBCHRONIC TOXICITY). This NOEL was used in the assessment of potential toxic nasal effects from direct subchronic exposure to cycloate via the inhalation route.

A NOEL of 0.02 mg/kg/day was calculated from the LOEL of 0.2 mg/kg/day based on brain damage (decreased brain weight) in a 15 exposure-day nose-only inhalation neurotoxicity study (Coombs, 1993) using a default uncertainty factor of 10 (presented in Section H. NEURO- TOXICITY). The DPR evaluation which indicated the lack of a NOEL for decreased brain weight was in agreement the US EPA review of the study (US EPA, 1994). The NOEL from a nose-only rat inhalation toxicity was considered most appropriate for evaluation of a systemic toxic effect (brain damage). Whole-body rat inhalation toxicity tests also involve considerable oral exposure from grooming activity, which would not be mimicked in potential human exposures.

A NOEL of 0.193 mg/kg/day was calculated from the LOEL of 1.93 mg/kg/day based on brain damage (decreased brain weight), in the 21-exposure day dermal toxicity study (Kinsey et al., 1991) using a default uncertainty factor of 10 (presented in Section C. SUBCHRONIC TOXICITY). Histological examination of the nervous system was not undertaken, but that did not prevent evaluation of brain weight change as an indicator of toxicity.

The inhalation and dermal neurotoxicity NOELs were used to evaluate potential combined human inhalation and dermal seasonal occupational exposure (Appendix A).

Chronic Toxicity

Nerve degeneration (neuromyopathy) was the toxicity end-point demonstrating the lowest NOEL from the chronic exposure to cycloate via the oral route in rats. The dose-related neuromyopathy with a NOEL of 0.5 mg/kg/day was observed in two chronic toxicity studies in rats fed technical cycloate in the diet for two years (Kundzins, 1979; Sprague, 1984), although the nervous system was not adequately examined due to the lack of perfusion tissue fixation (Stauffer Chemical Co., 1986). Dogs administered cycloate via gavage for one year exhibited brain and nerve damage, adrenal cortical hyperplasia/hypertrophy, focal myocardial degeneration/atrophy, degeneration of spermatogenic cells, and toxic effects in the liver and kidneys (Kurtz, et al., 1987). The NOEL was 0.5 mg/kg/day based on the hepatic, renal, adrenal, and myocardial effects. Thus a NOEL of 0.5 mg/kg was established from both the dietary studies in rats and the gavage study in dogs. The NOEL of 0.5 mg/kg/day was used to assess the toxic effects from potential human chronic dietary exposure.

Evaluation of the potential chronic exposure for workers considered the exposure frequency during a one year period (days per year)(Appendix A). Exposure days are likely to be grouped together and not spread-out during the year, and there is no evidence that cycloate accumulates in animal or human tissues. Therefore, potential continuous occupational chronic exposure to cycloate throughout the year is not expected to occur. Since potential continuous long-term occupational exposure to cycloate is not expected, the effects seen in long-term continuous-exposure animal experiments may not be relevant to workers. However, as damage to the central nervous system is generally considered to be non-reversible, one possible "long-term" effect of yearly use of cycloate would be any additive effects of potential nervous system toxicity from the yearly short-term exposures. Protection against toxic effects from potential short-term human exposures would also be expected to prevent any additive effects. For this reason, separate MOSs for potential annual average daily occupational exposure to cycloate were not determined.

B. EXPOSURE ASSESSMENT

Dietary Exposure

a. Tolerance

A tolerance is the maximum residue legally allowed for a specific pesticide, its metabolites, or break-down products in or on a particular raw agricultural commodity. The tolerance for cycloate on the raw agricultural commodities of sugar beets (tops and roots) and spinach was set at 0.05 ppm by the U. S. Food and Drug Administration in 1967 (Stauffer Chemical Company, 1967). The current tolerance for sugar beets (tops and roots), garden/table beets (tops and roots), and spinach was also set at 0.05 ppm (negligible residue)

by the U. S. Environmental Protection Agency (EPA, 1989a). The EPA has defined negligible residue as the amount of a pesticide remaining in or on a raw agricultural commodity or group of raw commodities that would result in a daily intake "regarded as toxicologically insignificant on the basis of scientific judgement of adequate safety data" (EPA, 1989b).

b. Anticipated Residues (Primary)

The tolerance level of 0.05 ppm was assumed for the theoretical dietary residue level of cycloate in table beets due to the lack of actual residue data. Residue data from spinach and sugar beets are summarized in Table 6. Residues of cycloate measured in spinach ranged from less than 0.02 ppm [the minimum detection limit (MDL)], to 0.028 ppm (Patchett and Barney, 1980). The calculated average residue level was 0.011 ppm. Residues in sugar beet tops ranged from less than the MDL (0.02 ppm) to 0.035 ppm (Patchett and Barney, 1980; Stauffer Chemical Company, 1976; Hawker and Patchett, 1965; Patchett and Hawker, 1965). The average residue calculated for sugar beets was 0.011 and 0.015 ppm for roots and tops, respectively (Patchett and Barney, 1980; Stauffer Chemical Company, 1976; Hawker and Patchett, 1965; Patchett and Hawker, 1965). Since sugar beet tops are not directly consumed by humans, this commodity was not included in the dietary assessment for human exposure to cycloate.

Cycloate was not detected in 30 samples of sugar beet roots collected in 1965, 1976, and 1980 (Table 6). Two MDLs, either 0.02 ppm or 0.05 ppm, were reported. For those samples having residue levels below the MDL, a mean value was calculated using one-half of the MDL as a default procedure

Table 6. Residues of Cycloate in Spinach and Sugar Beets

Commodity	Study Conditions	Mean Residue ± SD (ppm)	Reference
Spinach (n = 27)	4-12 lbs/acre 55-279 days (PHI)[a]	0.011 ± 0.005 (0.02-0.028)[b]	Patchet, 1980
Sugar Beets			
Roots (n = 30)	4-15 lbs/acre 97-185 Days (PHI)	0.011 ± 0.004 (<0.002-<0.05)	Patchet, 1980; Stauffer, 1976; Hawker & Patchet, 1965; Patchet & Hawker, 1965.
Tops (n = 31)	4-15 lbs/acre 97-185 Days (PHI)	0.0149 ± 0.007 (<0.02-0.035)	Stauffer, 1976; Hawker & Patchet, 1965; Hawker,1965.

[a] Pre-Harvest Interval [b] Range of residues detected.

c. Anticipated Residues (Secondary)

According to the U. S. Environmental Protection Agency (EPA), Subdivision O, sugar beets are used in animal feed in the form of "leaves", molasses, and dried pulp (Schmitt, 1982). Livestock may be fed beet tops from fields which were treated with cycloate. The maximum expected percentage of these feeds in animal diets was 60%, and 20% for beef/dairy cattle and turkeys, respectively. Swine and laying hens consumed sugar beet leaves and products as approximately 10% of their dietary source. Results from metabolism studies in rats, mice, and monkeys indicated that bioaccumulation of cycloate and its metabolites was minimal. Tolerances for meat and animal products (e.g. eggs, milk, etc.) have not been established. Consequently, the potential exposure to cycloate from ingesting meat and animal products was not addressed in this risk assessment.

d. Dietary Exposure Analyses

Dietary exposure analyses were conducted using the Technical Assessment System (TAS) software programs (TAS, 1990a,b). The dietary consumption estimates are based on data from the 1987-88 U. S. Department of Agriculture's (USDA) Nationwide Food Consumption Survey (USDA, 1987-88). The USDA survey was a probability survey of respondents 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.

(1) Acute Exposure

The TAS EXPOSURE 4 is a dietary exposure analysis system which estimates acute (short-term) exposure to constituents in foods comprising the diets of the U.S. population (TAS, 1990a). EXPOSURE 4 computes exposure only for those individuals who are "consumers". A "consumer" is defined as an individual who consumed at least one of the foods containing a residue of the chemical being evaluated. The maximum residues detected, the MDL (for those without detectable residue), or the tolerance level (for those without measuring data), were used for the evaluation of potential health effect from acute dietary exposure to cycloate. Table 7 shows the 95th percentile of the estimated acute exposure dosages of cycloate per user-day from ingesting commodities grown in the treated fields. Infants (< 1 yr) and children (1-12 yrs) had the highest potential acute exposures ranging from 0.060 to 0.078 ug/kg/day. The remaining subgroups had exposures of 0.029 to 0.046 ug/kg/day. The complete dietary exposure analysis is available from DPR Medical Toxicology Branch.

(2) Chronic Exposure

The assessment of chronic dietary exposure to cycloate used the TAS EXPOSURE 1 dietary analysis system (TAS, 1990b). The EXPOSURE 1 utilized the annualized average daily food consumption rates, and the mean residue levels are used as the anticipated residue levels in food. The estimated potential chronic exposure to cycloate from diets is shown in Table 7. Non-nursing infants (< 1 yr) had the highest potential chronic exposure dosage of 0.01 ug/kg/day. Nursing infants (< 1 yr) had the lowest potential chronic exposure dosage of 0.001 ug/kg/day. Other population subgroups had similar potential chronic exposure dosages of 0.003 to 0.006 ug/kg/day.

Table 7. Potential Dietary Exposure to Cycloate

Population Subgroup	Potential Exposure (ug/kg/day)	
	Acute[a]	Chronic[b]
U.S. - Overall	0.044	0.003
Western Region	0.037	0.003
Infants < 1 yr (nursing)	0.068	0.001
Infants < 1 yr (non-nursing)	0.078	0.010
Children (1-6 yrs)	0.078	0.006
Children (7-12 yrs)	0.060	0.005
Males (13-19)	0.046	0.004
Males (20+ yrs)	0.029	0.003
Females (13-19 yrs)	0.037	0.003
Females (20+)	0.029	0.003

[a] Acute exposure uses maximum detected residue levels and represents potential human exposure at the 95th percentile. The complete dietary exposure analysis including other subgroups and other percentiles is available from DPR Medical Toxicology Branch.
[b] Chronic exposure uses annualized mean residue levels

Occupational Exposure (Appendix A)

Application of Ro-Neet 6EC is commonly performed with a ground-boom type spray-rig followed by a tractor pulling a rotary harrow (or disk) for incorporation, or with a combined sprayer/rotary harrow pulled by a single tractor. Two work groups are hence potentially exposed to the herbicide during its application by the spray-rig method. These are tractor drivers and spray-rig applicators, the latter also working as mixer-loaders. In the combined sprayer/rotary harrow method, a single worker (herein simply referred to as the combined driver/applicator) will perform both the application and the incorporation of cycloate. Both application methods are used by commercial applicators, although growers are less likely to use the second combined method working primarily as both a tractor driver and an applicator. The work task with highest exposure potential is mixing and loading, which is expected to account for as much as 90% of the worker exposure in question (Appendix A).

