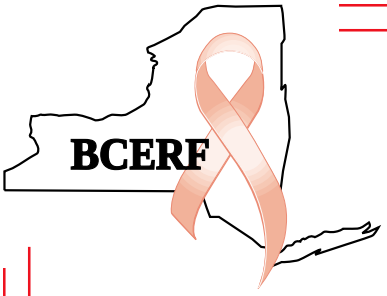

Cornell University
Program on Breast Cancer and Environmental Risk Factors in New York State (BCERF)*

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Critical Evaluation of Alachlor's Breast Cancer Risk

by

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Author's Note: The reader is encouraged to read Appendix B prior to reading this Critical Evaluation. Appendix B includes an explanation of the approach used in writing BCERF Critical Evaluations and an explanation of the BCERF Breast Cancer Risk Classification System.

I. Introduction

Alachlor (2-chloro-2',6'-diethyl-*N*-(methoxymethyl) acetanilide (IUPAC) (Meister, 1999) is a chloroacetamide herbicide that is used to prevent weed growth primarily on corn and soybean crops (Gianessi and Anderson, 1995b; Gianessi and Anderson, 1995a; Meister, 1999). While alachlor breaks down relatively quickly in soil through the action of bacteria, there is concern that levels of certain degradative products of alachlor in ground and surface water often far exceed levels observed for alachlor. Alachlor is one of the herbicides for which the Environmental Protection Agency (EPA) will require State Management Plans because of the mobility, persistence, and detection of alachlor and its degradative products in water supplies. This is one of the reasons why alachlor was nominated by the New York State (NYS) Department of Environmental Conservation (DEC) as a high priority pesticide to be evaluated by BCERF. Alachlor also has been identified as a carcinogen in long-term experimental animal feeding studies, including induction of nasal, stomach, thyroid, and lung tumors. There has been considerable debate regarding the mechanisms of tumor induction at these sites, and whether these mechanisms are relevant to humans. We will provide an analysis of these proposed mechanisms of tumor induction in this Critical Evaluation, as well as evaluating whether alachlor has the potential to affect the risk of breast cancer. In addition, sections on the environmental fate of alachlor and exposure in occupational settings provide information on the potential for human populations to be exposed to this herbicide and its degradative products.

A. History of Use and Uses:

Alachlor was first introduced by the Monsanto Co. in 1969 to control annual grasses and broadleaf weeds in crops (Stevens and Sumner, 1991; WSSA, 1994). This includes controlling annual grasses such as barnyardgrass, crabgrass, foxtail, panicum, millet, goosegrass, signalgrass, red sprangletop and witchgrass, as well as broadleaf weeds including carpetweed, galinsoga, jimsonweed, lambsquarters, purslane, black nightshade, pigweed, puslane, Florida pusley and waterhemp (Monsanto, 1999; WSSA, 1994). It is used both as a selective pre- and postemergence herbicide on a variety of agricultural crops, including corn, soybeans, peanuts, sorghum, dry beans and lima beans (Meister, 1999; Stevens and Sumner, 1991). It has been used to a lesser extent on sugarcane, sunflowers and tobacco (Gianessi and Anderson, 1995a; Stevens and Sumner, 1991). Non-agricultural uses have included weed control on ornamentals and turf (Wauchope et al., 1992).

The use of alachlor as a agricultural herbicide has declined dramatically in the last dozen years. In the mid- and late 1980s it was one of the most heavily used herbicides in the United States (US). In 1987, 55-60 million lbs of active ingredient (AI) was used annually in agricultural crop production. By 1993, use had declined to 45-50 million lbs of AI per year. More recent estimates indicate that alachlor use declined further with 19-24 million lbs of AI used during 1995 (Aspelin, 1997). Usage estimates of alachlor for NYS are available for 1990-93, with 610 thousand lbs of AI used annually (Gianessi and Anderson, 1995b).

B. Chemical Information:

Table 1. Chemical information on alachlor

Common Name: alachlor (Meister, 1999)
Chemical Name: 2-chloro-2',6'-diethyl-N-(methoxymethyl) acetanilide (IUPAC) (Meister, 1999)
Trade Names: See Appendix C.
Chemical Formula: ($C_{14}H_{20}NO_2Cl$) (Stevens and Sumner, 1991)
Chemical Family: Chloroacetamides (Ahrens, 1994)
CAS Registry Number: 15972-60-8 (Meister, 1999)

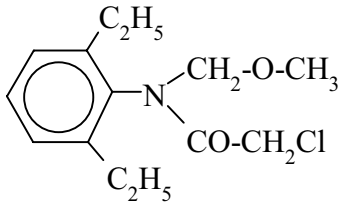

Figure 1. Chemical structure of alachlor

C. Metabolism:
A pathway for alachlor metabolism in rat hepatocytes has been suggested by Bonfanti et al. (1992). Some of these reactions are mediated by P-450 enzymes. The o-demethylation of alachlor to form 2-chloro-N-hydroxymethyl-N-(2,6-diethylphenyl)acetamide is followed by an N-dealkylation to form one of the most abundant metabolites, 2-chloro-N-(2,6-diethylphenyl)acetamide (CDEPA). An arylamide hydrolysis of CDEPA results in the formation of 2,6-diethylaniline. A variety of metabolites can be formed from the oxidation of 2,6-diethylaniline by aniline hydroxylase. 2,6-diethylaniline can be metabolized to 2,6-diethyl-N-hydroxy-aniline, and it has been suggested that it may be further metabolized to 2,6-diethylnitrosobenzene, which is mutagen (Kimmel et al., 1986). It should be noted, however, that in this study researchers could not identify the 2,6-diethylnitrosobenzene in the *in vitro* hepatic assay system. However, they were able to detect 2,6-diethylnitrobenzene. They also could not detect 2,6-diethyl-4-hydroxy-analine or 3,5 diethybenzoquinone-4-amine (DEBQI), the toxic metabolite that is hypothesized to bind to cellular proteins and induce cell death in nasal cells. It has been hypothesized that DEBQI can bind to cellular proteins and induce cell death in nasal passages and subsequently induce cell proliferation and induction of nasal turbine tumors in rats (Dapson and McMahon, 1996).

Results of experiments conducted in rats injected intraperitoneally (i.p.) with chloroacetanide pesticides, including alachlor and metolachlor, suggests that rats metabolize these compounds by hepatic mixed-function oxidase systems to anilines that are further metabolized to nitrosobenzenes, which are known carcinogens (Kimmel et al., 1986). However, there was not *in vivo* evidence of the formation of quinonimine metabolites in rats. Dialkylquinones have the capacity to induce sister-sister chromatid exchanges in human lymphocytes (Hill et al., 1997). Using a sensitive GS/MS/SIM analysis, Jefferies et al. (1998) has identified thiol adducts of dialkylquinoimines in the urine of alachlor, acetochlor and metolachlor treated Sprague-Dawley rats. This gives support to the hypothesis that the unstable dialkylbenzoquinones are alachlor metabolites in rats.

Studies using radiolabled alachlor have monitored the excretion of alachlor metabolites in the urine and feces of rodents and primates. When ^{14}C-alachlor was administered orally to Sprague-Dawley rats by gavage, an average of 49.9% was excreted via the urine and 35.4% was excreted via the feces over the 72 hour (hr) collection period (Davison et al., 1994). In contrast, primates appear to excrete alachlor metabolites primarily via the urine. Unpublished data from studies conducted in Rhesus monkeys indicate that an average of 87% of the administered dose of radiolabled alachlor was recovered in the urine, with 79% recovered in the first 24 hrs after dosing. The major urinary metabolites identified in the monkeys included mercaputrate (thioether), cysteinyl, thioacetic acid and glucoronide conjugates (Monsanto, 1985; Monsanto, 1984) as cited in (Sanderson et al., 1995a).

Very few studies have been devoted to determining the types of alachlor metabolites excreted in human urine. A study sponsored by the National Center for Environmental Health and the National Institute of Occupational Safety and Health (NIOSH) used mass spectrometry (LC-MS/MS) to determine alachlor metabolites in the urine of workers occupationally exposed to alachlor (Driskell et al., 1996). The major urinary metabolites identified in this study were 2,6-diethylaniline and alachlor mercapturate. Alachlor mercapturate has also been identified in monkey urine (Sanderson et al., 1995a). In contrast, alachlor-o-glucuronide, a urinary metabolite detected in Rhesus monkeys, was not observed in human urine. This and other studies have not detected 2,6-hydroxyethylethylaniline (HEEA) in human urine (Cowell et al., 1987; Driskell et al., 1996), while others have reported the identification of HEEA in human urine (Marcus, 1987).

II. Regulatory Status

A. Regulatory History:
Alachlor was first registered for use as a selective herbicide with EPA in 1969. A Registration Standard for alachlor was issued on November 20, 1984. This document stated that alachlor was classified as a carcinogen, additional data was needed on the leaching and mobility of alachlor and its potential to contaminate ground and surface water, further monitoring studies of ground and surface water were required, and additional studies were required on the toxicology, product chemistry, and residue chemistry (USEPA, 1998b). On January 9, 1985, EPA published a "Notice of Initiation of Special Review of Registrations of Pesticide Products Containing Alachlor" because of concerns about alachlor's carcinogenicity. A notice was issued on October 8, 1996, which stated that EPA would allow continued use of products containing alachlor, but this was subject to modification of the terms and conditions of its registration.

On December 31, 1987, EPA issued a notice entitled "Alachlor: Notice of Intent to Cancel Registrations, Conclusion of Special Review." This notice was also known as the Position 4 Document (PD-4). The PD-4 stated that the alachlor registration would be canceled unless alachlor products complied with terms set forth in the notice. Terms included: designation as a Restricted Use Pesticide due to its oncogenic effects in animal studies, labels would have a tumor hazard warning, a mechanical transfer system had to be used by mixer/loader/applicators who treated more than 300 acres annually, and additional groundwater monitoring data were required. These labeling changes were accepted by the registrant, and submitted in early 1988 (USEPA, 1998b).

A Cancer Review Committee convened by EPA reviewed the cancer causing potential of alachlor in 1996 (Dapson and McMahon, 1996). The cancer classification for alachlor by EPA is listed in Section III.C. of this report, "Carcinogen Classification by other Agencies."

The use of alachlor products is further restricted in some parts of the US. Alachlor can not be applied in Suffolk and Nassau Counties of Long Island, NY (Monsanto, 1999). This label restriction on alachlor's use was made at the request of the manufacturer, Monsanto (personal communication, Maureen Serafini, NYSDEC). This may have been requested because there were some detections of alachlor in Suffolk County groundwater wells on Long Island, NY.

