Public Health Goal for
ALACHLOR
in Drinking Water

Prepared by

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

December 1997

LIST OF CONTRIBUTORS

PHG PROJECT MANAGEMENT

Project Officer
Anna Fan, Ph.D.

Chemical Prioritization Report Outline
Joseph Brown, Ph.D.
Coordinator
David Morry, Ph.D.
Yi Wang, Ph.D.

Document Development
Michael DiBartolomeis, Ph.D.
Coordinator
George Alexeeff, Ph.D.
Hanafi Russell, M.S.
Yi Wang, Ph.D.

Public Workshop
Michael DiBartolomeis, Ph.D.
Coordinator
Judy Polakoff, M.S.
Organizer

Methodology/Approaches/Review Comments
Joseph Brown, Ph.D.
Robert Howd, Ph.D.
Coordinators
Lubow Jowa, Ph.D.
David Morry, Ph.D.
Rajpal Tomar, Ph.D.
Yi Wang, Ph.D.

REPORT PREPARATION

Author
Jolanta Bankowska, Ph.D.

Primary Reviewer
John Faust, Ph.D.

Secondary Reviewer
Michael DiBartolomeis, Ph.D.

Final Reviewers
Anna Fan, Ph.D.
William Vance, Ph.D.

Editor
Michael DiBartolomeis, Ph.D.

SUPPORT

Administrative Support
Edna Hernandez
Coordinator
Laurie Bliss
Sharon Davis
Kathy Elliott
Vickie Grayson
Michelle Johnson
Juliet Rafol
Genevieve Shafer
Tonya Turner

Library Support
Mary Ann Mahoney
Valerie Walter

Website Posting
Robert Brodberg, Ph.D.
Edna Hernandez
Laurie Monserrat, M.S.
Judy Polakoff, M.S.
Hanafi Russell, M.S.

We thank the U.S. EPA's Office of Water, Office of Pollution Prevention and Toxic Substances, and National Center for Environmental Assessment for their peer review of the PHG documents, and the comments received from all interested parties.

PREFACE

Drinking Water Public Health Goal of the Office of Environmental Health Hazard Assessment

This Public Health Goal (PHG) technical support document provides information on health effects from contaminants in drinking water. The PHG describes concentrations of contaminants at which adverse health effects would not be expected to occur, even over a lifetime of exposure. PHGs are developed for chemical contaminants based on the best available toxicological data in the scientific literature. These documents and the analyses contained in them provide estimates of the levels of contaminants in drinking water that would pose no significant health risk to individuals consuming the water on a daily basis over a lifetime.

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

1. PHGs for acutely toxic substances shall be set at levels at which scientific evidence indicates that no known or anticipated adverse effects on health will occur, plus an adequate margin-of-safety.
2. PHGs for carcinogens or other substances which can cause chronic disease shall be based solely on health effects without regard to cost impacts and shall be set at levels which OEHHA has determined do not pose any significant risk to health.
3. To the extent the information is available, OEHHA shall consider possible synergistic effects resulting from exposure to two or more contaminants.
4. OEHHA shall consider the existence of groups in the population that are more susceptible to adverse effects of the contaminants than a normal healthy adult.
5. OEHHA shall consider the contaminant exposure and body burden levels that alter physiological function or structure in a manner that may significantly increase the risk of illness.
6. In cases of scientific ambiguity, OEHHA shall use criteria most protective of public health and shall incorporate uncertainty factors of noncarcinogenic substances for which scientific research indicates a safe dose-response threshold.
7. In cases where scientific evidence demonstrates that a safe dose-response threshold for a contaminant exists, then the PHG should be set at that threshold.
8. The PHG may be set at zero if necessary to satisfy the requirements listed above.
9. OEHHA shall consider exposure to contaminants in media other than drinking water, including food and air and the resulting body burden.
10. PHGs adopted by OEHHA shall be reviewed periodically and revised as necessary based on the availability of new scientific data.

PHGs adopted by OEHHA are for use by the California Department of Health Services (DHS) in establishing primary drinking water standards (State Maximum Contaminant Levels, or MCLs). Whereas PHGs are to be based solely on scientific and public health considerations without regard to economic cost considerations, drinking water standards adopted by DHS are to consider economic factors and technical feasibility. For this reason PHGs are only one part of the

information used by DHS for establishing drinking water standards. PHGs established by OEHHA exert no regulatory burden and represent only non-mandatory goals. By federal law, MCLs established by DHS must be at least as stringent as the federal MCL if one exists.

PHG documents are developed for technical assistance to DHS, but may also benefit federal, state and local public health officials. While the PHGs are calculated for single chemicals only, they may, if the information is available, address hazards associated with the interactions of contaminants in mixtures. Further, PHGs are derived for drinking water only and are not to be utilized as target levels for the contamination of environmental waters where additional concerns of bioaccumulation in fish and shellfish may pertain. Often environmental water contaminant criteria are more stringent than drinking water PHGs, to account for human exposures to a single chemical in multiple environmental media and from bioconcentration by plants and animals in the food chain.

TABLE OF CONTENTS

SUMMARY

A Public Health Goal (PHG) of 0.004 mg/L (4 ppb) is developed for alachlor in drinking water. Review of the recent literature for alachlor did not identify any new studies that would be more appropriate for deriving a PHG for alachlor than studies used previously by the U.S. Environmental Protection Agency (U.S. EPA) to develop a Maximum Contaminant Level Goal (MCLG) and a Maximum Contaminant Level (MCL) of 0 ppb and 2 ppb, respectively. The basis for these levels is the carcinogenic potential of alachlor. Recent reevaluation of carcinogenic potential of alachlor supports a non-linear approach to risk assessment. Therefore, the Office of Environmental Health Hazard Assessment (OEHHA) determined that 0.5 mg/kg-day (no tumor response level for the nasal turbinate tumors), the lowest level among all no-observed-response-levels for adverse effects observed in animal studies, is the point of departure for calculating a PHG using the non-linear approach for carcinogenic risk assessment. Based on these data and methods, OEHHA calculates a PHG of 4 ppb for alachlor in drinking water.