A biological monitoring study was carried out in sugar beet growing fields in Idaho and Oregon during March 1994 (Meier, 1995). In this 1994 study, a total of 17 male workers who mixed, loaded, and applied Ro-Neet were monitored for their inhalation exposure to and systemic absorption of cycloate. For many of these workers, their application also included the immediate incorporation of the herbicide in to soil. The study period of each of the

workers was 4 days, which included a 24-hour baseline (pre-exposure) period followed by one day of exposure and then two further days of urine collection. The subjects were asked to refrain from any potential exposure to cycloate for a minimum of four days prior to application and two days after the exposure. Twenty-four hour urine samples were taken from the workers for each of the four study days. Air samples for inhalation exposure were collected on the day of exposure only. The urine samples were analyzed for the 4-hydroxycycloate, whereas air samples were analyzed for the parent compound cycloate. Absorbed daily doses calculated from those biological monitoring data for cycloate handlers in California are summarized in Table 8.

Table 8. Absorbed Daily Dosages for Workers Handling Cycloate in California, as Calculated from Biological Monitoring Data[a,b].

	Dermal[c]	Inhalation[d]	Total Daily[e] (ADD)	Total Seasonal[f] (SADD)
Range (n = 17)	0.33 - 74.5	0.05 - 12.7	0.53 - 87.0	0.45 - 74.6
Arithmetic Mean (±SD)	14.6 ± 19.2	1.57 ± 3.13	16.1 ± 22.1	13.8 ± 18.9
Geometric[g] Mean (±SD)	6.25 ± 5.77	0.46 ± 4.61	7.16 ± 4.26	6.14 ± 4.26

[a] from the study by Meier, 1985 (Appendix A); the absorbed dosages presented here, all in ug/kg/day, were normalized to an 8-hour work day and to the body weight measured.

[b] workers are best classified as tractor drivers/applicators, since on the day of exposure they were all involved in mixing, loading, applying, and incorporating cycloate in the field; three workers did spend, however, a couple of hours incorporating the herbicide that was already applied by another worker. Many of the workers applied cycloate under closed cab and wore at least normal work clothes and protective equipment as specified on the product label (see Appendix A for further detail).

[c] calculated from the difference between total daily and the dosage calculated for inhalation exposure.

[d] based on a respiration rate of 14 L/min, as common practice for this type of (light) work activity (WH&S Exposure Assessment Group, 1993), and on a default respiratory uptake of 50% (WH&S Exposure Assessment Group, 1993).

[e] total (i.e. accounting for both dermal and inhalation exposure) daily urinary levels were adjusted for incomplete recovery by addition of the level collected on the 3rd day post-exposure (see Appendix A for discussion).

[f] for all workers except growers who work fewer days than do commercial applicators or tractor drivers, the seasonal absorbed daily doses or dosages (SADD) were assumed to be 85.7% (i.e. 6 work per week) of the absorbed daily doses or dosages (ADD); the SADD for a grower was was assumed to be 3/14 of his ADD since the shortest time at which the subacute effects of concern could be seen in animals was 14 days.

[g] due to the adjustment for post day-3 urinary excretion, it is (more) difficult to show statistically whether the total daily urinary levels follow a lognormal distribution. The geometric means are hence presented here primarily for completeness as well as comparison purposes only (Appendix A).

C. RISK CHARACTERIZATION

The assessment of health effects resulting from the potential dietary and/or occupational exposure to cycloate is expressed as the margin of safety (MOS), which is the ratio of the NOEL identified in the most relevant animal study to the estimated dosage for human exposure.

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

For the assessment of nervous system effects (decreased brain weight) from potential seasonal dermal and inhalation occupational exposure to cycloate, a combined MOS approach (Hazard Index) was used. The calculation of a combined MOS is appropriate when potential exposure occurs by more than one route, and the toxicity end point for each route is the same (US EPA, 1989). The combined MOS takes into account the fact that the dose required to produce a specific toxic effect may vary with the exposure route.

$$\text{Margin of Safety (Combined)} = [1/MOS_{inhalation} + 1/MOS_{dermal}]^{-1}$$

Dietary Exposure

a. Acute Dietary Exposure

The highest estimated potential acute dietary exposure to cycloate for various population subgroups having user-days at the 95th percentile was less than 0.1 ug/kg/day (Table 7). This estimate for acute exposure during a single day is approximately five orders of magnitude lower than the NOEL of 20 mg/kg/day (or 20,000 ug/kg/day) for brain damage (neuronal necrosis) observed in animals having one day oral exposure to cycloate, producing an MOS of greater than 168,000.

b. Chronic Dietary Exposure

The estimated potential chronic dietary exposure to cycloate for various population subgroups was less than or equal to 0.01 ug/kg/ day (Table 7). This estimate for chronic dietary exposure is about four orders of magnitude lower than the NOEL of 0.5 mg/kg/day (or 500 ug/kg/day) for effects observed in animals having chronic dietary exposure to cycloate, producing MOS's of 49,000 to 470,000.

Occupational Exposure (Pre-plant use only)

a. Acute Single-Day Exposure

The estimated potential total acute single-day occupational exposure (i.e. the Absorbed Daily Dosage) to cycloate for the workers ranged from 0.53 to 87 ug/kg/day (Table 8). Using the acute single-day NOEL of 20 mg/kg (20,000 ug/kg), based on brain damage (neuronal necrosis) in an acute rat neurotoxicity study, the MOSs for the workers with the highest exposure was 230.

Any additional potential acute exposure to cycloate for agricultural workers from dietary sources would be insignificant compared to the occupational exposure and would not significantly affect the MOS estimates.

b. Seasonal Exposure

Cycloate is a pre-plant use herbicide, so the "season" for its use is limited, and does not extend over the entire growing year. Table 9 presents potential short-term occupational margins of safety for workers involved in the seasonal pre-plant application of cycloate to sugar beets.

For evaluation of potential inhalation effects on the nasal epithelium, a comparison of the estimated NOEL of 10 ug/kg/day from the 15 exposure-day whole body rat inhalation study (Parr-Dobranski, 1994), was made to the potential seasonal inhalation exposure for workers. The range of MOSs was from 1-234. The MOS for the arithmetic mean of exposures was 8, and the MOS at the geometric mean of exposure was 25.

Evaluation of the potential combined exposure for workers from both the dermal and inhalation routes considered the potential effect of brain damage present in a rat 3 week (15 exposure-day) inhalation neurotoxicity study (Coombs, 1993), and a 4-week (21 exposure-day) dermal toxicity study (Kinsey et al., 1991). A Combined MOS (Hazard Index) approach was used to calculate MOSs for the potential combined exposures (US EPA, 1989).

The neurotoxicity NOEL was 193 ug/kg/day in the dermal toxicity study, and 20 ug/kg/day in the inhalation toxicity study. Inhalation neurotoxicity MOSs ranged from 2 to 468. The MOS for the arithmetic mean inhalation exposure was 15, and the MOS at the geometric mean of exposure was 51. Dermal neurotoxicity MOSs ranged from 3 to 685. The MOS for the arithmetic mean of dermal exposure was 15, and the MOS at the geometric mean of exposure was 36.

The MOSs for brain damage following combined dermal and inhalation exposures ranged from 1 to 275. The MOS at the arithmetic mean of the two exposure routes combined was 8, while the MOS at the geometric mean of both exposure routes combined was 21.

Table 9. Neurotoxicity Margins of Safety for Potential Short-Term Occupational Exposure to Cycloate Liquid.

	Dermal Exposure[a]	Inhalation Exposure[b]	Combined Exposure[c]
Range of MOSs	4-685	2-468	1-279
Arithmetic Mean	15	15	8
Geometric Mean[d]	36	50	21

[a] MOS (brain damage following dermal exposure) = NOEL (193 ug/kg/day)/ dermal exposure dosage

[b] MOS (brain damage following inhalation exposure) = NOEL (20 ug/kg/day)/ inhalation exposure dosage

[c] MOS (brain damage following combined dermal and inhalation exposure) = $[1/MOS_{inhalation} + 1/MOS_{dermal}]^{-1}$

[d] Geometric mean presented for comparison purposes only (see Table 8).

b. Chronic Exposure

Evaluation of the potential chronic exposure for workers considered the exposure frequency during a one year period (days per year)(Appendix A). As previously mentioned, exposure days are likely to be grouped together and not spread-out during the year, and there is no evidence that cycloate accumulates in animal or human tissues. Therefore, potential continuous occupational chronic exposure to cycloate throughout the year is not expected to occur. Since potential continuous long-term occupational exposure to cycloate is not expected, the effects seen in long-term continuous-exposure animal experiments may not be relevant to workers. However, as damage to the central nervous system is generally considered to be non-reversible, one possible "long-term" effect of yearly use of cycloate would be any additive effects of potential nervous system toxicity from the yearly short-term exposures. Protection against toxic effects from potential short-term human exposures would also be expected to prevent any additive effects. For this reason, separate MOSs for potential annual average daily occupational exposure to cycloate were not determined.

V. RISK APPRAISAL

Risk assessment is the process used to evaluate the potential for human exposure and the likelihood that the adverse effects of a substance will 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. These, in turn, result in uncertainty in the risk characterization which integrates all the information from the previous three processes. Qualitatively, risk assessments for all chemicals have similar uncertainties. However, the degree or magnitude of the uncertainty can vary depending on the availability and quality of the data, and the types of exposure scenarios being assessed. Specific areas of uncertainty associated with this risk assessment for cycloate are delineated in the following discussion.