B. Drinking Water Standards and Health Advisories:

1. MCLG: The Maximum Contaminant Level Goal (MCLG) for alachlor is zero, because alachlor has been classified as a likely carcinogen by EPA (USEPA, 1996; USEPA, 1998b).

2. MCL: EPA has set Maximum Contaminant Level (MCL) for alachlor in drinking water at 0.002 mg/ L (= 2 µg/L)(USEPA, 1996). The MCL is an enforceable limit for the maximum allowable concentration of a chemical in public drinking water supplies.

3. HA: Health Advisory (HA)* levels for alachlor in drinking water are as follows:

10 kg child:
- One-day = 0.1 mg/L
- Ten-day = 0.1 mg/L
- Longer term, not established

70 kg adult:
- Longer term, not established
- Lifetime, not established

* *The HAs are non-enforceable limits of the concentration of the chemical in drinking water that are not expected to cause any adverse noncarcinogenic health effects when consumed for no more that the time period specified with a margin of safety (USEPA, 1996).*

C. Food Residue Tolerances:

The EPA sets tolerances for levels of alachlor residues in food. The Food and Drug Administration (FDA) and the US Department of Agriculture (USDA) are the Federal agencies responsible for monitoring the levels of alachlor residues and several metabolites in domestic and imported foods and animal feeds. Because of new legislation set forth in the 1996 Food Quality and Protection Act, the tolerances for alachlor may be reset according to the new guidelines. Until the tolerances are reset, tolerances set by EPA in 1998 for residues of alachlor and its metabolites in raw agricultural products and animal feeds are as follows: poultry, cattle, hog, sheep and horse meat, 0.02 ppm; poultry, cattle, hog, sheep and horse fat, 0.02 ppm; milk and eggs, 0.02 ppm; fresh corn (includes sweet corn) and peanuts, 0.05 ppm; lima beans, dry beans and sorghum grain, 0.1 ppm; soybean hay and corn for fodder or forage, 0.2 ppm; soybean for forage, 0.75 ppm; sorghum for fodder and forage, and peanut hulls, 1.5 ppm; and peanut hay and peanuts for forage, 3.0 ppm (USEPA, 1998a).

III. Overall All Evidence for Carcinogenicity (non-breast sites)

A. Human Studies

1. Case Studies:

While case studies do not provide sufficient evidence of a cause and effect relationship, such studies are useful for the generation of testable hypotheses. One study has reported an increased incidence of pediatric colon cancer during 1974-1976 in the Mississippi Delta (Caldwell et al., 1981) in populations possibly exposed to alachlor and other pesticides. The incidence of colon cancer observed in these children was about five times the expected rate for this age group. Of the 13 adolescent children that were treated for colorectal adenocarcinoma, ten of the patients reported living in rural areas of Mississippi, Tennessee and Arkansas where pesticide use was high. Soybeans and cotton were the two most common crops grown in these areas, and the majority of the cases reported living on farms, or living near farms that used pesticides. Serum residue levels of pesticides in cases and family members were analyzed for persistent organochlorine pesticides such as DDT, dieldrin and beta hexachlorocyclohexane. Some of the families did show higher levels of one or more of these pesticides in their blood. Although alachlor was commonly used as a herbicide on soybean crops in the Mississippi Delta region during this time period, there was no assessment of potential for exposure to this pesticide other than residence on a farm that grew soybeans treated with pesticides (Caldwell et al., 1981). Therefore, because of the potential for exposure to multiple pesticides, it is not possible to conclude whether or not these cases were definitely exposed to alachlor, or if the suspected exposure was related to their disease outcome.

2. Occupational Cohort Studies:

Two studies have followed a cohort of workers employed at the Monsanto plant in Muscaine, Iowa, which has manufactured alachlor since 1968, for the incidence of cancer (Leet et al., 1996). The first study followed a cohort of 943 white workers (82% men and 18% women) who had at least one year of cumulative employment from when alachlor was first processed at the plant in March of 1968 through December of 1990. During the follow-up period, 686 workers were still living or had died in Iowa, while 219 former workers no longer resided in Iowa. The State Health

Registry of Iowa was used to identify cancer cases. Eighteen of the workers (15 male, three female) were diagnosed with cancer during the period of 1970-1990. The standard incidence ratio (SIR) for all cancers was 1.5 (95% Confidence Interval [CI] 0.9-2.4) for all workers exposed to alachlor compared to Iowa residents; SIRs were only elevated for male and not female workers. The male workers that were exposed to "high" levels of alachlor had higher rates of colorectal cancer (three cases, SIR = 5.2; 95% CI 1.1-15.1) than residents of Iowa. Two cases of chronic myeloid leukemia were also reported in the alachlor exposed workers (SIR = 40.0, 95% CI 4.8-144.5). However, the authors stated that there was no trend with increased cumulative exposure to alachlor in these cancer cases. No mention was made if any confounding factors were considered or controlled for in this study.

A second study on cancer mortality and cancer incidence in the same alachlor manufacturing worker cohort extended the observations through 1993 (Acquavella et al., 1996). Of the 1,025 white males and females followed for cancer incidence, 24 workers (breakdown by gender not specified) were reported to have a cancer diagnosis. The State Health Registry for Iowa was the source for the incidence rates of cancer in the general population of Iowa. The SIR for all cancers for workers with exposure to alachlor was 1.4 (95% CI 0.9-2.1). Of the 1,025 alachlor workers, 68% (n = 701) were classified as having high exposures, either occupationally or to the drinking water (a contaminated well was identified in 1975 with an alachlor concentration of 2 mg/L). The SIR for all cancers in workers with high exposures to alachlor was 1.2 (95% CI 0.7-2.0). The cancer most frequently observed in those with a high exposure to alachlor was colorectal cancer, with three cases reported (SIR = 1.9, 95% CI 0.4-5.6). Two cases of chronic myeloid leukemia were also reported in the high exposure group (SIR = 18.6, 95% CI 2.3-67.2), but there were no reports of this type of cancer in those classified as having a prolonged exposure (at least five years) to alachlor. Other types of cancers reported in the high exposure group included non-Hodgkin's lymphoma (NHL) (SIR = 2.4, 95% CI 0.3-8.8; two cases, 0.5 cases expected) and melanoma (SIR = 1.9, 95% CI 0.2-6.7; two cases, 1.1 cases expected). We do not have an explanation as to why some of the SIRs differ in these two studies, other than the number in the cohort was increased by nearly 60 workers in the Acquavella study compared to the Leet study. Cancer mortality rates were also determined in this cohort by Acquavella et al. (1996). Among all alachlor exposed workers (n = 1,036), there were eight deaths due to cancer (Standard Mortality Ratio [SMR] = 0.9, 95% CI 0.4-1.7). However, there were no cancer deaths among workers with five or more years of high exposure and 15 or more years since first exposure to alachlor (SMR = 0, 95% CI 0-1.6).

Neither study reported a higher cancer incidence of the naval cavity, thyroid gland or stomach, as has been reported for alachlor exposed animals in cancer bioassays. However, there is not always direct concordance between the site of a cancer in animal bioassays and humans. Unfortunately, the age range of the cohort was not specified in either report. It is possible that an insufficient amount of time has elapsed between exposure and the time necessary for tumor development. Because the expected SIRs were low, it is possible that the age of this cohort is relatively young. This cohort should continue to be monitored to determine if there is an increased incidence of any other cancers as this cohort ages. Since individual exposure levels were not actually measured, but estimated from employment records, it is difficult to determine how accurate the estimates were of those who were highly exposed to alachlor. Despite its limitations, this study suggests that there is an elevated risk of myeloid leukemia in alachlor manufacturing workers. While the risk of other cancers such as colorectal, NHL and melanoma were elevated, the 95% confidence intervals included one, indicating that the cancer incidences in the alachlor manufacturing workers were not significantly different from the general population of Iowa. However, there is difficulty in interpreting these results, since the rural character of Iowa and its dependence on farming would mean that members of the general population may also have exposures to alachlor through ingestion from contaminated air, groundwater or surface water. It would have been more accurate to compare the alachlor manufacturing workers to a subset of the Iowa population that did not have evidence of other types of exposures to alachlor.

3. Case Control Study:

A case control study has also examined whether pesticide exposure was related to the incidence of multiple myeloma (MM) in a small cohort of 173 white men from Iowa with MM and 650 controls (Brown et al., 1993). This study included all cases of MM in white men in Iowa diagnosed during 1981-84 that were at least 30 yrs old. Diagnosis was confirmed by review of tumor pathology by a pathologist. Controls were identified through random digit dialing, Medicare records and state death certificate files. A standardized questionnaire was used to obtain detailed information on occupation, and potential for exposure to over 115 pesticides, duration of pesticide use, and protective equipment used while handling a pesticide. Cases and controls were matched for age by five year age group and vital status. While there was a slight, but non-significant elevation in the risk of MM among farm workers (odds ratio [OR] = 1.2, 95% CI 0.8-1.7), there were no associations between the use of any specific class of pesticide and the risk of this type of cancer. The OR of MM from mixing or handling alachlor was 0.9 (95% CI 0.5-1.7), based on 13 cases and 73 controls.

4. Ecological Study:

Ecological studies compare cancer incidence or mortality of populations using estimates of exposures for the entire population

to chemicals or classes of chemicals. Individual exposures are not measured. While these types of studies are most useful to generate a hypothesis, they are not valid for testing a hypothesis or for making conclusions about any cause and effect relationship. One ecological study in Minnesota compared cancer mortality rates in different regions of this state based on land use and crops grown (Schreinemachers et al., 1999). Cancer rates in three agricultural areas of the state (region 1, corn and soybeans; region 2, wheat corn and soybeans; and region 3, potato, wheat, and sugar beets) were compared to an urban/forested region of the state (region 4) for the years 1980-1989. Pesticide usage was based on a survey of farmers conducted in 1990 by the Minnesota Department of Agriculture, while cancer mortality rates were based on data collected by the National Center for Health Statistics. While alachlor was cited as one of the most frequently used pesticides on corn and soybeans (regions 1 and 2), many other pesticides were also cited as being used frequently on these crops. Use per acre or per region for the individual pesticides was not provided in the paper.