INTRODUCTION

The purpose of this document is to develop a PHG for alachlor in drinking water and examine the impact of the currently available data on alachlor's health risk assessment. Alachlor (2-chloro-2', 6'-diethyl-N-methoxymethyl acetanilide) is a pre-emergence herbicide widely used to control many broadleaf weeds and annual grasses. Most of alachlor use is on corn, soybeans and peanuts. Alachlor is marketed under the trade names Alanex, CP 50144, Lasso, and Lazo.

HUMAN EXPOSURE

Human exposure to alachlor may result from activities involving alachlor application, dietary exposure through treated commodities, and through alachlor residues in ground or surface water. The first two sources are not part of this evaluation, although the magnitude of dietary exposure could have an impact on the PHG estimate if adequate data exist to determine exposures from diet and from drinking water. In the absence of such data, exposure from alachlor from drinking water (occupational exposure is not taken into account) would be assumed to constitute 20% of the total exposure to the chemical.

When soil and climatic conditions are favorable, alachlor may contaminate drinking water by runoff into surface water or by leaching into ground water. This can occur following normal agricultural use of alachlor or due to contamination such as spills or runoffs. The available data indicates that alachlor residues in ground water attributable to leaching after normal use are rarely higher than 10 ppb and typically fall in the range of 0.2 to 2 ppb (U.S. EPA, 1987a).

METABOLISM

Production of nasal turbinate tumors in rats by alachlor is one of the most significant adverse effects caused by this chemical. U.S. EPA (1997) recently reported on the production of tumors in nasal mucosa. The Agency hypothesized that alachlor is metabolized in the rat to the glutathione conjugate, which is excreted through the bile into the gastrointestinal tract. In the gastrointestinal tract, enteric bacteria metabolize the conjugate to the thiol conjugate, with subsequent S-methylation of the thiol group. This product, the methyl sulfide, is reabsorbed into systemic circulation where conversion to the secondary sulfide occurs. Hydrolysis of the secondary sulfide

by arylamidase produces the diethylaniline metabolite of alachlor. Oxidation of the diethylaniline metabolite produces the putative toxic metabolite, diethylbenzoquinone imine (DEBQI). This metabolite binds to cellular protein, resulting in eventual cell death. Ensuing regenerative cell proliferation can then lead to neoplasia through "fixation" of spontaneous mutations.

TOXICOLOGY

Acute Toxicity

Alachlor demonstrates relatively low acute toxicity in mammals. The acute studies for alachlor revealed the following values: the oral LD_{50} in the rat is 0.93 g/kg (Wistar albino rat; 95% confidence limits, 0.81 to 1.05 g/kg), the dermal LD_{50} in the rabbit is 13.3 g/kg (New Zealand White rabbit, 95% confidence limits, 6.7 to 26.6 g/kg), and the inhalation LC_{50} in the rat > 5.1 mg/L (Monsanto, 1978a; 1981). The technical product has only slight skin and eye irritation potential following acute exposure to rabbits and guinea pigs (Monsanto, 1978b, 1984a).

Chronic Noncarcinogenic Toxicity

The main chronic toxic effects other than cancer are hepatotoxicity and ocular lesions. Below are summaries of the most significant studies pertaining to these effects.

Six-Month Dog Feeding Study

Dose-dependent hepatotoxicity was observed in a six-month dietary study in dogs at all tested doses of 5, 25, 50 and 75 mg/kg-day (Ahmed *et al.*, 1981). At all dose levels for males and at dose levels of 25 mg/kg-day and above for females, absolute and relative liver weights were significantly increased. Liver fatty degeneration and biliary hyperplasia were found in both sexes at dose levels of 25 mg/kg and greater. Alachlor-treated animals had high rate of mortality. All animals (11/12), except for one female, died at the 75 mg/kg-day level; 4/6 males and 3/6 females died following ingestion of 50 mg/kg-day; and one animal of each sex died at 25 mg/kg-day. No mortality was noted at 5 mg/kg-day. One male died "accidentally" in the control group.

One-Year Dog Feeding Study

In an one-year oral toxicity study, beagle dogs (six/sex/dose) were administered alachlor in gelatin capsules at levels of 0, 1, 3 or 10 mg/kg-day (Naylor *et al.*, 1984). The study identified a no-observed-adverse-effect-level (NOAEL) of 1 mg/kg-day and a lowest-observed-adverse-effect-level (LOAEL) of 3.0 mg/kg-day based on hemosiderosis in the liver, kidney and spleen. Hemosiderosis seen in the liver of three dogs at 10 mg/kg-day was correlated with red cell destruction. A diagnosis of hemolytic anemia was supported by reduced red blood cell counts, hematocrit and hemoglobin levels.

Two-Year Rat Feeding Studies

In a two-year rat feeding study in the Long-Evans strain (50 animals/sex/dose), alachlor was toxic at all doses tested (14, 42 and 126 mg/kg-day) (Daly *et al.*, 1981b). The main systemic effects were the development of hepatotoxicity and an ocular lesion, classified as uveal degeneration syndrome (UDS). This syndrome in its mildest form is characterized by free-floating iridial and

choroidal pigments in the ocular chamber and by pigment deposition on the cornea and lens. In its most severe form, the syndrome is characterized by bilateral degeneration of the iris and diminution in the size of the ocular globe with secondary total cataract formation. Besides systemic effects, the study revealed the carcinogenic potential of alachlor (this study is discussed in more detail under "Carcinogenicity").