A. HAZARD IDENTIFICATION

Interspecies Extrapolation

In the absence of appropriate data from human observations, results from animal studies were extrapolated to humans in the hazard identification process. It was assumed that the absorption, distribution, metabolism, and excretion of cycloate in humans were similar to that in animals. Animals exposed to cycloate showed brain and nerve damage, epithelial hyperplasia/hypertrophy of the nasal cavity, irritation of the skin, slightly decreased red blood cell numbers, adrenal hyperplasia/ hypertrophy, degeneration of spermatic cells, various liver and kidney effects, and myocardial degeneration/atrophy. Similar effects of brain and nerve degeneration, cardiomyopathy, and nasal lesions, were observed in animals administered EPTC (S-ethyl-dipropyl-thiocarbamate), another thiocarbamate herbicide which may be used as a tank-mix with cycloate in several mid-western states (Brammer, 1993; Daly and Knezevich, 1985; Dickie, 1987; Scott, et al., 1985; Sprague and Taylor, 1987; Tisdel, et al., 1984; Warner, et al., 1983; Tisdel, et al., 1986; ICI Americas, 1992). Animals exposed to EPTC via inhalation also showed hyperplasia/degeneration in the nasal epithelium and sinus (Scott, et al., 1985). The epidemiological survey on cycloate conducted by the registrant had major limitations in its study design, data collection, and data analysis/ interpretation (Palshaw, 1985). The negative conclusion by the author of that study could not be used to negate the results from animal studies. The summary of the Russian worker study did not provide sufficient detail for evaluation, and the more recent human pharmacokinetic study did not include post-dose neurological examinations. Therefore, it was assumed that cycloate has the potential to cause similar effects in humans.

Margins of safety calculated for brain damage from potential combined inhalation and dermal repeated-day exposures for workers were less than one in several instances. Such margins of safety indicate potential human exposure dosages equal to or greater than those received by experimental animals. Reports of mental defects in humans after cycloate exposure have not been received by DPR. Only one illness, consisting of upset-stomach and eye irritation, has been reported. This would indicate that humans may not be as sensitive as rats to the neurotoxic effects of cycloate, or that the estimated NOEL was inappropriately low and produced an underestimation of the MOS. An alternate hypothesis would be that

subtle defects in brain function may be difficult to detect without special testing, and years of exposure may be required before the progression of effects would become apparent.

Additional discussion is warranted regarding the lack of reports of brain or nerve dysfunction in humans exposed to cycloate. Obvious neurological problems such as tremors, numbness, tingling, headache, confusion, or blurred vision etc. would be more likely to result in inability to work than would more subtle effects in the nervous system. In California, worker illness reports generally follow worker complaints, medical examinations, or worker compensation claims (DPR, 1990b). Thus, any cycloate-induced neural damage which did not trigger one of those events would be less likely to be reported. Also, pre- and post-exposure examinations would be necessary to detect less obvious effects. This is not routinely done in agricultural situations, and was apparently not part of the registrant-supplied epidemiological survey.

Also of concern is the human pharmacokinetics study which indicated that 2 of 6 volunteers were slow excreters of cycloate (Marsh, 1993). They eliminated cycloate less than one half as fast as the other participants, with detectable metabolite levels still present 60 hours after a single dose. In theory, such a delay could place individuals at greater risk due to prolonged tissue levels of cycloate. This effect would be most pronounced following repeated exposures.

B. EXPOSURE ASSESSMENT

Dietary Exposure

Estimates of potential exposure to cycloate from dietary sources assumed that cycloate is applied to all commodities (spinach, sugar beets, and table beets) allowed for the registered use of this compound. It also assumes that the nature and concentration of cycloate in food does not change after harvesting, during the transportation/storage, or by cooking or food processing techniques. These conservative assumptions could result in an overestimate of the potential exposure. Assuming residue levels at half (50%) of the MDL for those samples of spinach and sugar beet tops having non-detectable residues could overestimate or underestimate the potential exposure. Using the tolerance level as the theoretical residues in or on table beets due to the lack of actual data overestimated the potential exposure from this commodity.

Occupational Exposure

The risk assessment for occupational exposure assumed that workers have a potential exposure to liquid cycloate for up to 14 days/year. The potential risk calculated in this assessment could be an underestimate or overestimate for a worker having an exposure duration/frequency which deviates from this assumption.

C RISK CHARACTERIZATION

In the risk characterization, data from rat dermal, oral, or inhalation studies were used to assess the potential effects derived from the exposure from dermal and inhalation routes for workers. A dermal absorption rate of 19.3%, as determined in experimental animal studies, was assumed to be the same for worker dermal exposure (Appendix A). The use of that assumption would lead to an overestimate of potential health effects if the human absorption rate was less. A pulmonary retention/absorption rate of 50% was used to evaluate the potential effects to laboratory animals and workers via the inhalation route of exposure (Raabe, 1986,1988; Appendix A). The use of such assumptions might lead to an

overestimate or underestimate of the potential health effects, although the MOS calculations would remain the same if the same percentage is used for both animals and humans. The daily exposure to cycloate for chemigation workers was estimated using surrogate exposure data for EPTC, a similar-use thiocarbamate herbicide. The physico-chemical properties and use characteristics of the two chemicals are quite similar, so the estimate was believed to be accurate.

Evaluation of the potential chronic exposure for workers considered the exposure frequency during a one year period (days per year)(Appendix A). Exposure days are likely to be grouped together and not spread-out during the year, and there is no evidence that cycloate accumulates in animal or human tissues. Therefore, potential continuous occupational chronic exposure to cycloate throughout the year is not expected to occur. Since potential continuous long-term occupational exposure to cycloate is not expected, the effects seen in long-term continuous-exposure animal experiments may not be relevant to workers. However, as damage to the central nervous system is generally considered to be non-reversible, one possible "long-term" effect of yearly use of cycloate would be any additive effects of potential nervous system toxicity from the yearly short-term exposures. Protection against toxic effects from potential short-term human exposures would also be expected to prevent any additive effects. For this reason, separate MOSs for potential annual average daily occupational exposure to cycloate were not determined.

Interspecies variability is another aspect that may affect the assessment of risk, most specially for the cycloate-induced epithelial hyperplasia of the nasal cavity. The increased complexity of the nasal turbinates of the rats, as opposed to humans, the straighter nasopharyngeal region, and the regional lower flow rates might alter the toxicity at given exposure concentrations (Schreider, 1986). Additional information on the deposition, reactivity, solubility, absorption, metabolism, and clearance of cycloate in the nasal cavity epithelium of animals and humans would permit additional consideration of interspecies dosimetric adjustment (Jarabeck, et al., 1990: EPA, 1990). Such information would also help define the relative contributions of regional versus systemically absorbed cycloate in production of nasal damage.

Another area of uncertainty is the biological significance of the persistent epithelial hyperplasia present in the nasal cavity. Hyperplasia is characterized by an absolute increase in the number of cells per unit of tissue due to an enhanced rate of cell proliferation. During hyperplasia the proliferating cells may appear less differentiated than their non-proliferating counterparts. This is believed to reflect the fact that the "cellular machinery" is dedicated to cell division rather than to production of gene products for normal function of the cell. In this manner, hyperplasia may alter the capabilities of the organ or tissue affected. Hyperplasia may regress when the stimulus causing it is removed, or the hyperplasia may persist as a cellular change, with organizational or cytological abnormalities, that is less subject to normal tissue regulatory mechanisms (Maronpot, 1991). This may be the case with cycloate, as hyperplasia was still present in nasal cavity of rats 10 weeks after the end of a 3-week inhalation exposure (Parr-Dobrzanski, 1994).

Published reports of inhalation toxicity studies of other chemicals have indicated that nasal cavity hyperplasia may be a preneoplastic change (Boorman et al., 1990: Feron et al., 1986; Haschek et al., 1991; and Sellakumar et al., 1983). It is not possible to predict, from the results of a subchronic study, whether hyperplasia will progress to neoplasia (Boorman et al., 1990). Current FIFRA guidelines state that carcinogenicity studies are required when hyperplasia is produced in a subchronic study (EPA, 1992).

The exposure to cycloate was evaluated for up to 3-14 days per year (Appendix A). The MOS for nasal damage was derived from an estimated NOEL from a 15 exposure-day rat inhalation study. The combined MOS for systemic neurotoxicity was derived from estimated NOELs from the 15 exposure-day rat inhalation neurotoxicity study and a 21-exposure-day rat dermal toxicity study. The resulting evaluation might overestimate the potential human risk if the toxic effect of the hyperplasia/hypertrophy of the nasal epithelium, or the neurotoxicity does not occur after 3-14 days exposure, is completely reversible, and/ or is not cumulative after each years exposure

Brain damage was also detected in numerous cycloate toxicity studies. Regeneration of neurons in the central nervous system is generally considered to be impossible, so potential reversibility of cycloate effects was not a consideration. The apparent lessening of neural effects (decreased brain weight) during the recovery phase of the second 15 exposure-day inhalation neurotoxicity study (Coombs, 1993) was most likely a manifestation of the proliferation of astrocytic cells to fill the space left by the dead neurons (Solleveld et al., 1990) Reversibility was also assessed in the subchronic neurotoxicity study in which rats were dosed for up to 27 days (Sprague and Thompson, 1988; Turner, 1988). A possible degenerative change in peripheral nerve seen at the end of treatment was not detected at the end of the recovery period. The biological significance of that effect was questionable, however

This risk assessment indicates that potential exposure to cycloate for consumers via dietary sources had MOS's of greater than 49,000 for chronic exposures to greater than 168,000 for acute exposures. On the other hand, the MOS's calculated for several work situations were less than 100.