This study did not find any significant elevation in cancers of the thyroid, nasal cavity or the stomach in regions of Minnesota that planted corn and/or soybeans (region 1 and 2) compared to urbanized areas of the state (region 4). These results and other trends are summarized below. In region 1 and 2, the Standardized Mortality Rate Ratio (SRR) in males for cancers of the stomach was close to one. Cancer mortality was slightly, but not significantly elevated for cancers of the nasal cavity, middle ear and sinuses in region 1 (SRR = 1.58; 95% CI 0.85-2.96), and for the thyroid cancer mortality in region 2 (SRR = 1.88; 95% 0.69-5.10), while in region 1 mortality rates from thyroid cancer were decreased (SRR = 0.60; 95% CI 0.29-1.22), but did not achieve statistical significance. For women, cancer mortality rates in regions 1 and 2 were not affected positively or negatively for cancers of the stomach, or in region 1 for cancer of the thyroid. Mortality from cancer of the thyroid decreased, but not significantly, in region 2 (SRR = 0.44, 95% CI 0.017-1.15), and for nasal and sinus cancers in region 1 (SRR = 0.62; 95% CI 0.32-1.19). This study did not show that there were higher mortality rates for cancers of thyroid, nasal passages or stomach, which are the cancer sites that have been associated with the carcinogenicity of alachlor in experimental animal cancer bioassays. However, the results of this study are difficult to interpret, because the usage patterns for pesticides assessed in 1990 would not reflect use patterns of ten to 20 years ago when many of the cancers with a long latency would have been initiated. To determine if there is a relationship between the use of certain pesticides and cancer mortality or cancer incidence, prospective studies are needed that assess pesticide use and individual exposures and then follow the occurrence of cancer over the next ten to 20 years.

B. Experimental Animal Studies:

The majority of the studies evaluating the oncogenicity of alachlor in experimental animals have been conducted on behalf of the registrant, Monsanto, in support of the registration of alachlor containing products. Most of these cancer bioassays and related mechanistic studies are not published in the peer-reviewed literature. Summaries of the studies included here are from EPA documents, including water criteria documents and health advisory reports (USEPA, 1987), toxicology reports used in support of establishing MCLGs and EPA Carcinogenicity Peer Review memorandums (Dapson and McMahon, 1996; Hauswirth, 1987; Mahfouz, 1984; Taylor, 1982). Studies have documented an increased incidence of lung tumors in mice, and stomach, thyroid and nasal turbine tumors in rats fed alachlor over an 18 to 24 month period. Further studies have attempted to determine the mechanism responsible for the alachlor-induced nasal turbine tumors and thyroid gland tumors and the relevance of the observed effects to humans.

1. Mice:

In a study conducted by Bio-Dynamics, Inc. (Daly et al., 1981a) as cited in (Dapson and McMahon, 1996; Marcus, 1987; USEPA, 1990b), technical grade alachlor (purity not specified) was fed via the diet to male and female CD-1 mice (50/sex/dose) at 0, 26, 78, or 260 mg/kg/day for 18 months. The levels of alachlor used in this study may have exceeded the maximum tolerated dose, as evidenced by an increased trend in mortality with increasing dose of alachlor in the female mice. In the female mice, there was 56% mortality in controls compared to 69% mortality in the high dose 260 mg/kg alachlor group. Mortality rates were not affected in the alachlor treated male mice. Significant dose-related trends ($p<0.05$) were observed for an increased incidence of lung bronchiolar-alveolar adenomas in high dose male mice 10/46 (22%) (observed number of tumors/number of animals examined; value as percent) compared to controls 6/47 (13%). There were, however, no significant differences between treated groups and control groups when pair-wise comparisons were made. In high-dose female mice there was a significant trend ($p<0.01$) for an increased incidence of bronchiolar-alveolar adenomas, as well as a significant difference in tumor incidence when pair-wise comparisons were made between the 260 mg/kg/day dose group (10/37, 27%, $p<0.01$) and controls (2/41, 5%). These results indicate that there was a dose-response effect. The inert vehicle used for the first 11 months of the study, 0.5% epichlorhydrin, had previously been shown to induce tumors in rats. Epoxidized soybean oil was used as a stabilizer for the remainder of the study. Although it was suspected that the epichlorhydrin may have been responsible for the cancer effects observed in this study, repetition of the study without the use of epichlorhydrin as the stabilizer resulted in lung tumors observed in the male, but not in the female mice (see below, 1994 EHL mouse study).

A second mouse alachlor oncogenicity study was conducted by the Environmental Health Laboratory (EHL) in 1994 (EHL, 1994) as cited in (Dapson and McMahon, 1996). CD-1 male mice (50/dose) received 0, 16.64, 65.2, or 262 mg/kg/day, while the female mice received 0, 23.73, 90.34 or 399.22 mg/kg/day of alachlor (purity not specified) in the diet for 18 months. There were significant differences in pair-wise comparisons for the incidence of bronchiolar-alveolar ademomas in male mice in the 16.64 mg/kg/day group (11/49, 22%; $p<0.05$); the 65.42 mg/kg/day group (15/50, 30%; $p<0.01$); and the 262.40 mg/kg/day group (12/49, 24%; $p<0.05$); compared to controls (4/48, 8%). While there was an increased incidence of lung tumors in all alachlor treated male mice compared to controls, there was not a demonstrated dose-response relationship, since the incidence of tumors did not increase with the alachlor dose. Also, since this type of lung tumor is a common tumor in the aging CD-1 mouse, a significance level of $p<0.01$ would more accurately reflect a treatment effect. By this criteria a treatment effect on the incidence of lung adenomas was only demonstrated in the male mice receiving 65.42 mg alachlor/kg/day. This is the only group that had some mice with malignant lung carcinomas (3/50, 6%). This does give some limited evidence of the ability of alachlor-induced benign lung tumors to progress to a carcinoma in treated animals.

Alachlor treatment did not significantly affect the incidence of bronchioalveolar tumors in the female mice, even though the doses of alachlor used in the EHL study were higher than the doses used in the Bio-Dynamics study which previously had found a higher incidence of lung tumors in the female mice in the 260 mg/kg/day alachlor treatment group (Daly et al., 1981a). There was no evidence of a significantly increased incidence of malignant lung tumors (carcinomas) with alachlor treatment in either the Bio-Dynamics or the EHL mouse bioassay in either gender.

Monstanto Co. noted that the incidence of lung tumors in the first mouse Bio-Dynamics study (Daly et al., 1981a) was within the historical range of 0-41% for lung tumors in the CD-1 mouse reported previously by Sher (Toxicology Letters 11:103-110, 1982). The average incidence of lung adenomas in the female alachlor treated rats in the Bio-Dynamics study (Daly et al., 1981a) was 17% which is within the historical range. However, an EPA peer review committee convened in 1987 noted that the Bio-Dyanamic alachlor oncogenicity study only had a duration of 18 months, while historical range studies usually followed mice for at least two years. This would make a comparison of the 18 month data and the historical range data based on longer durations invalid. The EPA peer review panel pointed out that concurrent controls would more accurately reflect any background incidence of the lung tumors in CD-1 mice (Hauswirth, 1987). But, debate on whether the lung adenomas were related to alachlor administration was also considered by the EPA peer review committee in 1996 (Dapson and McMahon, 1996). The 1996 peer review committee noted that there was a lack of reproducibility between the two mouse oncogenicity studies, with the first Bio-Dynamics study demonstrating lung adenomas in the females and not the male mice, while the later EHL study demonstrated statistically higher incidence of lung tumors only in the male mid-dose group and in none of the alachlor treated female mice. The committee noted that there was no evidence of progression to malignancy, however there is some evidence of progression to malignancy in the male mid-dose group in the EHL study, as was stated previously. It was also noted that alachlor treatment did not cause any tissue injury or cell proliferation in the lung in either study, indicating a lack of lung toxicity (Dapson and McMahon, 1996).

However, the 1996 EPA carcinogenicity peer review panel did not consider that alachlor may be acting as a lung tumor promoter. The tumor promoting capabilities of alachlor have been demonstrated in the rodents treated with the carcinogen DEN followed by alachlor treatment (Kurata et al., 1993). Alachlor may have the capability to promote these "common" lung adenomas in the CD-1 mouse. Because the CD-1 mouse is an outbred strain, it may explain the differences in the pattern of lung tumor incidence in the two mouse studies. Though alachlor is not genotoxic, others have demonstrated that some of its metabolites are genotoxic (Tessier and Clark, 1995). Although the mechanism by which alachlor is a lung carcinogen has not been identified, this is in itself not sufficient evidence to totally discount the data in these studies, which do provide limited evidence of the induction of lung adenomas in alachlor treated mice.

2. Rats:

In the first of a series of chronic exposure studies, Long-Evans rats (50/sex/dose) were fed 0, 14, 42, or 126 mg/kg/day of alachlor (as Lasso Technical, 92.5% pure) starting at 50 days of age in the diet for 117 weeks in males and for 106 weeks in females (Daly et al., 1981b) as cited in (Dapson and McMahon, 1996; Taylor, 1982; USEPA, 1990b). For the first 11 months, the diet contained 0.5% epichlorohydrin as a stabilizer, while for the last 16 months, the stabilizer was switched to epoxidized soybean oil. Control diet consisted of untreated Purina Lab Chow, R-5001. There was a significant trend for mortality to increase as the dose of alachlor increased ($p<0.05$) in the high dose group compared to controls for both sexes. This suggests that the 42 mg/kg/day dose of alachlor was probably the maximum tolerated dose. For male rats, there was a significant increase in nasal respiratory epithelium adenomas in the mid- (10/41, 24%; $p<0.01$) and high-dose groups (23/40, 58%; $p<0.01$) compared to controls (0/46, 0%). The incidence of nasal turbinate adenomas were also significantly increased in females in both the mid-dose (4/41, 10%; $p<0.05$) and high dose groups (10/41, 24%; $p<0.01$) compared to controls (0/47, 0%). Incidence of nasal adenocarinomas were not affected by alachlor treatment in either the male or female rats (Dapson and McMahon, 1996).

There were also significant increases in the incidence of mixed malignant gastric tumors in the high dose male group (11/41, 27%, $p<0.01$) compared to controls (0/43, 0%), and in stomach osteosarcomas in the high dose group (2/37, 5%, $p<0.05$) compared to controls (0/39, 0%). The incidence of leiomyosarcomas or gastric adenocarcinomas of the stomach in male rats was not affected by alachlor treatments when pair-wise comparisons were made. For the female rats, only the incidence of mixed gastric tumors was elevated in pair-wise comparisons of the high-dose and control groups (16/41; 39% in 126 mg/kg/day group; 0/45 in controls, $p<0.01$). The incidence of stomach leiomyosarcomas, osterosarcomas or gastric adenocarinomas, were not affected by alachlor treatment in the female rats in this study (Dapson and McMahon, 1996).