In order to further examine ocular effects, alachlor was administered to rat of the same strain (50/sex/dose) in two concurrent studies for two years (Stout *et al.*, 1984a, Stout *et al.*, 1984b). In the first study (Stout *et al.*, 1984a) animals consumed 0, 0.5, 2.5 or 15 mg/kg-day. In the second study (Stout *et al.*, 1984b) rats were administered an oral dose of 126 mg/kg-day. At the highest dose in the first study (15 mg/kg-day), there was a small increase in the number of animals exhibiting the initial stage of UDS, specifically, molting of retinal pigmentation. The NOAEL was determined be 2.5 mg/kg-day for UDS. In the second study, animals exposed to 126 mg/kg-day for different periods of time demonstrated that the UDS is an irreversible syndrome. Oncogenic effects of alachlor exhibited in these studies are discussed below (under "Carcinogenicity").

Reproductive Toxicity

In a three-generation reproduction study in Spraque-Dawley rats, alachlor was administered orally at 0, 3, 10 or 30 mg/kg-day (Schroeder *et al.*, 1981). An NOAEL of 10 mg/kg-day and an LOAEL of 30 mg/kg-day were determined for renal toxicity in F_2 adult males and F_3 pups (Schroeder *et al.*, 1981). The renal effects consisted of discoloration, chronic nephritis and increased absolute kidney weights.

Developmental Toxicity

In a teratology study in the rat, Rodwell and Tracher (1980) reported that alachlor did not produce teratogenic effects in rats when administered by gavage at dose levels of 50, 150 or 400 mg/kg-day. An NOAEL of 150 mg/kg-day was determined based on maternal and fetal toxicity.

In a developmental toxicity study by Schroeder (1988), female New Zealand white rabbits were administered 0, 50, 100 or 150 mg/kg alachlor by gastric intubation on gestation days 7 through 19. Maternal toxicity (not statistically significant decrease in body weight gains during the dosing period only) was observed at 150 mg/kg-day. No adverse effects were noticed at 100 mg/kg-day.

There are two additional rabbit teratology studies conducted by International Research and Development Corporation (Monsanto, 1980; 1984b) that used identical dose levels of 0, 10, 30 or 60 mg/kg-day. The main difference between these two studies was the use of different vehicles to suspend the technical alachlor. The first study used corn oil and the second used mineral oil. The studies had many technical flaws (including inadequate animal care, improper gavage technique, inadequate historical data and lack of individual fetal body weight and structural variations) and could not be used to evaluate the teratogenic potential of alachlor (U.S. EPA, 1990).

Genetic Toxicity

Most of the available mutagenicity studies indicate that alachlor may only be weakly mutagenic. Discussed below are the studies most relevant to characterizing mutagenic potential of alachlor.

Alachlor produced some weakly positive mutagenic effects in an *in vivo/in vitro* hepatocyte DNA repair assay in male F-344 rats (six groups of three animals) exposed to this compound up to 1 g/kg (Monsanto, 1985e).

Negative results were observed in assays performed by Shirasu *et al.*, (1980). A rec-assay was conducted in *Bacillus subtilis* strains M45 and H17 at six concentrations (20 to 20,000 µg/plate) and it showed no evidence of test-compound-induced inhibition. Also, negative were assays (both with and without metabolic activation) in *Escherichia coli* strain WP2 hcr and *Salmonella typhimurium* strains TA1538, TA1537, TA1535, TA98 and TA100 at concentrations ranging from 10 to 15,000 µg/plate.

The clastogenicity of alachlor has been demonstrated *in vitro* in several published studies, including cytogenetic studies from animal and human cells (Bagchi *et al.*, 1995; Surrales *et al.*, 1995; Ribas *et al.*, 1995). Most *in vivo* studies of micronuclei induction were generally negative at doses up to 1,000 mg/kg.

In contrast, two metabolites of alachlor, N-[2-ethyl-6-(1-hydroxyethyl)phenyl]-N (methoxymethyl)-2-(methylsulfonyl) acetamide, were positive both with and without microsomal activation in the Ames test with *Salmonella* strain TA100 (Kier, 1985). A third metabolite, N-2-ethyl-6 (1-hydroxylethyl) phenyl-2-(methylsulfonyl) acetamide, was weakly positive in TA100 in the presence of metabolic activation (Kier, 1985). Besides alachlor metabolites, Kier (1985) also studied urine and bile from Long-Evans rats treated with alachlor for mutagenic potential. Urine from the rats given a single oral dose of 700 mg alachlor/kg produced a weak mutagenic response in *Salmonella* strain TA1537 in the presence of both metabolic activation and β-glucuronidase; a similar effect was noted in TA98 exposed to β-glucuronidase without metabolic activation. These suggest that under certain conditions, not only alachlor's metabolites, but the parent compound by itself may produce weak mutagenic response in bacteria. In a series of Ames tests, bile from intravenously dosed Long-Evans rats (70 mg alachlor/kg) exhibited no mutagenic activity (Kier, 1985).

The data on mutagenicity as a whole suggest that genotoxic species of alachlor may be produced. However, it is proposed that genotoxic activity and cell proliferation are observed at doses of alachlor in which glutathione (GSH) depletion and/or saturation of protein-binding with alachlor has occurred. Therefore, the possible genotoxic activity of alachlor may not manifest itself at doses where GSH is not depleted. These data are supportive of a non-linear mode of action for tumor induction (U.S. EPA, 1997).

Carcinogenicity

Human Data

In two studies, mortality rates and cancer incidence were examined among manufacturing workers with potential exposure to alachlor (Leet *et al.*, 1996; Acquavella *et al.*, 1996). Mortality from all causes combined in workers believed to have high alachlor exposure was decreased in relation to the standardized mortality ratio, while cancer mortality was similar to the standardized mortality ratio for this cause of death. There were no cancer deaths among workers with five or more years of high exposure, and 15 years since first exposure. In the earlier report (Leet *et al.*, 1996) it was observed that alachlor-exposed workers had elevated rates of colorectal cancer and that the excess

occurred in male workers with five or more years in high alachlor exposure jobs. The major limitation of the study was the small size of the study population and minimal length of follow-up.