An MOS of 100 is generally considered sufficient for protection of human health when the NOEL is based on results from animal studies, with a comparable duration of exposure for humans. However, for serious, irreversible toxic effects, such as destruction of brain nerve cells, the acceptability of the standard 100-fold margin of safety may be questioned; and can be augmented by additional modifying factors based on scientific judgement (US EPA, 1988b, 1993d). An earlier review of the toxicity of cycloate was conducted for the US EPA by 2 recognized experts in neurotoxicity, Drs. Zoltan Annau of Johns Hopkins University, and Mohamed Abou-Donia of Duke University (US EPA, 1986). Drs. Annau and Abou-Donia agreed that the neurotoxicity of cycloate observed in rat studies was indicative of possibly serious effects on humans exposed to the compound.

An area of interest is the biological significance of the decreased absolute brain weight commonly seen in cycloate toxicity studies (Table 5). Both the US EPA and the World Health Organization have indicated that brain weight is an important aspect of neurotoxicity testing (US EPA, 1991b,1993e; WHO, 1986). It is also important to note that brain weight is relatively independent of decreases in body weight (Hayes, 1989; Scharer, 1977). This means that, even in animal studies with decreased body weights at higher dose levels, decreased brain weight would be considered a primary neurotoxic effect.

The weight of the whole brain is an insensitive indicator of local damage to various brain regions (e.g. hippocampus dentate gyrus) as was evident in the rat acute oral neurotoxicity study (Rattray, 1993). In that study, both decreased total brain weight and neuronal necrosis of several brain regions were observed at the highest dose level, while neuronal necrosis alone was detected at the two lower levels. The weight of various regions of the brain would have provided more useful information. However, regional brain weights were not included in the study protocol. The US EPA currently requires regional brain weight determinations only in developmental neurotoxicity testing (US EPA, 1991b).

Local damage in the brain represents a greater danger than local damage in other organs. This is due to the unique functions of brain regions (speech, memory, control of fine movements, etc.). In this manner, loss of a small percent of total brain cells could have profound effects.

Animal studies have also shown that cycloate causes brain damage at doses below those producing other toxic effects. The brain areas affected have been reported to be involved with learning and memory formation in rats, monkeys, and humans (Tilson et al., 1990; Willams et al., 1975; and Zola-Morgan, 1994). There was an apparent progression of effects, with more severe changes present after longer exposure periods. Also, cycloate, unlike some other neurotoxicants (e.g organophosphate insecticides) does not produce clinical signs of exposure, such as tremors or diarrhea, that would give warning of contamination and subsequent neural effects.

The severity of the toxicity (i.e. brain cell damage) noted in animal studies suggests that a higher MOS could be considered. However, the conservative estimation factors used to determine potential no-effect levels from animal studies (an extra uncertainty factor of 10 to account for lack of a NOEL) had already established an additional safety margin.

An interesting aspect of the neurotoxicity endpoints is the seemingly paradoxical situation of an estimated inhalation subchronic NOEL being lower than the oral chronic NOEL (0.02 mg/kg/day for subchronic vs 3.0 mg/kg day for chronic). An obvious explanation is that cycloate is more toxic via the inhalation route. This was shown in the four most recent neurotoxicity experiments (Horner, 1993; Rattray, 1993; Coombs, 1993, and Parr-Dobranski, 1994), in which the 3-week inhalation NOELs were two orders of magnitude lower than the 13-week oral NOEL. Another explanation is that the older chronic studies were unable to detect more subtle changes in the nervous system. First, perfusion fixation was not undertaken in those studies, as they were conducted before it was known that the procedure is necessary to reliably detect cycloate-induced neural damage (Stauffer Chemical Co., 1986). Second, the chronic studies were of aging rats. Aging rats have an increased background incidence of neural changes which hindered statistical analysis of the increased incidence present in cycloate-treated groups. The incidence of axonal atrophy in the two lower dose groups of the second rat chronic study (Sprague, 1984), were twice that of the control group, but did not reach statistical significance using Fisher's Exact test (Table 4).

Using the argument that the more sensitive shorter-term neurotoxicity studies were a more accurate reflection of cycloate toxicity, the subchronic estimated inhalation NOEL of 0.02 mg/kg/day could have been be used to evaluate potential chronic human dietary exposures. This risk assessment did not use the subchronic NOEL in that manner. Even if that number had been used, the chronic dietary MOSs would have all remained above 2000, and so would not have been the driving factor in determination of potential risk.

Cycloate is not the only thiocarbamate herbicide with adverse neurotoxic effects. Animal studies submitted to DPR and the US EPA (DPR, 1993b; US EPA, 1993d) identify four other members of this class of chemicals as producing similar neurotoxicity (EPTC, pebulate, vernolate, and molinate), and one member lacking this activity (butylate). A recently conducted rat acute oral neurotoxicity study of EPTC reported cell death in the same regions of the brain and at the same dose levels (Brammer, 1993). Although cycloate is considered the prototype of this neurotoxic class, molinate is neurotoxic in more species (four versus two) than any other member of the class, and may be active at lower dosages (US EPA, 1993d).

Margins of safety of less than 100 have been identified for brain, nerve, and other organ damage, for various potential occupational exposures.

A risk evaluation of cycloate for consumer (dietary) and occupational exposures has been submitted by the registrant (McFadden, 1986). The registrant concluded that "there is minimal risk to both applicators and consumers from the use of RO-NEET."

As presented in this Risk Characterization Document, the independent evaluation by DPR draws the conclusion that adverse effects are not expected from exposure to cycloate via dietary sources for consumers. However, DPR concluded that margins of safety less than 100 existed from occupational exposure to cycloate.

VI. PROPOSED RISK MITIGATION

The MOSs calculated for the Seasonal Average Daily Dosage (SADD) were lower than those calculated for acute exposure (ADD) and chronic exposure (AADD). Measures providing satisfactory mitigation for the SADD would also increase the level of protection for acute and chronic exposure. Consequently, this proposed risk mitigation highlights the resulting calculations for the SADD.

In order to reduce the Absorbed Daily Dosage (ADD) and resulting SADD, the recommended mitigation was to require that emulsifiable cycloate concentrate be mixed and loaded in a closed system in addition to wearing and using Personal Protective Equipment (PPE) specified on the current product label (Appendix B). Workers applying emulsifiable cycloate concentrate would sit in an enclosed cab, in addition to wearing and using PPE specified on the current product label. In lieu of using an enclosed cab, they could wear coveralls and a half-face respirator

For comparison purposes, the various seasonal MOSs in question before and after the proposed mitigation are presented in Table 10 below and in Appendix B Table B-2. Also included are the seasonal average daily dosages for driver/applicators calculated for the various routes of exposure.

Table 10. Margins of Safety Critical for Seasonal Exposure of Driver/Applicators Handling Cycloate and Their Seasonal Average Daily Dosage[a, b]

	Dermal Exposure[c]		Inhalation Exposure[d]		Combined Exposure[e]	
	Dosage	**MOS**	**Dosage**	**MOS**	**Dosage**	**MOS**
Under Current Label	12.5	15	1.35	15 (8)	13.8	8
Following Mitigation[f,g]	0.86	224	0.09	222 (111)	0.95	110

[a] The MOS listed here were for decreased brain weight, excepted those presented in parentheses (see footnote d). Dosages and MOSs from Appendix B Table B-2.

[b] The seasonal average daily dosages shown here, in ug/kg/day, are equivalent to 86% (i.e. 6 days per week) of those for single-day exposure presented in Table 1 in Appendix A (i.e., in the human pesticide exposure assessment for cycloate [HS-1556]); combined dosages were calculated by adding the dosages obtained from the corresponding dermal and inhalation exposures.

[c] These MOS were based on the NOEL of 193 ug/kg/day for decreased brain weight following dermal exposure.

[d] These MOS were based on the NOEL of 20 ug/kg/day for decreased brain weight following inhalation exposure; the MOS presented in parentheses are based on the NOEL of 10 ug/kg/day for nasal hyperplasia.

[e] For decreased brain weight based on both dermal and inhalation exposures, the MOS were calculated from the following hazard index approach: $[1/MOS_{inhalation} + 1/MOS_{dermal}]^{-1}$.

[f] Workers would use an effective closed system for mixing/loading, in addition to wearing and using Personal Protective Equipment (PPE) specified on the current product label.

[g] Workers would sit inside an enclosed cab, in addition to wearing and using PPE specified on the current product label; in lieu of using an enclosed cab, they could wear coveralls and a half-face respirator.

VII. CONCLUSION

Dietary Exposure

Estimates of potential dietary exposure to cycloate had MOS's of greater than 49,000 for potential chronic dietary exposure to greater than 168,000 for potential acute dietary exposure. An MOS of at least 100 is generally considered to be protective of human health when the NOEL is based on results from animal studies with a comparable duration of exposure for humans.

Occupational Exposure

Under current use patterns, the MOSs for potential single-day acute exposure to liquid cycloate were 230 or greater. For seasonal occupational exposure the neurotoxicity MOSs were quite variable, ranging from 1-275. The MOSs for the arithmetic and geometric mean of exposure were 8 and 21, respectively. Nasal damage MOSs ranged from 1-234. The MOSs for the arithmetic and geometric mean of exposure were 8 and 25, respectively.

A proposed mitigation involving use of an effective closed system for mixing and loading cycloate emulsifiable concentrate, label-required PPE, and use of an enclosed cab would lower potential exposures considerably producing MOS of greater than 100 for all potential use situations evaluated. In lieu of using an enclosed cab, applicators could wear coveralls and a half-face respirator.

In summary, the potential acute or chronic exposure to cycloate via the dietary sources is not expected to elicit adverse health effects. For potential occupational exposure to cycloate emulsifiable concentrates, some of the resulting neurotoxicity and nasal damage MOSs were less than 100. However, a proposed mitigation of exposure would produce MOS greater than 100. A risk assessment of the granular formulation was not conducted, as it is not registered for use in California.

VIII. TOLERANCE ASSESSMENT

A. BACKGROUND

A tolerance is the maximum, legal amount of a pesticide residue that is allowed on a raw or processed agricultural commodity, or in an animal tissue used for human consumption. The U.S. EPA 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 unapproved commodities). Tolerances are enforced by FDA, USDA, and state enforcement agencies (e.g. Pesticide Enforcement Branch of DPR).