Thyroid follicular cell adenomas were also significantly increased in the high dose male group (11/45, 24%; $p<0.01$) compared to controls (1/47, 2%), as well as in thyroid follicular cell carcinomas (high dose group 2/37, 5%; controls 0/42, 0%; $p<0.05$). Only the incidence of thyroid adenocarinomas was significantly elevated in the high dose female mice (2/38, 5% 126 mg/kg/day; 0/45, 0% in controls). The incidence of thyroid carcinomas was unaffected in the female rats (Dapson and McMahon, 1996).

While the incidence of liver tumors (adenoma plus hyperplastic nodules) increased in both the male and females in the mid- and high-dose groups, this effect was not statistically significant. However, in an EPA review of this study, it was suggested that this effect on liver lesions was dose-related, though no statistical tests were performed for a trend effect (Taylor, 1982). Alachlor did appear to induce toxic effects in the liver of both sexes of alachlor treated animals. Some of the pathological lesions included: an increased incidence of periportal hepatocyte hypertrophy, cytoplasmic laminated bodies, and central lobular hepatocyte necrosis (Taylor, 1982).

A second bioassay was conducted using much lower levels of alachlor (Stout, 1984) as cited in (Dapson and McMahon, 1996; Mahfouz, 1984; Taylor, 1984; USEPA, 1990b). Alachlor was administered in the diet as alachlor technical (94.13% purity) at 0. 0.5, 2.5, or 15 mg/kg/day to seven week old female and male Long-Evans rats (50/group/sex). However, even with these lower doses, in the females there was still a significant trend ($p<0.01$) of higher mortality in the 15 mg/kg/day group (72% mortality) compared to controls (56% mortality). Relatively high mortality rates in the male rats in the 15 mg/kg dose group (54% mortality) compared to lower dose groups (21% mortality) may not have achieved statistical significance because of the abnormally high mortality rate in the control males (66% mortality) (Mahfouz, 1984).

There was a significantly higher incidence of nasal respiratory epithelium adenomas in high dose alachlor treated male rats receiving 15 mg/kg/day (11/45, 24%; $p<0.01$) compared to controls (0/45, 0%). There were no other alachlor-related tumor incidences that were significantly elevated in the male rats. Pair-wise comparisons indicated that nasal respiratory epithelial adenomas in female rats were also significantly increased in the 15 mg/kg/day group (9/34, 26%; $p<0.01$) compared to controls (0/38, 0%) (Dapson and McMahon, 1996). [Author's note: a previous EPA memo dated 11/19/84 (Mahfouz, 1984), had different denominators when specifying tumor incidences for nasal turbinate adenomas for this study. Incidence in males were 15/45 in the 15 mg/kg/day group and 0/44 in controls; females 14/48 in the 15 mg/kg/day group and 0/42 in controls.] In addition to this neoplastic lesion, alachlor also induced non-neoplastic proliferative changes in the nasal submucosa gland (Mahfouz, 1984; Taylor, 1984). Induction of nasal submucosal gland hyperplasia was significantly elevated ($p<0.01$) in both male (incidence in treated 21/49 vs. controls 2/50) and female animals (incidence in treated 11/48 vs. controls 2/49) in the high dose 15/mg/kg/day groups compared with controls (Mahfouz, 1984).

A significantly increased incidence of benign adrenal pheochromocytomas were also observed in the females in the high dose group (5/48, 10%, $p<0.05$) compared to controls (1/42, 2%). The incidence of thymus malignant lymphosarcomas in male mice was significantly elevated in the 15 mg/kg/day alachlor treated group (3/43, 7%; $p<0.05$) compared to controls (0/48, 0%) (Mahfouz, 1984).

There was also an increase in the incidence of thyroid follicular cell tumors in the 15 mg/kg/day male group (13.3%) compared to controls (6.7%). In an evaluation of this report by the EPA, it was noted that while this effect was not statistically significant, this effect was considered to be biologically significant (Mahfouz, 1984). Gastric tumors were not induced in this study. This is in contrast to the previous rat study (Daly et al., 1981b), which did detect gastric tumors in animals fed the 126 mg alachlor/kg/day diet.

A third study was also conducted concurrently with the second study (Stout, 1984). The third study ran a "fourth" treatment group at 126 mg/kg/day in the diet. This study is of limited use for evaluating the oncogenic potential of alachlor, since its primary purpose was to use a variety of dosing regimes to determine the reversibility of alachlor ocular lesions (uveal degeneration syndrome). The animals receiving 126 mg/kg/day alachlor were divided into three groups (50 rats/sex/group): Group 1, was sacrificed after eight months treatment with alachlor in the diet (126 mg/kg/day); Group 2, treated with 126 mg alachlor kg/day in the diet for up to 5.5 months, followed by treatment with control diet for the remainder of the study; Group 3 received the 126 mg alachlor/kg/day in the diet for two years. There was bias in assignment of animals, since the second group of animals was

chosen primarily on the basis of having early ocular effects. The most significant finding of this study was a high incidence of nasal tumors in Group 2 which had only been exposed to alachlor for up to 5.5 months. This suggests that alachlor-induced nasal tumors appear during the first quarter of the rats' lives. Similarly, one of the females receiving 126 mg/kg/day developed an undifferentiated stomach sarcoma after only 5.5 months of treatment. The total incidence of stomach tumors in Study 3 was 19/31 in the females ($p<0.001$) and 3/68 in males (USEPA, 1990b). Since malignant stomach tumors were also observed in the first rat study conducted in 1981, it is likely that the gastric tumors in alachlor treated groups were not due to the epichlorohydrin stabilizer used for the first part of Study 1 (Marcus, 1987; USEPA, 1990b). Similarly, a high incidence of nasal turbine tumors were observed in the male (49/61) and female (13/25) rats in Study 3 when epichlorohydrin was not used as the stabilizer (Mahfouz, 1984). The EPA concluded that "This study (study 3) (Stout, 1984) indicates that the tumor response observed in the earlier study (Daly et al., 1981b) cannot be explained on the basis of the presence of epichorhydrin in the test material, and suggests that a partial lifetime exposure (approximately one-fourth the lifespan of the animals) can result in a tumor incidence similar to that of a lifetime exposure" (Marcus, 1987).

3. Discussion of Mechanisms of Tumor Induction

a. Mechanisms of Thyroid Tumor Induction:

It should be noted that thyroid tumors were not observed in the second rat alachlor bioassay at the lower dose levels of alachlor in either the male or female rats (Stout, 1984). This is in contrast with the elevated incidences of thyroid tumors that were reported in the first 1981 rat study in both the male and female high-dose 126 mg/kg/day alachlor treated groups (Daly et al., 1981b). Others have conducted studies to determine the mechanism of benign thyroid follicular cell tumor induction in alachlor treated Long-Evans rats (Wilson et al., 1996). Male Long-Evans rats were fed a diet containing 0 mg/kg/day (control) or 126 mg alachlor (94.6% purity) per kg bdwt/day for 7, 14, 29, 60 and 120 days (n=14 to 20 animals per group). An additional 20 animals were maintained on the alachlor diet for 60 days, and then were fed the control diet for an additional 60 days to determine if alachlor-induced effects were reversible. At the end of the indicated treatments, animals were killed and liver and thyroid weights, serum levels of triiodothyronine (T3), thyroxine (T4), and thyroid stimulating hormone (TSH), and hepatic uridine 5'-di-phosphate glycuronyl transferase (UDPGT) activity were determined.

Circulating levels of TSH, liver and thyroid weights, and hepatic UDPGT activity were all elevated for at least some of the time points in the alachlor treated rats, and some of these changes occurred as early as the seventh day of treatment. TSH levels were elevated at all time points in alachlor treated animals compared to controls, but this effect was statistically significant only for the 14, 29 and 60 day time points. T3 levels were elevated in alachlor treated animals compared to controls at all time points, except day 28. Levels of T4 were more variable in alachlor treated rats. T4 levels were significantly depressed at day seven, were significantly elevated on day 14, were lower in alachlor treated animals compared to controls on day 28, and were similar to control levels on days 60 and 120. UDPGT activity was higher in alachlor treated animals than in corresponding controls for all time points, though this effect was statistically significant only at the 28 and 60 day time points. The changes in T3, TSH, hepatic UDPGT activity and liver weights were reversible when animals were returned to a control diet for 60 days after prior treatment with alachlor. Thyroid weight, however, did not completely return to normal after alachlor administration ceased (Wilson et al., 1996).

The authors of this study noted that others have shown that chronic exposure of the rat thyroid gland to sustained levels of TSH can result in hyperplasia and neoplasia (Furth, 1968). The TSH levels may in turn be elevated in response to low levels of T3 and T4 through a negative feedback loop through the pituitary gland.

The authors hypothesized that the thyroid tumors observed in high-dose alachlor treated male rats result from the induction of TSH (Wilson et al., 1996). It is possible that alachlor may elevate hepatic UDPGT activity in the liver, with corresponding decrease in circulating T3 and T4 levels, and a subsequent increase in TSH. Elevated TSH levels may then result in a hyperplastic response in the thyroid leading to benign thyroid tumor formation. This suggests that the mechanism of thyroid tumor induction is a result of a non-genotoxic disruption of the pituitary axis (Dapson and McMahon, 1996; Wilson et al., 1996).

b. Mechanisms of Gastric Tumor Induction:

Gastric tumors in alachlor treated rats were only observed in the 1981 study at the highest dose of 126 mg alachlor/kg/day (Daly et al., 1981b), a level deemed by the EPA to be excessively toxic to both the male and female rats (Dapson and McMahon, 1996). Similar results were not observed in the later 1984 study that used 15 mg/kg/day as the highest dose of alachlor (Stout, 1984). A third study conducted concurrently also reported gastric tumors in male and female rats treated with a 126 mg/kg/day diet that did not contain epichlorohydrin as the stabilizer (Stout, 1984).

Studies have been submitted to EPA to elucidate the mechanism of the gastric tumors in alachlor treated rats. Monsanto has conducted a gastric tumor promotion study of alachlor in Long-Evans rats (as cited on page 23 of Dapson and McMahon, 1996; study conducted by Environmental Health Laboratory for Monsanto Co., Monsanto Study No. ML-93-137, Monsanto EHL Study # EHL 93049, Feb. 3, 1995). Male and female Long-Evans

rats (80/sex) were dosed by gavage with a known gastric tumor initiator N-methyl-N'-nitro-N-nitroso-guanidine (MNNG). The MNNG dosed animals were divided into four groups of 20 animals/sex. One group was not treated with alachlor (only treated with MNNG), a second group received 8,000 ppm catechol in the diet, and two groups received alachlor via the diet at 15 or 126 mg/kg/day for one year. An additional group of 20 animals/sex that were not MNNG treated received a single oral dose of DMSO followed by alachlor treatment at 126 mg/kg/day via the diet for one year. Near the end of the study an additional group of 15 animals/sex was obtained to serve as untreated controls, and to obtain blood samples for baseline serum gastrin levels. At the end of the study, stomach pH, gastric acid secretion over four hours, and serum gastric levels were determined in the MNNG initiated animals (no alachlor promotion) and the DMSO-alachlor treated animals. No data were available on the serum gastrin levels or gastric pHs of MNNG-alachlor treated animals.