The latter report (Acquavella *et al.*, 1996) provided some follow-up that lessened the observed to expected ratio reported for colorectal cancer. The findings in both reports suggested no appreciable effects of exposure to alachlor on workers mortality or cancer incidence. Nevertheless, the authors suggested that further monitoring of the incidence of colorectal cancer and other cancers among those workers with high exposure to alachlor is important, since the cohort is still relatively young and the follow-up period relatively short.

It should be noted that none of the animal studies with alachlor demonstrated any increased incidence of colorectal cancer.

Animal Data

Laboratory animal feeding studies with alachlor demonstrated oncogenic effects in two species, mice and rats. In mice, alachlor produced lung tumors, and in rats, stomach, thyroid and nasal turbinate tumors.

A statistically significant increase ($p < 0.05$) in lung bronchiolar tumors was demonstrated at 260 mg/kg of alachlor administered to female mice of the CD-1 strain (Daly *et al.*, 1981a). This was the highest dose of alachlor tested in an 18-month feeding study of 0, 26, 78 or 260 mg/kg-day. The increase of lung tumors in male mice was not significant at any dose. Alachlor used in this study contained 0.5% epichlorohydrin for the first 11 months. However, in the remainder of the study period another inert compound was used. Epichlorohydrin has been shown to produce both nasal turbinate tumors and stomach tumors in rats (Konishi *et al.*, 1980; Laskin *et al.*, 1980).

In a second carcinogenicity study using CD-1 mice (Monsanto, 1994), male mice received dietary alachlor at 0, 16.64, 65.42 or 262.4 mg/kg-day for 79 weeks. For the same period of time female mice received dietary alachlor at 0, 23, 73, 90.34 or 399.22 mg/kg-day. A significant pair-wise difference for the incidence of bronchioalveolar adenomas and adenomas and/or carcinomas combined at all dose levels in comparison to control was observed in male mice. There were no significant increase in the incidences of compound-related tumors observed in female mice in this study.

After considering all of the available biological and statistical information from the two mouse studies, there is some question as to whether alachlor causes an increase in lung tumors in mice. The concerns include: lack of reproducibility of results, non-linear dose response, lack of progression and morphological similarity of the produced tumors to the spontaneous age-associated neoplasms in CD-1 mice.

Three additional chronic feeding studies are available, all conducted in Long-Evans rats. In the first study by Daly *et al.* (1981b), 50 rats/sex/dose were administered 0, 14, 42 or 126 mg/kg-day alachlor for two years. As in the mouse study, the technical grade alachlor was stabilized with 0.5% epichlorohydrin during the first year of the study, and another inert compound was used in the second year of the study. Dose-dependent increases ($p < 0.05$) were noted for nasal turbinate adenomas in both sexes for the mid- and high-doses. Also, in the high dose group in both sexes ($p < 0.001$) there were statistically significant increases in the incidence of malignant stomach tumors, described by the authors as neoplasms pluripotent in ability to form mixed carcinoma-type tumors.

In addition, in both sexes at the high-dose level, follicular tumors of the thyroid (adenomas plus carcinomas) were increased with the increase being significant in males ($p < 0.001$).

Two other feeding studies were performed by Stout *et al.* (1984 a, b). Originally these two studies were conducted as one study with a common control group. The first study (Stout *et al.*, 1984a) included four groups of 50 male and 50 female Long-Evans rats exposed to 0, 0.5, 2.5 or 15 mg alachlor/kg-day in the diet for two years. The second study (Stout *et al.*, 1984b) consisted of a "fourth" treatment group that received 126 mg/kg-day alachlor in the diet for various periods of time. In neither study was epichlorohydrin used as an inert ingredient in the technical product. The main purpose of the second study was to investigate the nature and reversibility of the ocular lesions (i.e., UDS) that developed in rats following exposure to alachlor, as previously described in this memorandum.

Stout *et al.* (1984 a, b) demonstrated a statistically significant ($p < 0.001$) increase in nasal epithelial adenomas in both males and females administered alachlor at 15 and 126 m/kg-day in the diet for two years. In the first study (Stout *et al.*, 1984a) an increase was noted in the incidence of thyroid-follicular cell adenomas in males and in a rare stomach tumor (malignant mixed gastric tumor) in both sexes. Nasal adenocarcinomas were noted only at 126 mg/kg-day both in males (7/61) and females (2/25). A high incidence of nasal tumors was also produced in males (10/17) and females (19/46) exposed to 126 mg/kg-day for only five to six months.

The usefulness of the second study (Stout *et al.*, 1984b) for the quantitative assessment of alachlor carcinogenic potential is limited because of the biased selection process in the design according to which rats were administered 126 mg/kg-day alachlor in the diet for 5.5 to 24 months. However, the results are valuable in the qualitative assessment of the weight-of-the-evidence for the oncogenicity of the new technical product not stabilized with epichlorohydrin. These results indicate that the tumor response observed in the earlier study by Daly *et al.* (1981b) cannot be explained on the basis of the presence of epichlorohydrin in the test material. The study suggests also that a partial exposure (approximately 25% of the life-span of the animals) can result in tumor incidence rates at the end of a two-year period that are similar to the incidence rates following a lifetime exposure.

Structure-Activity Relationships

Structure-activity relationships play an important role in the evaluation of the weight-of-evidence for carcinogenic potential. Alachlor is structurally related to acetochlor, allidochlor, butachlor, metolachlor, propachlor, diphenamide and SAN 582H. The U.S. EPA Health Effects Division Carcinogenicity Peer Review Committee (CPRC) in 1997 concluded that some of these chemicals have been shown to induce tumors at one or more of the same sites as alachlor (U.S. EPA, 1997). Statistically significant increases in nasal tumors have been reported for acetochlor and butachlor in rats. Nasal tumors have also been reported in rats maintained on a diet with 3,000 ppm of metolachlor; however the results were not statistically significant. Nevertheless, nasal turbinate tumors are considered rare and these results are supportive of a neoplastic response at that site by these compounds (U.S. EPA, 1997).