Currently, pesticide tolerances are generally set at levels that are not expected to produce deleterious health effects in humans from chronic dietary exposure. Typical data requirements for establishing a specific tolerance include 1) toxicology data for the parent compound, major metabolism or degradation products and impurities, 2) product chemistry, 3) analytical method(s) that are readily available, accurate and precise, 4) measured residues in crops used for animal feeds, 5) measured residues in animal tissues (e.g. meat, milk, eggs) from direct or indirect (feed) applications, 6) measured residue levels from field studies. The minimum requirements for the field study include: 1) an application rate at or above the highest rate on the product label, 2) the greatest number of allowable repeat applications, 3) the shortest pre-harvest interval listed on the product label. Generally the registrant of a pesticide requests a commodity-specific tolerance, which is equal to the highest measured residue, or some multiple of that value, from the field trial using the specific pesticide.

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 dietary exposure assessment using the residue level equal to the tolerance was conducted for each individual label-approved commodity. The TAS Exposure-4 software program (TAS, 1990a) and the USDA consumption data base (USDA) were used in this assessment. The acute tolerance assessment does not routinely address multiple commodities at the tolerance levels since the probability of consuming multiple commodities at the tolerance decreases as the number of commodities included in the assessment increases.

Cycloate is used as a selective pre-plant herbicide to control weeds in fields of sugar beets, table beets and spinach. Tolerances of 0.05 ppm have been established for sugar beets (roots and tops), garden/table beets (roots and tops), and spinach (EPA, 1991b).

Results

Based on the 95th percentile of the theoretical consumption rate estimate (exposure dosage), the lowest acute MOS of over 22,000 was for non-nursing infants consuming table

beets. All other population subgroups had greater MOS's. All MOS's were greater than 100 and considered sufficient for protection of human health. Margins of safety for other population subgroups and percentiles are on file in the Medical Toxicology Branch of DPR.

C. CHRONIC EXPOSURE

A theoretical chronic exposure assessment using residues equal to the established tolerances for individual or combinations of commodities was not 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 the DPR pesticide monitoring programs which indicate that less than one percent of all sampled commodities have residues at or above the established tolerances (DPR, 1990, 1994).

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IX. APPENDICES

A. HUMAN OCCUPATIONAL EXPOSURE ASSESSMENT

B. MITIGATION PROPOSAL FOR EXPOSURE TO CYCLOATE (RO-NEET™)

HUMAN PESTICIDE EXPOSURE ASSESSMENT

CYCLOATE
(RO-REET® 6EC for Use as Herbicide)

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HS 1556 November 26, 1991; Revised February 20, 1996

ABSTRACT

Cycloate (Ro-Neet®) is a selective thiocarbamate herbicide which has been registered in California for control of weeds in sugar beets, table beets, and spinach for more than a decade. This active ingredient is a toxicity Category III pesticide and is classified as a moderate skin irritant. Only one dermal absorption study for cycloate was reported, in which single doses applied on the rat skin were noted to be greater than field worker exposure. The dermal absorption for 10 h of exposure was shown to be 19.8% in that rat study. Data on comparative metabolism suggest that cycloate is rapidly excreted in mammals following a single dose, and that differences in metabolism are likely to play a significant role in its disposition in many animal species. Spray rig applicators, tractor drivers, driver/applicators, and growers were the four work groups identified among workers handling cycloate in agricultural fields. Exposure data from both a passive dosimetry and a biological monitoring study submitted by the registrant were used to estimate the absorbed daily dosages for these workers in California. Passive dosimetry data are considered to afford comparatively less accurate exposure estimates and were included here for comparison purposes only. The average (arithmetic mean) absorbed daily dosage calculated from the biological monitoring data was 17.1 µg per kilogram of body weight for driver/applicators, who were the only work group monitored in the study. Data from the passive dosimetry study showed that among the four work groups considered, driver/applicators were the ones experiencing the most exposure to cycloate. This technical report was prepared as Appendix A (i.e. Human Pesticide Exposure Assessment) to the Department's risk characterization document for use of cycloate as a selective herbicide. Cycloate is in risk assessment under SB-950 because of its adverse effects observed in animal tests for oncogenicity, teratogenicity, and delayed neuropathy.

APPENDIX A

HUMAN PESTICIDE EXPOSURE ASSESSMENT

CYCLOATE
(Ro-Reet® 6EC for Use as Herbicide)

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Cal/EPA Department of Pesticide Regulation

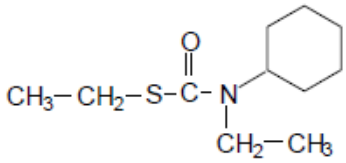
November 26, 1991; Revised February 20, 1996

I. INTRODUCTION

Cycloate is a selective thiocarbamate herbicide which has been registered in California for control of weeds in sugar beets, table beets, and spinach for more than a decade. The assessment of worker exposure for this active ingredient (AI) was first performed by the Worker Health and Safety Branch (WH&S) of the Cal/EPA Department of Pesticide Regulation (DPR) in 1991. At that time the exposure assessment was based on patch dosimetry data from a worker exposure study and on the percutaneous absorption observed in a rat study, both of which were submitted by the registrant. Absorption data from animal studies normally afford less accurate exposure estimates, in that many chemicals tend to have lower skin penetration in humans than in many test animals including rodents. Less accurate estimates could also result from extrapolating the patch residues observed in limited areas to a much greater body surface area, since this approach would magnify any errors inherent in the measurement. In light of these limitations, the registrant recently resubmitted another worker exposure study in which urine contents were monitored on 17 workers handling cycloate in sugar beet growing fields. There is the notion that compared with dosimetry data, urinary levels tend to relate more accurately the internal dose that causes a toxic effect. This present revised document hence contains a reassessment of the worker exposure with data obtained from the biological monitoring study. As with the earlier version, this reassessment is written to be an integral part of the Department's risk characterization document for use of cycloate in California as a selective herbicide under the reregistration request. Results and information contained in this exposure reassessment document again may be used as the starting point for developing mitigation measures for the exposure involved if it is found to cause excessive risk. The toxicological endpoints of main concern for cycloate here are nasal hyperplasia and brain damage, which result primarily from short-term exposure (Meierhenry, 1994).

II. PHYSICAL AND CHEMICAL PROPERTIES

Cycloate (Ro-Neet®, S-ethyl cyclohexylethylthiocarbamate, CAS Registry No. 1134-23-2, molecular weight 215.37, molecular formula $C_{11}H_{21}NOS$) is a selective herbicide used in California to control broadleaf and grassy weeds in sugar beets, table beets, and spinach. This compound is commercially available as a clear, amber liquid. It is a relatively non-reactive substance and forms an emulsion with water. The vapor pressure of cycloate is 830 mPa at 25^{o}C, with a specific gravity of 1.016 at 30^{o}C and a boiling point of 145 - 146^{o}C at 13 mbar (RSC, 1990). Although cycloate has low solubility in water (< 1 g/L), it is miscible with most common organic solvents (e.g. acetone, benzene, kerosene). The following is the chemical structure of cycloate:



III. FORMULATION/INTENDED USE PATTERN

All pesticides containing the cycloate active ingredient are manufactured solely by Zenica (formerly ICI Americas) under the trade name Ro-Neet® . Ro-Neet is labeled as a pre-sowing selective herbicide restricted to being incorporated immediately with soil because of its high volatility. Although the product is available in the 10G (granular) and the 6EC (liquid) formulation, the latter is the only formulation of cycloate that has been registered for use in California. Each gallon of the liquid formulation contains 6 lb of the cycloate active ingredient. The label specifies that a maximum of 4 lb AI be applied per acre of clay (heavy) soil.

IV. U. S. EPA/CALIFORNIA STATUS

Ro-Neet has been federally registered for use to control weeds in sugar beets, table beets, and spinach since July 1967. Throughout the years, the product has undergone several registration amendments to broaden its use. On March 2, 1982, the U. S. Environmental Protection Agency (USEPA) approved additional claims for cycloate that included its impregnation into dry bulk fertilizer for more effective use on sugar beets. The only data call-in notice issued by the USEPA for cycloate was on March 28, 1984. As a result of this issuance, the registrant agreed to conduct and submit a dog chronic feeding study within the 48-month USEPA time frame (i.e., no later than March, 1988). The chronic effects in this dog study were evaluated by the Medical Toxicology Branch in March, 1988 (Luthra, 1988).

V. USAGE IN CALIFORNIA

Ro-Neet is not a restricted pesticide in California. As such, only licensed pest control operators were

required to report its usage prior to 1990. According to the latest annual pesticide use reports (DPR, 1993; 1994; 1995b), 627 applications of cycloate were made in California in 1991, yielding a total of 45,638 lb of the active ingredient used in that year. In 1992, the number of applications was reduced to 525, although the annual usage was increased to 59,368 lb. The 1993 figures (524 and 51,715 lb) were very comparable to those seen in the preceding year. (Later statistics are not yet available to WH&S, as they are currently being compiled and verified.)

VI. LABEL PRECAUTIONS

Ro-Neet is a toxicity Category III pesticide product labeled with the signal word CAUTION. The label requires workers to wear chemical-resistant gloves, long-legged pants, shoes plus socks, and a long-sleeved shirt when handling the herbicide. The statement of practical treatment advises that large amounts of water be given to the victim if he or she accidentally swallows the product. For eye and dermal contacts, the label recommends flushing the affected areas with large quantities of running water for at least 15 minutes. If poisoning is through inhalation, the victim should be immediately removed from the contaminated atmosphere. In all cases, medical attention should be sought as soon as possible.

VII. WORKER ILLNESSES

To this date (through 1993 annual cases reported in 1995 (DPR, 1995a)), there has been only one occupational illness that has been reported by California physicians as directly related to exposure to cycloate (DPR, 1989). This one case occurred in Oxnard, California in October, 1983. The patient (an irrigator) was reported to have been exposed to spray drift during an application of Ro-Neet on spinach (presumably while moving sprinkler irrigation pipes in an adjacent field). The worker later became ill and complained of upset stomach and burning eyes.