Alachlor was found to promote the development of glandular stomach tumors in females and to a more limited extent in male rats. There were significantly more male rats with glandular fundic tumors in the MNNG-high dose alachlor group ($n = 6$) compared to the animals that received only MNNG and no alachlor ($n = 0$). In females, 14 animals were reported with glandular fundic tumors in the MNNG-high dose alachlor group, compared to no animals with these types of tumors in the MNNG controls. No stomach tumors were reported in the MNNG-low dose alachlor treated rats in either sex. The male rats treated with DMSO and alachlor but no MNNG treatment did not have any stomach tumors, while three mixed gastric fundic tumors and one fibroma was reported in the females treated with DMSO and alachlor alone. In the DMSO-high dose alachlor treated females, serum gastric levels were significantly elevated (678.3 pg/ml, $p < 0.01$) compared to MNNG controls (38.5 pg/ml); gastric pH was also elevated in the DMSO-alachlor females (pH 4.5, $p<0.01$) compared to MNNG controls (pH 2.87). In males, while serum gastrin levels were elevated in DMSO-alachlor treated animals compared to controls, gastric pH was unaffected. The mucosa in the fundus was also atrophied in the MNNG-high dose alachlor animals and the DMSO-high dose alachlor group compared to MNNG-low dose alachlor and MNNG alone groups (Dapson and McMahon, 1996).

However, no data on gastrin levels or gastric pH were provided for the MNNG-alachlor treated animals, so it is not known if gastric pH or gastrin levels were similarly affected in the animals where gastric tumors were detected. This was a serious flaw in the design of the study. Another structurally related pesticide, butachlor, also has the ability to promote gastric tumors in MNNG initiated Sprague-Dawley rats. Monsanto contends that both products do not initiate the development of gastric tumors, but promote tumors through a non-genotoxic mechanism by affecting gastrin levels and altering gut pH.

There are some flaws in these conclusions. The promotional studies were only of one year duration, not two years that is used for a cancer bioassay. The shorter duration of these promotional studies may explain why no gastric tumors were observed in the DMSO-alachlor treated animals, and why alachlor-induced tumors were observed in the previously conducted two year long alachlor rat cancer bioassays that did not treat the animals with any known gastric tumor initiator (Daly et al., 1981b). Although alachlor itself does not appear to be genotoxic, some of its metabolites have been identified as being genotoxic (Tessier and Clark, 1995). It is possible that alachlor has the capacity to be both an initiator and a promoter of gastric tumors when given at high levels in experimental animal feeding studies. It should be noted, however, that the high levels of alachlor needed for gastric tumor induction have been deemed by the EPA as being excessively toxic to the animals. The lack of gastric tumors at lower doses of alachlor does suggest a possible threshold-effect of gastric tumor induction, which may be associated with the dose-dependent alachlor induced mucosal atrophy. Alternatively, it is possible that at higher doses, mutagenic intermediates are formed to a greater extent, and these metabolites may be responsible for the induction of the gastric tumors in alachor treated animals.

c. Mechanism of Nasal Turbine Tumor Induction:

Studies have also been submitted to EPA in support of a non-genotoxic mechanism of alachlor-induced nasal turbine tumors in Long-Evans rats. The registrants argue that the Long-Evans rat is particularly susceptible to the induction of nasal turbine tumors because of differences in the metabolism of alachlor in the rat compared to mice or primates. The registrants for alachlor have hypothesized that in the rat, alachlor is metabolized to glutathione (mercapturic acid) conjugate, which is excreted via the bile into the intestine. The conjugate may be metabolized to the thiol conjugate, and then with S-methylation, the methyl sulfide is formed, reabsorbed and the secondary sulfide is hydrolyzed by arylamidase to produce the 2,6-diethylanaline metabolite. When oxidized, the diethylanaline metabolite forms diethylbenzoquinoine imine (DEBQI). This toxic metabolite may then bind to cellular proteins and induce cell death, followed by tissue regeneration, cell proliferation, and formation of spontaneous mutations. Studies supporting this hypothesis were available in summary form in an 1996 EPA Alachlor Carcinogenicity Peer Review memorandum (Dapson and McMahon, 1996). These studies are unpublished. Several of the more important studies will be summarized below and will be identified by their MRID numbers when available. However, we cannot offer a full critique of these studies since details of the experimental design and complete results were not available in the 1996 Alachlor Carincinogenicity Peer Review memorandum (Dapson and McMahon, 1996).

In vitro studies submitted by Monsanto have demonstrated that the rat has the capacity to produce many of the intermediates that

can lead to the formation of DEBQI. Studies comparing the *in vivo* metabolism of alachlor in different tissues of the Long-Evans rat (MRID# 42852110, as cited in Dapson, 1996) have demonstrated that arylmidase activity is present in liver and nasal tissue with the formation of the 2,6 diethylaniline metabolite. Further studies (MRID# 42852111, as cited in Dapson and McMahon, 1996) demonstrated that the velocity of the nasal aryl amidase reaction was 14 to 32 times higher in the rat nasal tissues compared to the velocity in mouse nasal tissues, suggesting that one of the key enzymes involved in the formation of DEBQI is more active in the rat mucosa than in the mouse mucosa. Mice do not form nasal tumors in response to alachlor treatment. Other studies have also demonstrated (MRID# 43482301, as cited in Dapson and McMahon, 1996) that the velocity of several reactions, including the glutathione conjugation, hydrolysis of the secondary sulfide, and diethylanaline hydroxylation was higher in sub-cellular fractions (cytosolic, microsomal) from rat nasal tissue compared to human nasal tissue.

Whole body autoradiography studies (MRID# 42852103, as cited in Dapson and McMahon, 1996) using ^{14}C-alachlor were conducted to determine the localization of alachlor in the Long-Evans rat compared to the CD-1 mice and squirrel monkeys. These studies found that location of radiolabled alachlor in nasal turbines was highest in the rat, less prominent in the mouse and absent in the monkeys (actual levels and statistical significance not available). More radioactivity was found in the intestines of rats than in mice or monkeys, suggesting a slower elimination from the mouse or monkey gut, perhaps because of comparatively greater enterohepatic circulation of alachlor metabolites in the Long-Evans rat. Other studies (MRID# 42852105, as cited in Dapson and McMahon, 1996) have shown that in rodents orally dosed with radiolabled alachlor, nasal tissue localization of radioactivity was higher in Long-Evans rats compared to Sprague-Dawley rats or Fischer 344 rats, and was absent in hamsters.

Other studies have investigated alachlor's capacity to induce cell proliferation in the nasal mucosa of Long-Evans rats compared to CD-1 mice (MRID# 42852102, as cited in Dapson, 1996). Alachlor did not induce cell proliferation in the nasal mucosa of CD-1 mice, while alachlor produced a dose-related increase in cell proliferation in the nasal tissue of Long-Evans rats, and this cell proliferation effect was reversible after alachlor treatment was stopped.

These studies do suggest that alachlor can produce a reversible cell proliferation in the nasal turbines of the Long-Evans rat that is not seen in CD-1 mice. Although CD-1 mice appear to have many of the enzymes involved in the putative formation of the toxic DEBQI intermediate, the velocities of the reactions involved appear to be higher in the Long-Evans rat than in the mouse or in the primates. There may be differences in the metabolism of alachlor compared to mice or monkeys, suggesting that alachlor may be more extensively metabolized in the rat. These studies do support the general finding that the Long-Evans rat is particularly sensitive species to the formation of alachlor nasal-turbine tumors. However, there were no studies that showed that the reactions leading up to DEBQI formation could not occur in humans; these reactions just appear to occur at a higher velocity in the rat compared to human tissues based on *in vitro* studies.

There are other aspects of the Monsanto hypothesis that are not well supported by the existing data. There were no studies that attempted to localize DEBQI in nasal tissues in the *in vivo* or *in vitro* studies conducted by Monsanto. Therefore, there is no evidence that DEBQI is actually present in the nasal passages, nor were any studies conducted to show that this intermediate induces a higher rate of apoptosis prior to the induction of cell proliferation in the rat nasal passages.

It was also argued by the registrant that the hypothesized DEBQI-related mechanism of nasal tumor induction in the rat is probably non-genotoxic. However, others have found that certain alachlor metabolites are weakly mutagenic in tester strains of bacteria. As will be discussed in the genotoxicity section of this evaluation, three alachlor degradative products (2-hydroxy-2',6'-diethylacetanilide, 2-chloro-2',6'-diethylacetanilide and 2',6'-diethylacetanilide) are weakly mutagenic to *S. typhimurium* strain TA 100 (Tessier and Clark, 1995). The mutagenic effects of 2, 6-diethylacetanilide were only observed at the highest dose tested. It is possible that the tumor induction in the nasal turbines of the male Long-Evans rat may occur by multiple mechanisms, including a mutagenic effect that is most evident at high doses of alachlor, and by the hypothesized enhanced cell proliferation induced in response to cell regeneration after induction of cell death by a toxic alachlor metabolite. However, there is not convincing evidence that humans do not have the capacity to form nasal tumors by either mechanism; the evidence presented primarily has focused on identifying the Long-Evans rat as a particularly sensitive animal for the induction of nasal turbine tumors.

C. Current Classification of Carcinogenicity by Other Agencies

1. IARC Classification: not classified

2. EPA Classification: EPA classified alachlor as "likely" to be a human carcinogen at high doses, but "not likely" at low doses (USEPA, 1998b). It was agreed that a "margin of exposure" (MOE) approach (indicative of a non-linear dose response) should be used in the cancer risk assessment. Based on the MOE approach, the No Observable Effect Level (NOEL) for dietary (food and water) exposure was set at 0.5 mg/kg/day for nasal tumors and 14 mg/kg/day for stomach tumors. However, the EPA felt that the use of

MOE in making regulatory decisions had not been fully developed, and then recommended that both a MOE approach and a Q_1 approach be taken for cancer risk assessment. The Q_1 approach assumes that any exposure could lead to cancer, and is indicative of a linear dose-response relationship. The Q_1 for alachlor has been set at 0.08 mg alachlor/kg/day from food and water. EPA has estimated that the carcinogenic risks from exposure to food and water range from 7.8×10^{-7} to 1.4×10^{-6} in adult women and men using the Q_1 approach, and range from 2.9×10^{-4} to 1.4×10^{-6} using the MOE approach (USEPA, 1998b).