Stomach tumors and thyroid follicular cell tumors have also been reported for acetochlor and butachlor in rats. Male Sprague-Dawley rats administered diphenamide in the diet for 104 weeks showed trend and pairwise statistically significant epithelial hyperplasia of the stomach. Stomach lesions were observed in both female and male CD-1 mice administered propachlor in the diet for

18 months at 0, 100, 500, 1,500 or 6,000 ppm. Male mice at the highest dose exhibited erosion and ulceration of the glandular mucosa of the stomach.

The above data support the evidence that nasal turbinate tumors, stomach tumors and thyroid follicular tumors are related to exposure to these structurally-related compounds, including alachlor.

DOSE-RESPONSE ASSESSMENT

Noncarcinogenic Endpoints

U.S. EPA's Approach

The following describes the process used by U.S. EPA in deriving a reference dose (RfD) and a Drinking Water Equivalent Level (DWEL) based on noncarcinogenic adverse health effects produced by alachlor. A DWEL is a medium-specific (i.e., drinking water) lifetime exposure level, assuming 100% exposure from that medium, at which adverse, noncarcinogenic health effects would not be expected to occur. The DWEL is derived from the multiplication of the RfD by the assumed body weight of an adult and divided by the assumed daily water consumption of an adult.

Calculation of RfD

$$\text{RfD} = \frac{1 \text{ mg/kg-day}}{100} = 0.01 \text{ mg/kg-day}$$

The one-year feeding study in dogs by Naylor *et al.* (1984) was selected by U.S. EPA as the basis for the RfD calculation because the NOAEL of 1 mg/kg-day for increased hemosiderosis in liver and spleen represents the highest NOAEL for noncarcinogenic effects in laboratory animals. An uncertainty factor (UF) of 100 was applied in accordance with U.S. EPA guidelines for use of a NOAEL from animal study for risk assessment.

Calculation of DWEL

$$\text{DWEL} = \frac{0.01 \text{ mg/kg-day} \times 70 \text{ kg}}{2 \text{ L/day}}$$

$$= 0.35 \text{ mg/L} = 0.4 \text{ mg/L (rounded)} = 400 \text{ ppb.}$$

The body weight of an adult male (70 kg) and the volume of water consumption for an adult of 2 L/day were assumed in the calculation of the DWEL.

Health Advisories

The majority of federal drinking water standards are established by the Office of Drinking Water, U.S. EPA. Health advisories (HA), which serve as informal guidance to assist federal, state and local officials responsible for protecting public health when emergency spills or contamination occur (US EPA, 1988, 1990), depict non-regulatory concentrations of drinking water contaminants

at which adverse health effects would not be expected to occur over specific exposure time. HAs may be developed for one-day, 10-day, longer-term (approximately seven years) and lifetime exposures if adequate data are present for identifying a sensitive noncarcinogenic endpoint of toxicity.

For alachlor, short-term studies adequate to derive these short-term advisories for alachlor are not available. Therefore, a 10-day HA values was developed based on a longer-term study. The calculation of the 10-day HA (for a 10 kg child) of 0.1 mg/L is based on an NOAEL of 1 mg/kg-day for mild hepatotoxic effects observed in a one-year dog feeding study (Naylor *et al.*, 1984). This study was adequately conducted and the NOAEL reflected absence of hemosiderosis of the liver and spleen of treated dogs.

A lifetime HA for alachlor was not calculated by U.S. EPA because a high incidence of nasal turbinates tumors were produced in male and female rats exposed to alachlor (126 mg/kg-day) in the diet for less than five and one-half months during their normal life-span (two-year study) (Stout *et al.*, 1984b). The lifetime HA was not recommended by U.S. EPA because alachlor is a Group B2 - probable human carcinogen. This classification might be changed in the future based on the most recent fourth Health Effects Division CPRC evaluation (U.S. EPA, 1997).

Carcinogenic Endpoint

OEHHA evaluated the carcinogenic risk for alachlor using in its assessment both non-threshold and threshold approach. In both approaches carcinogenic risk assessment was based on the development of nasal turbinate tumors in Long-Evans rats of both sexes in two studies by Stout *et al.* (1984 a, b). Calculations were performed by using the multistage model TOX_RISK computer program with a body weight scaling factor of $(BW)^{3/4}$ (Crump *et al.*, 1993). The dataset used for this analysis reflects the combined incidences of nasal turbinate tumors in male and female rats exposed to up to 15 mg /kg-day alachlor (Stout *et al.*, 1984 a, b). The following are key data and estimates obtained from the analysis:

Doses	=	0, 0.5, 2.5 and 15 mg/kg-day
Responses	=	0/87, 0/89, 1/91 and 29/93
TOX_RISK (Q2)	=	1.662283E-003
q_1^*(human)	=	4.6921E-002 $(mg/kg\text{-}day)^{-1}$ (scaling factor $BW^{3/4}$)
MLE	=	1.532E-004 $(mg/kg\text{-}day)^{-1}$ (computed for *de minimis* risk of 10^{-6})
Chi-square	=	0.04
p	=	0.98
MLE_{10}	=	1.0585E+005 ppb
LED_{10}	=	1.8 mg/kg-day
CSF (human)	=	0.056 $(mg/kg\text{-}day)^{-1}$

Table 1 presents the number of tumors observed as reported by Stout *et al.* (1984a, b) and the number of tumors predicted by the model at particular experimental doses. The numbers in the fourth column are derived from TOX_RISK (multistage model) program and present risks predicted at certain dose levels.

Table 1. Combined Incidences of Nasal Turbinate Tumors in Male and Female Rats

DOSE (mg/kg-day)	# Responses Observed	# Responses Predicted	95% LED (Predicted @ Risk) (mg/kg-day)
0	0	0.00	0.00228 (0.0001)
0.5	0	0.04	0.0228 (0.001)
2.5	1	0.94	0.229 (0.01)
15	29	29.02	1.77 (0.1)

Based on the performed analysis and the results presented as well as on the p value of 0.98, it is apparent that the predicted curve fits the observed data very well. The 95% LED is approximately linear with risk from 10% to 0.01%.