In 1983 the registrant also conducted an epidemiologic survey (Palshaw, 1985), in which 1,070 of its employees at three manufacturing and packaging plants were evaluated for neurological disorders related to cycloate exposure. That survey was unable to link any observed (clinical) neurological disorders to exposure to cycloate at those plants.

VIII. ACUTE DERMAL AND RELATED TOXICITY

Two acute rabbit studies on cycloate were submitted by the registrant for review of dermal toxicity and dermal irritation. It was these two studies that provided the basic evidence for classifying Ro-Neet as a moderate skin irritant. The first study was performed in 1965 (Woodard Research Corporation, 1966) in which six groups, each consisting of 10 albino rabbits, were treated with either a solvent control or one of the pre-determined daily doses of cycloate. Severe skin irritation was noted in groups receiving

solvent (kerosene) control or undiluted formulation, but not in those treated with water or water-diluted formulation. The undiluted formulation originally was given to a group of the test animals at the rate of 2.5 ml/kg (i.e., ~ 2,500 mg/kg). However, two rabbits in this group were killed following a single dose at this level. The remaining rabbits hence were taken off the study for five weeks before they were treated again with a lower dose. Dermal LD_{50} for Ro-Neet in rabbits was nonetheless reported elsewhere to be greater than 4,640 mg/kg (RSC 1990, ICI Americas, 1981a).

The second study, which is believed to have been completed in the late 1960s, was formally *reported* by the registrant in 1981 (ICI Americas, 1981b). The test procedure was reported to have complied with the procedures outlined in the USEPA guidelines for pesticide registration in the United States (USEPA, 1978). Primary skin irritation was studied using a patch-test technique on the abraded and intact skin of six New Zealand albino rabbits. Half of a milliliter of technical cycloate was introduced to the animal skin under a one-inch square gauze patch. The patches were secured in place by adhesive tape and wrapped with rubberized damming for a 24-hour period. After 24 h of exposure, the patches were removed, and the resulting reactions were given a score based on the evaluation criteria described by Draize (1959). Remissible erythema or edema was observed in all rabbits treated with the test material.

Another study (ICI Americas, 1981c) submitted for evaluation of acute toxicity also indicated that cycloate was a moderate eye irritant in rabbits. There were no studies submitted for the skin sensitization of cycloate. However, according to an earlier draft of the cycloate risk characterization document (Meierhenry, 1994), the herbicide was considered not to be a skin sensitizer in guinea pigs. There is no sensitization statement specified on the product label.

IX. DERMAL ABSORPTION

A dermal absorption study was submitted in which over 150 Charles River male CD rats were used as test animals (Research Triangle Institute, 1986). For the most part, the study was conducted in accord with the study guidelines for mammalian dermal absorption (Zendzian, 1989), although the doses used in the study were found to be substantially higher than those expected to occur in agricultural workers exposed to cycloate. Rats were divided into groups for dosing at the rates of approximately 0.1, 0.2, 1, and 10 mg/cm^2; this is equivalent to dose levels ranging from 3 to 393 mg per rat. The mean animal weights for the various groups including the controls ranged from 234 to 249 grams.

Dermal absorption of ^{14}C-Ro-Neet 6E (with radiopurity > 99%) was studied in the animals whose backs were treated with either the undiluted formulation or one of the 1:10, 1:50, and 1:100 aqueous dilutions. The undiluted formulation and the various aqueous dilutions were prepared by mixing unlabeled and Ro-Neet with ^{14}C-Ro-Neet. Distilled water was then added to the mixture to yield the required dilution. Studies with control animals and a repeat dose of the 1:50 aqueous dilution with 4 animals were also conducted. Each dose was applied over the 4.9 cm x 6 cm area of the animal's back from which the fur had been removed. Protective, totally-occluding devices were attached to the rats to

isolate the dosed area. Groups of 4 animals were sacrificed at time points of 0.5, 1, 2, 4, 10, and 24 h after dosing. The remaining dose was then washed off the skin immediately before sacrifice (through anesthetization with ketamine/xylazine). Excreta, carcass, the skin in the dosed area, and a blood sample were assayed for radioactivity. The skin wash and protective coverings were radioassayed for the nonabsorbed radiolabel. The recovery of ^{14}C-cycloate from all sources averaged 99.9% of the applied dose.

Absorption was found to be most rapid during the first 0.5 h after dose application, ranging from 8% (including mostly skin-bound ^{14}C) of the applied dose for the highest dose group to 17% for the lowest dose group. The individual average absorption at the final, 24-hour time point ranged from 24% of the dose administered for the 1:100 dilution to 33% for the undiluted Ro-Neet formulation. At 10 h roughly equivalent percentages ranging from 17 to 22% of all dose levels were absorbed, with an overall average absorption rate of 19.8%.

The 19.8% absorption rate in 10 h was used by the registrant to calculate the absorbed daily dosages in their risk evaluation (McFadden, 1986). They presumed that a slight increase in the observed rate might have occurred as a result of *occlusion* of the dose site and, hence, adjusted the aforesaid rate to 19.3% in their exposure calculations. WH&S also used this adjusted rate here in its exposure calculations, under the contention that a further (unnecessary) increase in the observed rate could have occurred as a result of, if any, an acute inflammation of the treatment site that might have been caused by the occulsion. (It is important to note that wherever feasible, WH&S would rather use the dermal absorption rate from a dose that is comparable to field worker exposure.)

X. ANIMAL AND HUMAN METABOLISM

Two studies were provided in which the metabolic fate of cycloate was investigated in three animal species. Experimental designs in both of these studies appeared to have conformed to the USEPA study protocol published then for mammalian metabolism (Arne, 1983). The first animal study was conducted to determine the comparative metabolism and pharmacokinetics of cycloate in male Sprague-Dawley rats and CD mice (Chin, 1983). Animals of each species were administered ^{14}C-Ro-Neet (with radiopurity > 99%) at dose levels of 10 (i.v.), 40 (p.o.), or 160 (p.o.) mg/kg. Urine, feces, expired air, and tissues, as well as serial blood samples, were collected and analyzed for radioactivity. Metabolites of cycloate were then isolated, quantified, and identified in the urine of both species.

No dose-dependent kinetics were observed at the dose levels studied. The disappearance of radioactivity from the plasma of both species was found to be rapid, biphasic, and to follow first-order kinetics. The rate constants (of the terminal phase) for elimination of plasma radioactivity were significantly different between rats and mice; they averaged 0.012 and 0.022 h^{-1}, respectively. These (elimination) rate constants correspond to a half-life of approximately 60 h for rats and 30 h for mice. The shorter half-life observed in mice suggests that this species is more capable of removing cycloate (and its metabolites) from plasma than are rats.

Urinary excretion was found to be the primary route for the elimination of cycloate and its metabolites in both rats (95% of the total applied dose) and mice (65%). In mice, fecal excretion accounted for 25% of the dose administered, whereas in rats it represented only 8%. Biliary excretion appeared to have played a role in both species in the fecal excretion of cycloate and its metabolites. Radioactivity remaining in the tissue of rats and mice at the end of day 8 after dosing was found to be less than 1% of that administered, indicating that the bioaccumulation of cycloate and its metabolites was minimal in both species.

Cycloate was found to be extensively metabolized by rats and mice prior to excretion in the urine. There were 13 metabolites isolated in rat urine (accounting for 93% of the urinary radioactivity) and 12 metabolites isolated in mouse urine (accounting for 72% of the urinary radioactivity). *N*-ethylcyclohexylamine was identified as a major metabolite of cycloate in both rats and mice (42.8% of the dose administered, 50.6% of urinary ^{14}C; species not specified). Cyclohexylamine (a desethyl derivative) was also identified in the rat and mouse urine, although in both species it accounted for only 1% of the urinary radioactivity. Hydroxylation of the cyclohexane moiety of cycloate resulted in the formation of both structural and conformational isomers of the hydroxylated derivatives of cyclohexylamine and of *N*-ethylcyclohexylamine. Cycloate was also metabolized to 1-(*N*-ethylamino)-3,4-cyclohexanediol by both rats and mice. There was evidence showing that this metabolite was formed via the intermediate 1-(*N*-ethylamino)-3, 4-cyclohexene oxide.

The second animal study was conducted to determine the pharmacokinetics and metabolism of cycloate in male cynomolgus monkeys (Chin, 1984). The animals were administered a single dose of the radiolabeled Ro-Neet (with radiopurity > 99%) at levels of 10 (i.v.), 40 (p.o.), or 160 (p.o.) mg/kg. The test procedure followed closely that used for the earlier comparative metabolism study.

The data related to the plasma concentration-time profile likewise indicated that the disappearance of radioactivity was rapid and biphasic and followed first-order kinetics in monkeys. Although the average elimination rate constant varied from dose to dose, no dose-related effects were evident. A plasma half-life for cycloate-derived radioactivity was calculated to be approximately 55 - 65 h.

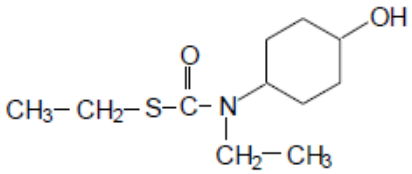
Urinary excretion was again found in this monkey study to be the primary route for the elimination of cycloate and its metabolites. Approximately 88% of the dose administered was seen to have been excreted in the urine at the end of day 8 after dosing. A majority of that eliminated in the monkey urine was seen to have been excreted within the first 24 h (after dosing). The recovery of radioactivity in the urine was consistent at all dose levels. Radioactivity recovered in the tissues at the end of the study was less than 1% of the dose administered.

As in rats and mice, cycloate was extensively metabolized in monkeys prior to urinary excretion. A total of 27 metabolites were isolated from the urine (75% of the urinary radioactivity). No unchanged cycloate was found in the monkey urine receiving a single dose at 160 mg/kg. *N*-ethylcyclohexylamine was again identified as a major metabolite (23.8% of the dose administered, 27.9% of urinary radioactivity).