3. NTP Classification: not classified (USDHHS, 1998).

IV. Critical Evaluation of the Evidence for Breast Cancer Risk

A. Human Studies

1. Occupational Cohort Study:

Cancer incidence and mortality has been evaluated in a cohort of 1,169 alachlor manufacturing workers (including 245 women) that were employed for at least one year at the Monsanto plant in Muscaline, Iowa (Acquavella et al., 1996). Exposure to alachlor was through occupational exposures or through the drinking water (a contaminated well was identified in 1975 with an alachlor concentration of 2 mg/L). Cancer mortality rates were assessed from 1968-1993, and cancer incidence rates were assessed from 1969-1993. For the entire cohort, there was one case of breast cancer observed and 1.2 cases expected. There were no breast cancer cases reported and 0.2 cases expected for those employed more than five years. For breast cancer mortality, there were zero cases observed and 0.3 cases expected. While the results of this study suggests that alachlor work related exposures are not related to breast cancer risk, the size of the female portion of this cohort is small, and it is not possible to make a definitive conclusion about the results of this study. Additonal efforts should be made, if possible, to expand the size of this cohort to increase the statistical power of the study. The age-range of this cohort was not specified. Therefore, follow-up of this cohort should continue, because of the long latency for the development of breast cancer, and the increased incidence of breast cancer with age.

2. Case-Control Study:

Case-control studies which have evaluated breast cancer incidence in alachlor exposed populations compared to non-exposed populations have not been located.

3. Ecological Study:

There is one ecological study which has compared cancer mortality from 1980 to 1989 in several agricultural regions of Minnesota to an urban-forested region of this state (Schreinemachers et al., 1999). The agricultural areas were divided into three regions based on crops: region 1 (corn and soybeans); region 2 (wheat corn and soybeans); and region 3 (potato, wheat and sugar beets). The urban-forested region of the state was designated as region 4. A 1990 survey conducted by the Minnesota Department of Agriculture provided information on pesticide use by county clusters that had similar geology and crops. While alachlor was one of the most frequently used pesticides on corn and soybean crops, many other pesticides were also used on these crops. Other pesticides used included 2,4-D, atrazine, bromoxynil, cyanazine, dicamba, s-ethyl dipropylthiocarbamate and metolachlor on corn crops, while bentazon, imazethapyr and trifluralin were used on soybeans. Cancer mortality data was obtained from the National Center for Health Statistics database. Age-standardized mortality ratios (SRRs) were calculated comparing regions 1, 2 and 3 to region 4. The SRR for breast cancer mortality in region 1 (SRR = 1.01; 95% CI 0.96-1.07) and region 2 (SRR = 1.00; 95% CI 0.89-1.12) were not affected positively or negatively, while the SRR for region 3 was significantly decreased (SRR = 0.85; 95% CI 0.75-0.97).

Ecological studies are used primarily for the generation of a hypothesis and not for testing a hypothesis, since by their nature individual exposure estimates of suspect chemicals are not made. The results of this study suggest that breast cancer mortality rates are similar or even decreased in areas with higher use of agricultural pesticides compared to urban areas (Schreinemachers et al., 1999). However, there are flaws in this study. No attempt was made to survey urban pesticide use in non-agricultural regions of the state; pesticide use estimates were only based on agricultural pesticides. Also, since breast cancer mortality typically accounts for only 25% of all breast cancer cases, cancer incidence rates rather than cancer mortality rates may have more accurately reflected if there was a relationship between pesticide use, including alachlor use, and the development of specific types of cancer.

B. Experimental Animal Studies of Mammary Carcinogenicity

1. Mice:

There is no mention of an increased incidence of mammary gland tumors in any of the alachlor mouse cancer bioassays that have been conducted to date as cited in the EPA 1996 Carcinogenicity Peer Review of alachlor (Dapson and McMahon, 1996).

2. Rats:

Rat cancer bioassays provide little evidence to support an oncogenic effect of alachlor in the mammary gland. In one rat bioassay, male and female Long-Evans rats (50/sex/dose) were fed alachlor technical (92.5% purity) at 0, 14, 42, or 126 mg/kg/day starting at 50 days of age for 117 weeks in males and for 106 weeks in females (Daly et al., 1981b) as cited in (Dapson and McMahon, 1996; USEPA, 1990b). For the first 11 months, the diet contained 0.5% epichlorohydrin as a stabilizer, while for the

last 16 months, the stabilizer was switched to epoxidized soybean oil. The control diet consisted of untreated Purina Lab Chow, R-5001. The number of mammary gland adenomas were higher in the treated female rats compared to controls, but this effect was not statistically significant as assessed by one sided Fisher exact test (Taylor, 1982). The incidence of mammary gland tumors was 7/50 (14%) in controls; 14/50 (28%) in the 14 mg/kg/day group; 12/50 (24%) in the 42 mg/kg/day group; and 14/50 (28%) in the 126 mg/kg/day group. The only mammary gland carcinoma reported was found in the control group (1/50); no mammary carcinomas were found in the alachlor-treated female rats (Taylor, 1982). A later EPA evaluation of this study by the 1996 EPA Alachlor Carcinogenicity Peer Review panel noted that when pair-wise comparisons were made, mammary gland adenofibromas were significantly higher ($p<0.05$) in the high dose 126 mg/kg/day alachlor-treated female rats compared to controls. The actual tumor incidence rates were not stated in this EPA document. The panel, however, concluded that the 126 mg/kg/day dose of alachlor used in this study was excessively toxic, and did not further discuss the mammary gland tumor data (Dapson and McMahon, 1996).

In a later rat bioassay using doses of alachlor (as alachlor technical, 94.13% purity) ranging from 0.5 to 15 mg/kg/day, the incidences of mammary gland adenomas were slightly, but not significantly higher in some of the alachlor treated groups of female Long-Evans rats compared to controls (Stout, 1984) as cited in Mahfouz, 1984. The incidence of mammary gland adenomas was 19/42 (45%) in controls; 23/44 (52%) in the 0.5 mg/kg/day group; 23/47 (49 %) in the 2.5 mg/kg/day group; and 20/48 (41.6 %) in the 15 mg/kg/day dose group. The incidence of mammary gland carcinomas was variable, and did not show a dose response relationship in alachlor-treated animals compared to controls. There were no significant differences in mammary gland carcinoma incidence rates between controls and alachlor treated female rats. Incidences of mammary gland carcinomas were 4/42 (9.5%) in controls; 3/44 (6.8%) in the 0.5 mg/kg/day group; 6/47 (12.7%) in the 2.5 mg/kg/day group; and 0/48 (0%) in the 15 mg/kg/day group (Mahfouz, 1984).

These long-term rat and mouse feeding studies indicate that alachlor is not a mammary carcinogen in experimental animals.

C. Other Relevant Data on Breast Cancer Risk

1. Evidence of Estrogenicity:

Few studies have evaluated the estrogenicity of alachlor. We could find no *in vivo* tests of estrogenicity, such as uterotropic wet weight gain in ovariectomized animals. Studies have indicated that in estrogen-dependent human breast tumor cell lines, or in yeast cells transfected with human estrogen receptor (hER), alachlor was not estrogenic, or a very weak estrogen, respectively. There is some evidence that alachlor may be a weak estrogen mimic and interact with non-mammalian estrogen receptor in other species such as the American alligator. These studies are summarized below.

Soto et al. (1995) was unable to demonstrate that alachlor was an estrogenic xenobiotic in the E-SCREEN assay which measures cell proliferation in an estrogen-dependent MCF-7 human breast tumor cell line. There are several *in vitro* studies which have documented that alachlor is a weak estrogen in non-mammalian species. Reproductive abnormalities in alligators residing in Lake Apopka, Florida, have been traced to contaminants known to be endocrine disrupting chemicals. Researchers have investigated the ability for a variety of environmental chemicals, including alachlor and many other pesticides, to compete with radiolabeled 17β-estradiol for binding to the alligator estrogen receptor (aER) (Vonier et al., 1996). These competitive binding assays indicated that alachlor is a very weak estrogen. Alachlor had an IC_{50} of 27.5 μM, which is over 3,500 fold higher than the IC_{50} of 0.0078 μM for 17β-estradiol (IC_{50} is the concentration necessary to inhibit [^{3}H] 17β-estradiol binding by 50%).

These researchers further evaluated the estrogenicity of alachlor in a series of *in vitro* assays (Klotz et al., 1996). The yeast based YES assay (yeast strain BJ2407 transformed with the pSCW231-hER expression plasmid, and the YRPE2 reporter plasmid that contains two estrogen response elements [EREs] linked to the *lacZ* β-glactocidase reporter gene), was used as first a series of *in vitro* estrogenicity screening tests. The fold induction of β-glactocidase was measured in response to treating the transfected yeast to various environmental chemicals. Alachlor was weakly estrogenic in the YES assay; a concentration of 10 μM alachlor induced a 40-fold induction of β-glactocidase. In comparison, only 0.0001 μM estradiol was necessary to induce a 50-fold induction of β-glactocidase in the YES assay. Alachlor was then tested for its ability to inhibit binding of [^{3}H] 17β-estradiol to hER. Alachlor was a very weak inhibitor of tritiated estradiol binding, and did not even achieve 50% displacement when tested at the maximum concentration of 100,000 nM. In the same assay the IC_{50} of cold 17β-estradiol was 1 nM. In the final assay, MCF-7 human breast cancer cells were transfected with a plasmid containing two EREs linked to a luciferase reporter gene. While only 100 pM of 17β-estradiol were necessary to achieve a 46-fold induction of luciferase activity above controls, 1 μM concentration of alachlor only achieved a 23-fold induction of luciferase activity, while 10 μM alachlor did not further increase activity, indicating a lack of a dose-response relationship.

In conclusion, at best, alachlor is a very weak environmental estrogen. Its mild estrogenic effects are more apparent in non-mammalian wildlife species such as the alligator, than in mammals.

2. Reproductive and Developmental Toxicology:
Studies have not demonstrated that alachlor has an adverse effect on reproduction in mammals. Experimental animals studies have not found either reproductive or developmental problems in rodents exposed to a variety of pesticide contaminants, including alachlor, commonly found in Iowa groundwater. Ecological studies in humans have suggested that there is an increased incidence of birth defects in the offspring of pesticide applicators in Minnesota, though these studies did not measure exposures to specific pesticides. These studies are discussed below.

a. Reproduction Studies:
In an unpublished three-generation reproductive study by Schroeder et al. (1981) as cited by (Marcus, 1987), the only adverse effect of administering alachlor at 3, 10 or 30 mg/kg/day was renal toxicity observed in F_2 males and F_3 pups that received the highest dose of alachlor. Adverse affects on fertility or reproductive endpoints were not reported.