CALCULATION OF PHG

Calculation of the PHG for alachlor heavily depends on the weight-of-evidence for its carcinogenic potential and its relevance to humans. Alachlor was classified as a Group B2 - probable human carcinogen by U.S. EPA (U.S. EPA 1984; 1986; 1987b). At the third Peer Review of Alachlor in 1996, classification was deferred pending promulgation of the new Guidelines for Carcinogenic Risk Assessment (U.S. EPA 1996b). At the fourth Peer Review in June 1997 (U.S. EPA, 1997) the Health Effects Division CPRC classified alachlor according to U.S. EPA's Proposed Guidelines for Carcinogen Risk Assessment (U.S. EPA, 1996b) as "likely" at high doses but "not likely" at low doses to be a human carcinogen by all routes of exposure. CPRC agreed that a non-linear approach (margin of exposure) should be used for the purpose of risk assessment for alachlor.

This decision resulted from the re-evaluation of the weight-of-evidence for alachlor carcinogenic potential. A significant role in this re-evaluation can be attributed to the new data provided by the registrant on a new mouse carcinogenicity study, additional mutagenicity studies, mechanistic data and toxicology data from a related compound, butachlor. The following are the major points of this analysis: 1) tumors of the nasal epithelium, stomach and thyroid were observed only at higher doses in rats, 2) there are quantitative differences in species sensitivity of the response and 3) there is evidence for a nonlinear dose-response for tumor induction at each site.

CPRC recommended that the margin of exposure (MOE) for establishing a safe level of exposure outside the observable range for the nasal tumors be determined with 0.5 mg/kg-day as the "point of departure," as no tumor response was seen at this dose level (Stout *et al.*, 1984b).

Non-linear Approach

OEHHA currently supports the non-linear approach recommended by U.S. EPA to estimate the carcinogenic risk of alachlor. Consequently, for calculating a PHG for alachlor, we used 0.5 mg/kg-day (no tumor response level for the nasal turbinate tumors), the lowest level among all no

response levels, for adverse effects observed in animal studies. A public health-protective concentration (C, in mg/L) can be calculated using the general equation for a carcinogen assuming a non-linear approach:

$$C = \frac{POD \times BW \times RSC}{UF \times L/day} = mg/L$$

where,

POD = Point of departure used for a non-linear approach, for alachlor this is for no tumor response (0.5 mg/kg-day)
BW = Body weight for an adult male (a default of 70 kg)
RSC = Relative source contribution (a default of 20% or 0.2)
UF = Uncertainty factor of 1,000 (10-fold for inter-species variation, 10-fold for human variability, and 10-fold for severity of endpoint)
L/day = Volume of water consumed daily by an adult (a default of 2 L/day).

Therefore,

$$PHG = \frac{0.5 \text{ mg/kg-day} \times 70 \text{ kg} \times 0.2}{1{,}000 \times 2 \text{ L/day}}$$

$$= 0.0035 \text{ mg/L} = 0.0035 \text{ mg/L (rounded)} = 4 \text{ ppb.}$$

Linear Approach

A public health-protective concentration (C, in mg/L) can be calculated using the general equation for a carcinogen assuming a linear approach:

$$C = \frac{BW \times R}{CSF \times L/day}$$

where,

BW = Adult male body weight (70 kg)
R = *De minimis* theoretical excess individual cancer risk level for lifetime (10^{-6})
CSF = Cancer slope factor [0.056 $(mg/kg\text{-}day)^{-1}$]
L/day = Volume of water consumed daily by an adult (2 L/day).

Therefore,

$$C = \frac{70 \text{ kg} \times 10^{-6}}{0.056 \ (mg/kg\text{-}day)^{-1} \times 2 \text{ L/day}}$$

$$= 6.25 \times 10^{-4} \text{ mg/L} = 0.625 \ \mu g/L = 0.6 \ \mu g/L \text{ (rounded)} = 0.6 \text{ ppb.}$$

OEHHA calculates a PHG of 4 ppb for alachlor in drinking water based on the non-linear approach and the Stout *et al*. (1984) data. It should be noted that the PHG for alachlor would be almost seven times lower (0.6 ppb versus 4 ppb) if the calculation was performed assuming linear

response of carcinogenic effects caused by alachlor. The PHG of 4 ppb is 2-fold higher than the federal MCL.

RISK CHARACTERIZATION

Sources of uncertainties involved in the development of the PHG for alachlor in drinking water include areas specific to this chemical (such as species differences in alachlor's metabolic activity and mode of action leading to production of tumors and the relevance of tumor production in different species to humans), and areas related to the risk assessment in general (such as inter- and intra-species extrapolation) and the use of default value for the estimation of the relative source of contribution.

The health risk assessment of alachlor is based on its carcinogenic potential. Alachlor was studied in both rats and mice. Studies in Long-Evans rats demonstrated increased incidence of nasal epithelial tumors, glandular stomach tumors and thyroid follicular cell tumors. Studies in CD-1 mice showed evidence of bronchioalveolar adenoma and combined adenoma/carcinoma, but the data were inconclusive.

Evaluation of the mechanism for carcinogenic response was critical in the risk assessment of alachlor. Carcinogenic responses caused by alachlor are considered by the scientific community to be non-linear. It was demonstrated that nasal tumor production is directly related to the diethylbenzoquinone imine metabolite of alachlor. Although, both rats and humans possess a similar mechanism for formation of the diethylbenzoquinone imine metabolite, there are large quantitative differences between rats and humans for formation of this metabolite and the nasal response is best characterized by a non-linear mode of action.