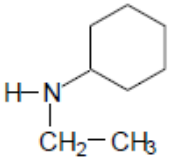
The results from both of these animal metabolism studies support the hypothesis that cycloate is rapidly excreted in mammals following a single (i.v. or oral) dose, and that differences in metabolism are likely to play a significant role in many animal species in the disposition of cycloate.

Three additional metabolism studies (Bratt and Davies, 1991; Chin and Clement, 1990; Lappin and Trivedi, 1991) were reviewed by the Medical Toxicology Branch in 1991 (Leung, 1991). These studies, while all on rats, were submitted as supplements to the above two earlier studies whose data were found by that same branch to be incomplete or less than acceptable (Patterson, 1986). The results of these additional studies, as summarized in the 1991 review, were found very similar to those presented above.

A human metabolism study was submitted more recently, in which 5 mg of cycloate was administered orally to 6 volunteers as a solution in corn oil (Marsh *et al*. 1993). In that human study, no cycloate was detected in any blood samples. Peak urinary excretion of the metabolite 4-hydroxy cycloate (as a conjugate) was seen to occur within the first 2 h and was almost completed by 24 h. The amount of this metabolite excreted in human urine ranged from 31 to 65% of the applied dose equivalent, with a mean of 50 ± 11%. Peak excretion of *N*-ethylcyclohexylamine, which was the major metabolite identified in the animal studies, was seen to occur within the first 24 h. This cyclohexylamine metabolite was detectable in the human urine for up to 120 h post-administration, but accounted for only 8 - 9% of the applied dose. Thus, it was the 4-hydroxy cycloate metabolite that was actually measured in the recently-submitted biological monitoring study whose data were used extensively in the current reassessment. The following are the chemical structures of these two major metabolites:



4-hydroxy cycloate



N-ethylcyclohexylamine

XI. EXPOSURE ASSESSMENT

Application of Ro-Neet 6EC is commonly performed with a ground boom type followed by a tractor pulling a rotary harrow (or disk) for incorporation, or with a combined sprayer/rotary harrow pulled by a single tractor. Two work groups are hence potentially exposed to the herbicide during its application by the spray rig method. These are tractor drivers and spray rig applicators, the latter also working as mixer/loaders. In the combined sprayer/rotary harrow method, a single worker (herein simply referred to as the combined driver/applicator) will perform both the application and the incorporation of cycloate in the field. Both application methods are used by commercial applicators, although growers are likely to use only the second combined method working primarily as both a tractor driver and an applicator. The work task with highest exposure potential in both methods is mixing and loading, which is expected to account for as much as 90% or more of the worker exposure in question (Rutz and Krieger, 1992).

XI.1. Calculated from Dosimetry Data

A worker exposure study (Knarr, 1982) was conducted in 1981, in which the daily dermal and inhalation exposures were monitored on workers handling cycloate in sugar beet fields located in the Magic Valley of central Idaho, at the time when the ambient temperatures were between 30 - 50ºF. Workers wore normal work clothing, as specified on the product label; but many did put on extra clothing such as coveralls. This 1981 study was submitted by the registrant to provide estimates of the daily dermal and inhalation exposures for workers handling cycloate in California. Potential dermal exposures were estimated by the procedure of Durham and Wolfe (1962) using gauze pads taped to the clothing. Inhalation exposure was estimated from air samples collected in the worker's breathing zone. A few of the underlying assumptions (e.g. for respiration rate, for body surface, etc.) used in the registrant's worker exposure study were found to be inconsistent with common practice and hence were modified accordingly in this exposure assessment.

Table 1. Daily Exposures and Absorbed Daily Dosages for Workers Handling Cycloate in California, as Calculated from Dosimetry Data[a].

Job Class	Days Exposed per Year[b]	Exposure (mg/person/day) Dermal[c]	Inhalation[d]	Dosage (µg/kg BW/day) Daily[e]	Seasonal[f]
Applicator	14	79.3	1.561	211.6	181.4
Tractor Driver	14	1.2	0.047	3.4	2.9
Driver/Applicator	14	145.3	0.528	372.4	319.2
Grower	3	145.3	0.528	372.4	79.8

[a] from a worker exposure study submitted by the registrant (Knarr, 1982), in which the applicators were seen to have driven their rig away from any spray that remained airborne (while they were sitting inside a closed cab on their rig that had a 50-foot spray boom mounted 3 to 4 feet above ground level).

[b] ranging from 10 - 18 days for the first three job classes, as provided in the worker exposure study.

[c] based on an 8-hour work day; exposures for the covered areas were calculated using the body surfaces given in the USEPA Pesticide Assessment Guidelines, Subdivision U (USEPA, 1986). The clothing penetration fraction used in the exposure calculation was assumed to be 43% and 29% for spray rig applicators (including tractor drivers) and for driver/applicators (including growers), respectively, as provided in the 1981 worker exposure study.

[d] based on an 8-hour work day and on a respiration rate of 14 L/min for this type of (light) work activity, as common practice within WH&S (Thongsinthusak *et al*., 1993).

[e] based on a dermal absorption of 19.3% (*see* Section IX); on a default respiratory uptake of 50% (Thongsinthusak *et al*., 1993); and on a default average male body weight (BW) of 76 kg, since only male workers were involved in the 1981 worker exposure study.

[f] for all workers except growers who work fewer days than do commercial applicators or tractor drivers, the seasonal absorbed daily doses or dosages (SADD) were assumed to be 85.7% (i.e., 6 work days per week) of the absorbed daily doses or dosages (ADD); the SADD for a grower was assumed to be 3/14 of his ADD since the shortest time at which the subacute effects of concern could be seen in animals was 14 days.

Daily exposures and absorbed daily dosages calculated from the dosimetry data for cycloate handlers are summarized in Table 1. As shown in this table, driver/applicators appeared to have experienced the largest absorbed dosage. There are limitations inherent in the use of dosimetry data for worker exposure assessment; this point was further discussed below in the Exposure Appraisal subsection. With this realization, the registrant recently resubmitted a biological monitoring study (Meier, 1995) in an effort to provide more accurate estimates of the daily exposure in question. That resubmission hence led to the current reassessment of worker exposure for cycloate, as presented below.

XI.2. Calculated from Biological Monitoring Data

The biological monitoring study was carried out in sugar beet growing fields in Idaho and Oregon during March, 1994 (Meier, 1995). In this 1994 study, a total of 17 male workers who mixed, loaded, and applied Ro-Neet were monitored for their inhalation exposure to and systemic absorption of cycloate. For many of these workers, their application also included the immediate incorporation of the herbicide into soil. The study period for each of these workers was 4 days, which included a 24-hour baseline (pre-exposure) period followed by one day of exposure and then two further days of urine collection. These subjects were asked to refrain from any potential exposure to cycloate for a minimum of 4 days prior to application and 2 days after the day of exposure. Twenty-four hour urine samples were taken from the workers for each of the four study days. Air samples for inhalation exposure were collected on the day of exposure only. The urine samples were analyzed for the 4-hydroxy cycloate (*versus* the *N*-ethylcyclohexylamine) metabolite, for reasons as stated in Section X, whereas air samples were analyzed for the parent compound cycloate. Absorbed daily dosages calculated from these biological monitoring data for cycloate handlers working in California are summarized in Table 2 below.

As shown in Table 2, the *average* total daily absorbed dosage calculated from the biological monitoring data was 17.1 µg per kilogram of body weight for driver/applicators handling cycloate in California. This (arithmetic) mean estimate is approximately 22 times less than that calculated from the dosimetry data. It was stated earlier (*see* Section I) that dosimetry data normally afford less accurate estimates of exposure than do biological monitoring data. Exposure estimates calculated from the dosimetry data were nonetheless included here for comparison purposes only.

As footnoted in Table 2, the workers in the biological monitoring study are best classified as tractor driver/applicators, since on the day of exposure they were all involved in mixing, loading, applying, and incorporating cycloate in the field. Like those in the 1981 study, the workers in this 1994 study all had prior experience in pesticide applications to sugar beets. Many of them wore at least label-specified normal work clothing which included gloves, pants, socks plus shoes, and a long-sleeved shirt. Five subjects did not wear any gloves, however. There were also two subjects who wore only a short-sleeved shirt but with a jacket on. The spray solutions were prepared by dilution of the required quantity of product with water (typically via a nurse truck). The workers either poured the formulated product directly into the spray tank, or measured the desired amount in a calibrated container prior to transferring the product to the spray tank. Application was made with typical commercial ground boom equipment and, for the majority (11 out of 17) of the cases, under a closed cab.

Table 2. Absorbed Daily Dosages for Workers Handling Cycloate in California, as Calculated from Biological Monitoring Data[a,b].

	Dermal[c]	Inhalation[d]	Total Daily[e]	Total Seasonal[f]
Range (n = 17)	0.33 – 77.3	0.04 – 12.5	0.53 – 89.8	0.45 – 77.0
Arithmetic Mean	15.5 ± 20.0	1.57 ± 3.13	17.1 ± 22.8	14.6 ± 19.6
Geometric Mean	6.69 ± 4.75	0.46 ± 4.62	7.62 ± 4.32	6.53 ± 4.32

[a] from the study by Meier (1995); the absorbed dosages presented here, all in µg/kg BW/day, were normalized to an 8-hour work day and to the body weight (BW) measured.

[b] workers are best classified as tractor driver/applicators, since on the day of exposure they were all involved in mixing, loading, applying, and incorporating cycloate in the field; three workers did spend, however, a couple of hours incorporating the herbicide that was already applied by another worker. Many of these workers applied cycloate under a closed cab and wore at least normal work clothes and protective equipment as specified on the product label (*see* text for further detail).

[c] calculated from the difference between total daily and the dosage calculated for inhalation exposure.

[d] based on a respiration rate of 14 L/min, as common practice for this type of (light) work activity (Thongsinthusak *et al*., 1993), and on a default respiratory uptake of 50% (Thongsinthusak *et al*., 1993).

[e] total (i.e. accounting for both dermal and inhalation exposure) daily urinary levels were adjusted for incomplete recovery by addition of the level collected on the 3rd day post-exposure (*see* text for discussion).