A pesticide-fertilizer mix representative of groundwater samples taken from Iowa was developed to test the toxicity and reproductive effective of multiple pesticides, including alachlor (Heindel et al., 1994; Heindel et al., 1997). The low dose for alachlor, termed "1X" was 0.9 ng/ml, and was representative of the median level of alachlor found in Iowa groundwater. The pesticide mix was administered in the drinking water at 1X, 10X and 100X to Swiss CD-1 mice to assess reproductive toxicity using the National Toxicology Program's Reproductive Assessment by Continuous Breeding (RACB) assay. There were no effects on the body weights or vaginal cyclicity of the dams, nor effects on sperm indices in the male breeders in the F_0 mice who received treated water prior to and during mating. Measures of fertility, including number of breeding pairs producing per litter, litters per pair, live pups per litter, sex and sex ratio of pups, and pup weights were unaffected by the treatments. There were no effects on organ weights, histopathology, sperm indices in males (epididymal sperm concentration, percentage motile sperm, percentage abnormal sperm, and testicular spermatid head count), or estrous cyclicity as measured by vaginal cytology in females in the second generation of mice exposed to the pesticide mixture.

b. Developmental Studies:
Pregnant female Sprague-Dawley rats received a mixture of pesticides in their drinking water representing levels of pesticides commonly found in Iowa groundwater to assess developmental effects in the second generation of pups. The mixtures were administered at 1X, 10X and 100X of the median level of the pesticides present in Iowa groundwater; the 1X concentration of alachlor in the rat drinking water was 0.9 ng/ml. The dams demonstrated no signs of toxicity at any of the dose levels tested, and there were no effects on fetal malformations, litter size, or fetal body weight, indicating no adverse effects on development or toxicity in the pups (Heindel et al., 1994).

The frequency of birth abnormalities in pesticide applicator and non-applicator families have been compared in different crop-growing regions of Minnesota (Garry et al., 1996). These regions included the region 1, northeastern forest/urban area; region 2, south and south central corn and soybean crop area; region 3, western wheat, corn and soybean growing area; and region 4, northwestern potato, wheat and sugar beet growing area. In general, pesticide applicator families had significantly higher rates of children born with circulatory/respiratory anomalies (OR=1.62, p=0.05), musculoskeletal/integumental anomalies (OR=1.49, p=0.02), and urogenital anomalies (OR=1.61, p=0.02) compared to non-applicator families. Similar patterns of excess birth defects in these categories was also observed in areas where corn and soybeans were grown (region 1 and 2), and in wheat, sugar beets and potato-growing regions (region 3). Although alachlor is used as an herbicide on corn and soybean crops, the authors did not provide any information on whether use of alachlor was associated with areas that had higher incidences of birth defects in pesticide applicator families. It should be noted that other herbicides are used on many of these agricultural crops, including the triazine herbicide atrazine on corn, and the phenoxy herbicide 2,4-D on wheat. Hence, with multiple use of pesticides in different regions, it is difficult to attribute the observed patterns of congenital birth defects to parental exposure to a particular pesticide.

3. Genotoxicity:
A compound may be genotoxic either by being a mutagen or a clastogen. Mutagens cause small changes in the nucleic acid sequence of DNA, while clastogens cause damage or gross changes to the chromosome as a whole. Most of the mutagenicity tests conducted on alachlor and its degradative products, have found these compounds to be negative or weakly positive mutagens in tester strains of bacteria or yeast. Other studies evaluating the genotoxicity of alachlor have yielded variable results. While the majority of the studies have demonstrated evidence of clastogenicity, not all have demonstrated a dose-response relationship. In several of the studies, clastogenicity was only observed at the highest tolerated dose where cytotoxic effects were also observed. Other studies have not found evidence of genotoxicity of alachlor or its degradative products. These studies are discussed below.

a. Mutagenicity Studies:
Shirasu et al. (1976) tested the mutagenicity of alachlor in the *Escherichia coli* strain WP2 *hcr*, *Salmonella typhimurim* strains TA1538, TA1537, TA1535, TA98 and TA100, and the rec-assay using the H17 Rec+ and M45 Rec- strains of *Bacillus subtilis*. Alachlor tested negative for mutagencity in all test systems.

Eisenbeis et al. (1982) found no evidence of mutagenicity when alachlor, as the full strength commercial preparation (Lasso®) or as an aqueous solution, was tested for mutagenic activity in the same strains of *S. tyrphimurium*. In a later unpublished study authored by Shirasu and colleagues, alachlor mutagenicity was retested in strains of *S. typhimuirum* and in *E. coli.* All mutagenicity tests, conducted with and without S9 metabolic activation, yielded negative results (Shirasu, 1980) as cited in (Marcus, 1987).

Others have hypothesized that plant activation may be necessary for alachlor to be mutagenic. To test this hypothesis Gentile et al. (1977) tested the ability of alachlor-treated corn plant extracts to induce mitotic gene conversion at the *ade* and *trp* loci of the D4 strain of *Sacchromyces cerevisiae*. Plant extracts treated with alachlor had four times the gene conversion compared to control levels. Plewa et al. (1984) tested for alachlor-induced mutations in strains of bacteria (*S. typhimurium*) and yeast (*S. cerevisiae*) with liver microsome activation, plant activation and no activation. Alachlor was also tested for inducing mutations at the *wx* locus of *Z. mays*. The only positive results observed were for a commercial formulation of alachlor tested in *S. cerevisiae* without activation, and in a technical grade formulation of alachlor tested in plant-activated *S. cerevisiae* . There was no evidence for mutagencity in *S. typhyimiun* or *Z. mays* with or with out biological activation. Treatment of animals with alachlor at 50 mg/kg bdwt for five weeks does not yield mutagenic products in the animals urine in *S. tyrphimurium* tester strain TA98 (George et al., 1995).

Others have found some alachlor degradation products and metabolites to be weakly mutagenic. The mutagenicity of alachlor and five degradation products found in groundwater were tested using the *Salmonella*/microsome assay (Tessier and Clark, 1995). Two of the alachlor degradative products, 2-hydroxy-2',6'-diethylacetanilide and 2-chloro-2',6'-diethylacetanilide, were weakly mutagenic to *S. typhimurium* strain TA 100 in at least two of the tested doses. Another degradation product, 2',6'-diethylacetanilide, also tested positive, but only at the highest dose tested (1,000 micrograms/plate). The TA100 strain tests for mutagens that cause base-pair substitutions. All of these alachlor degradative products are secondary acetanlides, suggesting that a structure-activity relationship may underlie the mechanisms of mutagenicity. The other degradative products, 2-hydroxy-2',6'-diethyl-*N*-(methoxymethyl)acetanilide, 2,6-diethyl-*N*-(methoxymethyl)aniline, and the end product of alachlor metabolism, 2,6-diethylaniline, were not found to cause significantly higher reversion rates in the *Salmonella* /microsome assay. It was hypothesized that the degradative products that did test positive exerted a mutagenic effect by acting as toxic electrophiles. To test this hypothesis, glutathione was added to the assay system to induce glutathione *S*-tranferase, a detoxifying enzyme which conjugates electrophilic toxins with glutathione. When a reduced form of glutathione was added to the assay, the mutagenicity of 2,-hydroxy-2',6'-diethylacetanilide was reduced, suggesting that this degradation product may be an electrophillic toxin. Others have hypothesized that a purported alachlor metabolite diethylbenzoquinoneimine (DEBQI), may be responsible for observed oncogenic effects of alachlor. The mutagenicity of DEBQI was tested in *S. typhimurium* strains TA98 and TA100, and was weakly positive in the TA100 strain, indicating the induction of base-pair substitution mutations (Hill et al., 1997).

b. Clastogenicity Studies:

In *in vivo* tests of genotoxicity, most studies have not been able to demonstrate an effect of alachlor treatment. Gebel et al. (1997) dosed 7-12 week old NMRI male (n = 4 per group) and female mice (n = 3 per group) with 50, 59, 100, and 115% of LD_{50} values for males, and 80 to 100% LD_{50} values of alachlor for the females. After 48 hrs, bone-marrow from the femurs were harvested to conduct a micronucleus assay. Alachlor had no genotoxic effect in alachlor-treated male or female animals.

In another study, the extent of cytogenic damage was determined in female B6C3FI mice exposed to levels of pesticides contained in drinking water that simulated the levels found in Iowa groundwater (Kligerman et al., 1993). Exposures were at 1X, 10X and 100X groundwater levels, and included the pesticides alachlor, atrazine, cyanazine, metribuzin, metolachlor and the fertilizer ammonium nitrate. There was no evidence of statistically significant cytogenic damage in spleens assayed for sister-chromatid exchange, chromosome aberrations or micronuclei.

Whether alachlor can induce DNA damage has been evaluated in rats. Taningher et al. (1993) could not find any evidence of alachlor-induced DNA damage by the alkaline elution test in livers of rodents treated with sub-lethal doses of alachlor. Georgian et al. (1983) determined the incidence of chromosomal aberrations in the bone marrow of male Wistar rats given a single i.p. injection of 0, 1.25, 2.5 or 5 μg alachlor per gram bdwt. The highest dose level proved fatal to the animals, the lowest dose had no effect, while at the mid-dose, animals did display an increase in chromosomal aberrations. However, no genotoxic effects were observed in rats (n = 4) orally dosed with 200 ppm alachlor for 280 days. The small number of animals and the lack of multiple doses limits the usefulness of this study. A mouse feeding study by Meisner et al. (1992), which evaluated the *in vivo* cytogenic effects of alachlor and atrazine administered in the drinking water, was also of limited value because the treated animals were only administered one dose of each herbicide (20 mg/kg/day). No cytogenetic effects were observed in the bone marrow cells of male and female mice dosed with alachlor or atrazine alone, while a statistically significant increase in break frequency and percent cells with aberrations was observed in the mice that received both atrazine and alachlor in the drinking water. A similar study with multiple doses of alachlor above the 20 mg/kg/day level needs to

be conducted to see if there is a dose-response relationship between alachlor and induction of clastogenic effects in rodents. Neither alachlor, nor any of its degradation products were found to be clastogens by the micronucleus assay (Tessier and Clark, 1995).