The increase in gastric tumors was statistically significant only at the highest dose tested, 126 mg/kg-day. Some hazard potential for gastric tumors may exist for humans after intense exposures. Clarification of the similarity and dissimilarity of the relevance of the rat stomach tumor would shed light on this uncertainty. At present these tumors should be considered relevant to humans. As to the mode of action stomach tumors are believed to be produced by a direct contact effect, a non-genotoxic mechanism. At doses below which gastric pathology is observed one would not expect a tumorigenic response. The use of the MOE approach for human cancer assessment is consistent with U.S. EPA's Proposed Guidelines for Carcinogenic Risk Assessment (U.S. EPA, 1996).

Thyroid tumors were observed only at excessive dose levels (42 and 126 mg/kg-day). The mechanism of thyroid tumorigenesis observed with alachlor is consistent with the mechanism of thyroid tumorigenesis observed with other chemicals causing a disruption of thyroid hormone balance, which is a threshold-dependent, non-genotoxic mechanism.

The evaluation of the mechanism for alachlor carcinogenicity led to the use of the non-linear approach for the purpose of risk assessment and consequently for the PHG calculation. In this calculation we used the level of 0.5 mg/kg-day (no tumor response level for the nasal turbinate tumors), an RSC of 20% (0.2), a UF of 1,000 and 2 L/day as a default value for water intake by adult person. The uncertainty factor of 1,000 accounts for inter and intra-species differences, and for carcinogenicity as a severe toxic effect.

For PHGs, our use of the relative source contribution (RSC) has, with a few exceptions, followed U.S. EPA drinking water risk assessment methodology. U.S. EPA has treated carcinogens differently from noncarcinogens with respect to the use of RSCs. For noncarcinogens, RfDs (in mg/kg-day), DWELs (in mg/L) and MCLGs (in mg/L) are calculated using UFs, body weights and water consumption rates (L/day) and the RSC, respectively. The RSC range is 20% to 80% (0.2 to 0.8) depending on the scientific evidence.

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

1. if Group A and B carcinogens (i.e., strong evidence of carcinogenicity) MCLGs are set to zero,

2. if Group C (i.e., limited evidence of carcinogenicity), either an RfD approach is used (as with a noncarcinogen) but an additional UF of 1 to 10 (usually 10) is applied to account for the limited evidence of carcinogenicity, or a quantitative method (potency and low-dose extrapolation) is used and the MCLG is set in the 10^{-5} to 10^{-6} cancer risk range,

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

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

The RSC of 0.2 used in the calculation of a PHG using the non-linear approach reflects the assumption that drinking water makes up 20% of the exposure to alachlor relative to other sources of exposure to alachlor. The other 80% comes mainly from the residues of alachlor in diet which is the most significant source of exposure to this chemical.

OEHHA determined that the non-linear approach to the risk assessment for alachlor reflects current status of knowledge on this chemical and its potential for human exposure. However, the relevance of tumors produced by alachlor in animal studies to humans cannot be dismissed. There are still uncertainties related to both the risk assessment procedures in general (such as inter and intra-species extrapolations) and to the specific features of alachlor toxicity and the lack of a more accurate value for an RSC. For these reasons we also presented another approach in calculating the PHG for alachlor. This approach reflects linear mode of action for alachlor's carcinogenic activity which results in more health-conservative value of 0.6 compared to 4 ppb. For risk management purposes, if the linear approach was used rather than the non-linear approach, public health-protective concentrations (C) of 6 and 60 ppb could also be calculated assuming cancer risk levels of 10^{-5} and 10^{-4}, respectively.

REGULATORY STANDARDS AND CRITERIA

U.S. EPA's MCLG and MCL are 0 ppb and 2 ppb, respectively, based on the carcinogenic potential of alachlor. The California MCL is 2 ppb.

REFERENCES

Acquavella JF., Riodan SG., Anne M., *et al.* (1996). Evaluation of mortality and cancer incidence among alachlor manufacturing workers. *Environ. Health Perspect.* **104**: 728-733.

Ahmed, F.E., Tegeris, P.C., Underwood *et al.** (1981). Alachlor: Six-month study in the dog. (Prepared by Pharmacopathics Research Laboratories, Inc.) PRL, Inc. report No. 7952; Monsanto report No. PR-80-015. St. Louis, MO: Monsanto Co. CDL: 246229-A; 246293.

Bagchi, D., Bagchi, M., Hassoun E.A. and Stohs, S.J. (1955). In vitro and in vivo generation of reactive oxygen species, DNA damage and lactate dehydrogenase leakage by selected pesticides. *Toxicology* **104**: 129-140.

Crump, K.S., Howe, R.B., Van Landingham, C., and Fuller, W.G. (1993). TOX RISK Toxicology Risk Assessment Program. Clement International Corporation, K.S. Crump Division. Ruston, Louisiana.

Daly, I.W., Hagan, G. K., Plutnik, R. *et al.** (1981a). An eighteen-month chronic feeding study of alachlor in mice. Project No. 77-1064. St. Louis, MO: Monsanto Co. CDL: 070168-A; 070169.

Daly, I.W., MccCandless, J.B., Jonassen, H. *et al.** (1981b). A chronic feeding study of alachlor in rats. (Prepared by Bio-Dynamics, Inc.) Project No. 77-2065. St Louis, MO: Monsanto Co. CDL: 070586-A; 070587; 070588; 070589; 070590.

Kier, L.D. (1985). Ames/Salmonella mutagenicity assays of synthesized alachlor metabolites. Study No. 850002, 850007, 850052, 840088, 840070. St. Louis, MO: Monsanto Co.

Konishi, Y., Kawabata, A., Denda, A., Ikeda, T., Katada, H., Maruyama, H. and Higashiguichi, R. (1980). Forestomach tumors induced by orally administered epichlorohydrin in male Wistar rats. *Gann* **71**, 922-923.