[f] seasonal daily dosage and seasonal period are as defined in footnote *f* in Table 1.

It is also footnoted in Table 2 that the total daily urinary levels presented here were adjusted for incomplete recovery of cycloate. This adjustment was made because the biomonitoring data showed that more excretion could take place *after* day 3 post-exposure. For 13 of the 17 volunteers, their urinary excretion of cycloate on day 3 (i.e., on the last day of sample collection) was found to be at least 50% of that observed on day 2, thus suggesting that more urinary excretion of the chemical or its metabolite could take place after day 3. As further footnoted in Table 3, the adjustment for incomplete recovery was made by adding the amount excreted on day 3 to the amount totaled over the first three days post-exposure. This amount was added to the total because in the absence of any evidence to the contrary, it was expected that the amount excreted on day 4 would also be at (the rate of) ≥ 50% of the amount excreted on day 3 and that the amount excreted on day 5 would be at ≥ 50% of the amount excreted on day 4 and so forth. Because of this adjustment, it is (more) difficult to show statistically whether the total daily urinary levels as calculated follow a lognormal distribution. The geometric means are hence presented in Table 2 primarily for completeness as well as for comparison purposes only. The dosages for inhalation exposure were calculated, however, primarily from the air samples. A statistical (normality) test showed that these inhalation dosages followed a lognormal rather than a normal distribution, thus supporting the use of the geometric mean for dosages from this respiratory route.

Table 3. Statistical Summary of Daily Urinary Excretion of 4-Hydroxy Cycloate Observed in the Biological Monitoring Study (Meier, 1995)[a].

	Day 1	Day 2	Day 3	Total[b]
Range (n = 17)	0.07 – 32.3	0.07 – 4.55	0.04 – 2.81	0.07 – 42.4
Arithmetic Mean	4.15 ± 7.85	1.33 ± 1.41	0.95 ± 0.84	6.94 ± 10.3
Geometric Mean	1.28 ± 4.45	0.74 ± 3.16	0.62 ± 4.98	2.66 ± 5.36

[a] the daily urinary output presented here, all in µg/kg BW, were normalized to the body weight (BW) measured; the following multiplier M was used in the biological monitoring study to convert the 4-hydroxy cycloate dosages to (the parent compound) cycloate equivalents: $M = (MW_1) \times (MW_2)^{-1} \times (R)^{-1}$, where MW_1 = molecular weight of cycloate (215), MW_2 = molecular weight of the major metabolite 4-hydroxy cycloate (231), and R = 50% = average % of the applied dose recovered as 4-hydroxy cycloate in the study up to 72 h post-dosing (*see* also Dong, 1996).

[b] to adjust for incomplete urinary excretion (*see* text for rationale and further discussion), the total urinary excretion of 4-hydroxy cycloate for each of the 17 volunteers was calculated by adding the amount excreted on day 3 to the amount accumulated during the three days post-exposure; note that day 1 in the biological monitoring study was referred to as the baseline (pre-exposure), rather than the exposure, period.

The absorbed dosages given in Table 2, all in µg/kg BW/day, were normalized to an 8-hour work day and to the body weight (BW) measured. Table 4 below summarizes the body weights measured on the 17 workers (in the biological monitoring study) and the number of hours for which these individuals worked with cycloate on the exposure day. The body weights of the 17 workers ranged from 55.8 to 109 kg, with an arithmetic mean of 91.7 ± 16.7 kg. The number of hours they each worked with cycloate ranged from 1.4 to 11 h, with an arithmetic mean of 5.6 ± 2.2 h. Table 3 also lists the ranges and the arithmetic means for two other related exposure factors, the number of acres treated and the amount of the cycloate active ingredient handled by these 17 workers. These two other factors were not used to normalize the absorbed dosages in this exposure assessment simply because they each alone would not be sufficient to account for the actual worker exposure to cycloate. The use of the total acres treated by the workers would not include the time that they spent for mixing/loading. The study document on the other hand reported only the amount of cycloate that each worker handled during mixing/loading, and not also during application or incorporation. Yet there were several workers who applied a tank of spray solution that was mixed/loaded earlier by another worker, in addition to those prepared by themselves.

X1.3 Exposure Appraisal

In using the absorbed dosages calculated in this exposure assessment, it is important to note that there was a high degree of conservatism (i.e., tendency to overestimate exposure) built into the process that might not be immediately apparent to the risk assessor or the risk manager. Such conservatism is very real, but typically hidden and therefore seldom acknowledged. Below is a brief account of the conservatism inherent in the most important factors used that are likely to overestimate the exposures calculated with the biomonitoring and, more so, with the passive dosimetry data. In fact, a couple of

these factors can be readily used to account for the 22-fold or so difference between the absorbed dosages calculated from the two sets of exposure data.

Table 4. Statistical Summary of Related Exposure Factors in the Biological Monitoring Study (Meier, 1995).

	Body Weight[a]	Hours Worked[b]	Cycloate Handled[c]	Acres Treated[d]
Range (n = 17)	55.8 – 109	1.43 – 11.0	7.5 – 225	10.0 – 79.5
Arithmetic Mean	91.7 ± 16.7	5.55 ± 2.16	55.8 ± 58.5	25.4 ± 17.1

[a] in kilograms, with ages ranging from 21 to 53.

[b] on the exposure day as mixers, loaders, applicators, and/or incorporators.

[c] amount of the active ingredient (AI) in pounds prepared by workers on the exposure day.

[d] with spray tank capacity ranging from 100 to 200 gallons at a target rate of 3 lb AI per acre of preplant; the actual application rates ranged from 1.7 to 3.2 lb AI per acre.

A) Dermal versus Oral Plasma Levels. Dosage is expressed as a single *static* value both in worker exposure and animal toxicology studies. The rate of dermal absorption is always lower than the rate of oral absorption in animals used for toxicology testing. Adverse effects occur only when plasma levels in the target organ exceed a critical level; yet dermal acquisition takes place over the entire work day. Since dermal absorption is slower than oral absorption, plasma levels for the same total absorbed dosage thus will not be nearly as high from a dermal versus oral exposure. In other words, a dermal dose acquired over the entire work day produces peak plasma levels much lower than the bolus oral feeding dosage acquired by animals in seconds to minutes. Because the effect used for risk assessment is highly dependent on plasma level, treating an 8-hour dermal acquisition as though it were a bolus (i.e., summing the entire dermal dose) is so conservative that it outweighs any perceived source of dose underestimation. The net effect of assuming both instantaneous dermal dose acquisition and absorption is an overestimate of peak plasma level by several fold for the same absorbed dose when compared to the oral route (*see*, e.g., Auton *et al*., 1993). It is also important to note that the lower the dose, the more pronounced this difference becomes. This difference is particularly pertinent when comparing the doses used in a toxicology study versus those to which a human would be exposed. Lower urinary metabolite concentrations (i.e., an indication of lower peak plasma concentrations) have been seen with dermally applied pesticides when compared with the urinary metabolite concentration observed following oral dosing (Krieger *et al*., 1991).

B) Partial vs. Full Workday Exposure Monitoring. Another source of dose overestimation comes from monitoring worker exposure for less than a full day's work. There is evidence (Spencer *et al*. 1995) showing that if an estimate of full day exposure were extrapolated from 1/3 day (four bins picked), the exposure would be overestimated by more than 50 - 80% and from 1/2 day (six bins picked), 20 - 40%. Shorter monitoring periods are encouraged because it allows an investigator to

obtain two or more replicates per individual per day of monitoring. There is evidence that hand residues remain virtually constant after exposure for the first couple of hours, indicating that they rapidly come into equilibrium with their environment. Thus summing hand washes taken throughout the day grossly overestimates actual dose. This same principle is operative for studies involving exposure to pesticide handler. One important factor producing high exposure estimates for handlers is the tendency of passive dosimetry to overestimate dermal dose (Maddy *et al*., 1989; Spencer *et al*., 1995). Another factor related more to partial day monitoring is the influence of serial hand washes and other "incomplete or fragmented" patch data (the same factor operating in reentry worker exposure) that often take place in a passive dosimetry study, such as in the 1981 worker exposure study (Knarr, 1982), from which the data were used to calculate the absorbed dosages presented in Table 1.

C) Dermal Absorption: Animal > Human. Skin is the primary route of worker exposure (Wolfe, 1976) accounting on average for up to 99% or more of the potential pesticide exposure for pesticide handlers. One additional significant factor that contributes to overestimation of dermal exposure is the difference between animal and human dermal absorption. As in the case for cycloate, the rat is the most commonly used model to estimate dermal absorption. This is because rats are relatively inexpensive and most of the toxicology is done with them. Many pesticide registrants also have an aversion to using humans for the determination of dermal absorption, even though they are the species for which risk assessment is intended. At any rate, dermal absorption observed in rats or in many other animal species tends to overestimate human dermal absorption by two- to ten-fold. Such a great disparity in dermal absorption between humans and other animal species has been demonstrated in approximately a dozen different compounds tested (Feldmann and Maibach, 1974; Sanborn, 1994; Shah and Guthrie, 1983; Thongsinthusak,1994; Wester and Maibach, 1977; Wester and Maibach, 1993).

D) Temporal Effect: Animal > Human. The aforementioned great disparity in dermal absorption seen between humans and other animal species is due at least in part to a phenomenon often referred to as, for lack of a better term, *temporal effect*. That is, the cause of this disparity is more than physiological in nature. It has to do with a temporal artifact whose effect is seldom acknowledged or accounted for in the risk assessment process. For example, when rats are exposed for 10 *human* hours prior to their being sacrificed for determination of dermal absorption, in reality it means that the animals are dosed for the equivalent of more than 350 h of *their own* life time (which has the equivalent of a little over *two* human years of human life). By the same token, an adverse health effect seen in, say, a 21-day rat inhalation study should be considered more correctly as that effect which would occur in humans only if a person were exposed to the same absorbed daily dosage (after normalization for body weight) *for a period of two or more human years* in his or her life time. Otherwise, it would be a fallacy to assert, as in an oncogenicity study, that dosing the rat daily to a chemical for two human years is like exposing a human to the same for *life*.

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