Laskin, S., Sellakumer, A.R., Kuschner, M., Nelson, N., LaMendula, S., Rusch, G.M., Katz, G.V., Dulak, N.C. and Albert, R.D. (1980). Inhalation carcinogenicity of epichlorohydrin in noninbred Spraque-Dawley rats. *J. Natl. Cancer Inst.* **65**: 751-757.

Leet T., Acquavella J., Lynch C., *et al.* (1996). Cancer incidence among alachlor manufacturing workers. *American J Industrial Medicine* **30**: 300-306.

Monsanto Co.* (1994). (Conducted by the Environmental Health Laboratory) Project Nos. MSL-13847 and EHL 91166; Study No. ML-92-001; MRID # 43507601.

Monsanto Co.* (1985e). Unscheduled DNA synthesis in rats. St. Louis, MO: Monsanto Co. Accession No. 253308.

Monsanto Co.* (1984a). Dermal sensitization--guinea pig. St. Louis, MO: Monsanto Co. CDL: 252772.

Monsanto Co.* (1984b). A teratology study in rabbits. (Prepared by International Research and Development Corp.) Study No. 401-208. St. Louis, MO: Monsanto Co. CDL: 25270.

Monsanto Co.* (1981). Acute inhalation LD_{50}--rat. (Prepared by Bio-Dynamics, Inc.) St. Louis, MO: Monsanto Co.

Monsanto Co.* (1980). A teratology study in rabbits. (Prepared by International Research and Development Corporation) Study No. 401-060. St Louis, MO: Monsanto Co. CDL: 242369.

Monsanto Co.* (1978a). Acute oral-rat, acute dermal rabbit. (Prepared by Bio-Dynamics, Inc.) St. Louis, MO: Monsanto Co. CDL: 241273.

Monsanto Co.* (1978b). Primary eye and primary dermal irritation--rabbit. (Prepared by Bio-Dynamics, Inc.) St. Louis, MO: Monsanto Co. CDL: 241273.

Naylor, M., Ribelin, W.E., Thake, D.E., Stout, L.D. and Folks, R.M.* (1984). Chronic study of alachlor administered by gelatin capsule to dogs. Study No.820165. St. Louis, MO: Monsanto Co. Accession No. 252570.

Ribas G., Frenzilli G., Barale R., and Marcos R. (1995). Herbicide-induced DNA damage in human lymphocytes evaluated by single-cell gel electrophoresis (SCGE) assay. Mutat. Res. **344**: 41-54.

Rodwell, D.E., and Tracher E.J.* (1980). Teratology study in rats. (Prepared by International Research & Development Corp.) IRDC No. 401-058; IR-79-020. St. Louis, MO: Monsanto Co. CDL: 252570.

Schroeder, R.D., Hogan, G.K., Smock, M.E. *et al.** (1981). A three-generation reproduction study in rats with alachlor. (Prepared by Bio-Dynamics, Inc.) Project No. 77-2066. St. Louis, MO: Monsanto Co. CDL: 070177-A.

Schroeder, R.E.* (1988). A teratogenicity study in rabbits with alachlor. (Prepared by Bio-Dynamics, Inc.) Project No. 87-3169 (BD-87-83). St. Louis, MO: Monsanto Co. MRID Nos. 405794-01; 40579402.

Shirasu *et al.** (1980). Microbial mutagenicity study. (Prepared by Institute of Environmental Toxicology, Kodira, Japan) St. Louis, MO: Monsanto Co. CDL: 248053.

Stout, L.D. *et al.** (1984a). A chronic study of alachlor administered in feed to Long-Evans rats. (Prepared by Environmental Health Laboratory) ML-80-186. St. Louis, MO: Monsanto Co. CDL: 252496 and 252497.

Stout, L.D. *et al.** (1984b). A chronic study of alachlor administered in feed to Long-Evans rats. (Prepared by Environmental Health Laboratory) ML-80-224. St. Louis, MO: Monsanto Co. CDL: 252498.

Surrales J., Catalan J., Creus A. *et al.* (1995). Micronuclei induced by alachlor, mitomycin-C and vinblastine in human lymphocytes: presence of centromeres and kinotochores and influence of staining technique. Mutagenesis **10**: 417-423.

U.S. EPA (1997). U.S. Environmental Protection Agency. Fourth alachlor carcinogenicity peer review of February 5, 1997. Office of Prevention, Pesticides, and Toxic Substances, U.S. EPA, Washington, D.C.

U.S. EPA (1996a). U.S. Environmental Protection Agency. Third alachlor carcinogenicity peer review of February 21, 1996. Office of Prevention, Pesticides, and Toxic Substances, U.S. EPA, Washington, D.C.

U.S. EPA (1996b). U.S. Environmental Protection Agency. Proposed Guidelines for Carcinogen Risk Assessment. Federal Register Vol. 61, No. 79 / Tuesday, April 23, 1996 17960-18011.

U.S. EPA (1990). U.S. Environmental Protection Agency. Quantification of toxicological effects for alachlor. Criteria and Standards Division, Office of Drinking Water, U.S. EPA, Washington, D.C.

U.S. EPA (1988). U.S. Environmental Protection Agency. Alachlor Health Advisory. Office of Drinking Water. Washington, D.C.

U.S. EPA (1987a). U.S. Environmental Protection Agency. Alachlor; Notice of Intent to Cancel Registrations; Conclusion of Special Reveiw. Federal Register Vol.52, No.251 49480-49503.

U.S. EPA (1987b). U.S. Environmental Protection Agency. Alachlor. Special review position document 4. Office of Pesticide Programs, Office of Pesticides and Toxic Substances. Washington, D.C.

U.S. EPA (1986). U.S. Environmental Protection Agency. Alachlor. Special review position document 2/3. Office of Pesticide Programs, Office of Pesticides and Toxic Substances. Washington, D.C.

U.S. EPA (1984). U.S. Environmental Protection Agency. Alachlor. Special review position document 1. Office of Pesticide Programs, Office of Pesticides and Toxic Substances. Washington, D.C.

---

* Proprietary studies