Few studies have been conducted to determine evidence of DNA damage in cells obtained from humans occupationally exposed to alachlor. Yoder et al. (1973) obtained lymphocytes from blood samples obtained in the mid-winter (no pesticides being applied) and during the peak period of summer pesticide application in 42 pesticide applicators and 16 non-applicators. Applicators were exposed to a wide variety of insecticides and herbicides, including alachlor. The results of the study are difficult to interpret because of the lack of statistical analysis of the results. In general, mean levels of chromatid gaps in applicators exposed to herbicides was higher in the summer (1.38 ± 0.22) compared to the off-season (0.38 ± 0.10). Similarly, chromatid breaks in herbicide applicators were higher in the summer (1.81 ± 0.35) than in the off-season (0.07 ± 0.05) (lesions were reported per person per 25 cells scored). Chromatid gaps and breaks for control subjects did not show any seasonal variation.

Possible clastogenic effects of alachlor have been determined in cultures of human lymphocytes, rodent hepatocytes, Chinese lung fibroblasts, Chinese hamster ovary (CHO) cells, and in the root tips of seeds. A dose-dependent increase in the frequency of sister chromatid exchanges (SCE) was observed in cultured human lymphocytes only in assays without an S9 rat liver microsome activation system. A dose-dependent increase in DNA single strand breaks was also observed in V9 Chinese lung fibroblasts using the alkaline elution assay. In contrast, these same researchers could not demonstrate DNA single strand breaks in rat hepatocytes incubated with up to 0.2 μM alachlor (Dunkelberg et al., 1994). Others have demonstrated alachlor-induced single strand breaks by alkaline elution in hepatocytes at much higher alachlor concentrations in the range of 200 to 400 μM. This type of DNA damage was only observed after metabolic activation with rat hepatocytes. Alachlor concentrations at or above 600 μM were cytotoxic (Bonfanti et al., 1992).

The induction of chromosome aberrations has also been demonstrated in CHO cells, but this effect was not observed in S9 metabolically activated CHO cells (Lin et al., 1987). Ribas et al. (1995) demonstrated DNA damage in alachlor-treated (1, 5, 10, or 20 μg alachlor/ml) human lymphocytes both with and without bioactivation (S9 mix) using the single-cell gel electrophoresis (SCGE) assay. However, in a later study Ribas et al. (1996) reported an increase in SCE in human lymphocytes only in the alachlor-treated cells without metabolic activation. The treatment of the lymphocytes with the S9 fraction decreased the induction of SCE by alachlor. A significant increase ($p<0.001$) in the induction of SCE was also demonstrated in human lymphocyte cultures treated with 10 μg/ml alachlor (Ribas et al., 1996). The induction of SCE in cultured CHO cells and in human lymphocytes exposed to alachlor and several metabolites was evaluated by Hill et al. (1997). Alachlor, at 10 μM, and diethylaniline at 3 or 30 μM induced a significant elevation in SCE in CHO cells. However, no induction of SCE was observed in CHO cells for another alachlor metabolite, ethylmethylanaline. Alachlor did induce SCE in human lymphocyte cultures in the highest dose tested (10 μM), but not at 3 or 1 μM. Micronuclei were induced in human lymphocytes treated with 5 to 20 μg/ml alachlor. Using antikinetochlor antibodies and fluorescence *in situ* hybridization (FISH), researchers found that alachlor produced kenetochlor- and centromere-negative micronuclei, suggesting that alachlor acts as a clastogen without aneugenic activity (Surrallés et al., 1995).

The induction of micronuclei in *Vicia faba* root tips was found to be dependent on the organic matter content of alachlor-treated soil (De Marco et al., 1990). When tested in Vico soil that has a high organic matter content, no significant increase in micronuclei was observed, while in San Pastore soil with a lower organic matter content, there was a strong association between induction of micronuclei and the dose of alachlor. The authors hypothesized that the Vico soil absorbed the alachlor, making it less available to the root tips.

Using the *Drosophila* wing spot assay, Torres et al. (1992) demonstrated that alachlor was genotoxic at the four concentrations tested (1, 2, 5 and 10 mM). Genotoxic effects of alachlor have also been demonstrated using *E. coli*. in the Microtitration SOS Chromotest (Xu and Schurr, 1990).

Several studies have investigated whether alachlor or its metabolites have the capacity to form DNA adducts. Researchers have (Brown et al., 1988) determined the extent to which alachlor, and two metabolic products 2-chloro-N-(2,6-diethylphenyl)acetamide (CDEPA) and diethylaniline, form adducts with DNA. This study used ^{14}C-labeled phenyl and methoxy-carbons of alachlor. A four-fold higher labeling to calf thymus DNA was observed from the ^{14}C-methoxy than ^{14}C-phenyl-alachlor. Therefore, the authors suggested that the methoxy group may be responsible for the carcinogenic effects of alachlor. Other researchers, using ^{32}P postlabeling analysis, have demonstrated that alachlor or CDEPA can form covalent DNA-adducts *in vitro* (Ross and Nelson, 1994). Other have synthesized and characterized adducts of alachlor and CDEPA with 2'-deoxyguanosine, thymidine, and their 3'-monophosphates (Nesnow et al., 1995). Results indicated that alachlor and CDEPA had the capacity to form N-1 adducts with 2'-deoxyguanosine and N-3 adducts with thymidine, and alachlor formed N-7 adduct with 2'-deoxyguanosine. In addition, N-1 adducts were described for alachlor and CDEPA with 2'-deoxguanosine 3'phosphate, and N-3 adducts with thymidine 3'-monophosphate.

In conclusion, while there is little evidence for the mutagenicity of alachlor, there is some evidence that its degradative transformation products may be weakly mutagenic, especially at high doses. In addition, there is some evidence that alachlor degradation products have the capacity to induce SCE in CHO cells and human lymphocytes. Both alachlor and CDEPA have the capacity to form DNA adducts.

4. Tumor promotion:
The ability of alachlor and other pesticides to act as liver tumor promoters were evaluated in male F344 rats (Kurata et al., 1993). Rats were injected i.p. with 200 mg/kg with the liver carcinogen diethylnitrosomine (DEN), and two weeks later were treated with alachlor. On week three, animals were subjected to a 2/3 partial hepatectomy, and were killed on week six. Carcinogenic potential was evaluated by comparing areas with positive GST-P positive foci in treated animals given alachlor and DEN versus those given DEN alone. Alachlor was rated as "positive". This paper was only available as an abstract, and further details were not available. It should be noted that alachlor has not been observed to induce hepatic tumors in animal cancer bioassays.

5. Immunotoxicity:
A functioning immune system is an important part of the body's defense system against cancer. Studies in experimental animals have demonstrated that chemical-induced immune dysfunction can be associated with an increased incidence of cancer in laboratory animals (Ward et al., 1984).

Studies that have investigated whether alachlor's carcinogenicity is mediated by changes in the immune system have not been able to demonstrate an adverse effect of alachlor administration. Flaherty et al. (1992), in a study sponsored by Monsanto Agricultural Company, exposed immunocompetent mononuclear cells from human peripheral blood to analytical alachlor (99% pure), alachlor conjugated to human serum albumin, or to the commercial formulation of alachlor, Lasso®, over a concentration range of 0.01 to 1.0 μM. There were no significant effects of the alachlor preparations on lymphocyte proliferation, antibody synthesis of IgG or IgM isotypes in pokeweed stimulated mononuclear cell cultures, cytotoxic T cell proliferation or lysis of target cells by natural killer cells (NK) or lymphokine activated killer cells.

A series of *in vivo* studies on male Fischer 344/N rats conducted by The National Institute for Occupational Safety and Health (NIOSH) also could not demonstrate an adverse effect of alachlor on the immune system (Biagini et al., 1993). Ten week old rats (n = 12) were treated with i.p. injections of alachlor in propylene glycol at 1.25, 2.5 and 3.75 mg/kg bdwt on days 6, 13 and 14 of the study. Alachlor had no significant effect on delayed hypersensitivity reactions to keyhole limpet hemocyanin (KLH), on the production of KLH IgG antibodies, production of NK cells, or on the number of granulocytes, and lymphocyte/monocyte ratio, or on body weight, spleen weight or thymus weight. The authors noted, however, that while rats metabolize alachlor to over 23 metabolites excreted 1:1 in the urine and feces, monkeys only excrete five metabolites primarily via the urine. Therefore, they concluded that while there is little effect of alachlor on immunotoxic assays in rats, it is not known if there is potential for primate-specific metabolites to have an effect which would not be detected in rodent immunoassays.

V. Other Relevant Information

A. Environmental Fate of Alachlor and its Degradation Products:
The environmental fate of alachlor has been extensively reviewed by others (Chesters et al., 1989). The purpose of this section is not to give a comprehensive review of the subject, but rather an overview of research on the environmental fate of alachlor, and especially the more recent research on the fate of its degradation products.

1. Persistency in Soil:
One of the most common degradation products of alachlor detected in both surface and groundwater is 2-[(2',6'-diethylphenyl)-methoxymethyl)amino]-2-oxoethanesulfonic acid. This degradation product is also called alachlor ethanesulfonic acid (ESA). ESA may result from the formation of an alachlor glutathione conjugate (Thurman et al., 1996). The first step is thought to be the loss of a chlorine on the alachlor side chain with the subsequent formation of a sulfur linkage to glutathione. Glutamic acid is removed by a glutamyltranspeptidase, and the glycine moiety is cleaved by carboxypeptidases. The sulfur-carbon bond is cleaved by cystetine-beta-lyases, with the sulfur further oxidizing in soil to the ESA of alachlor. Other transformation products of alachlor identified in soil include: 2-chloro-2',6'-diethylacetanilide, 2,6-diethylaniline, 2',6'-diethylacetanilide, chloroacetic acid, 2'6'-diethyl-N-methoxymethylaniline, 1-chloroacetyl-2,3-dihydro-7-ethylindole, [2-(2',6'-diethylphenyl) (methoxymethyl)amnio]-2-oxoacetic acid (OAA), 8-ethyl-2-hydroxy-1-(methoxymethyl)-1,2,3,4-tetrahydroquinoline, 7-ethyl-1-hydroxyacetyl-2,3-dihydroindole, 2'6'-diethyl-2-hydroxy-N-(methoxymethyl)acetanilide, and 9-ethyl-1-5,-dihydro-1-(methoxymethyl)-5-methyl-4,1-benzoxazepin-2-(3H)-one (Chesters et al., 1989; Gan et al., 1995).

While photodegradation and volatilization contribute to the dissipation of alachlor, the primary route of degradation is through microbial metabolism. The alachlor degradation products known to be produced through microbial degradation are listed in Table 2.

The half-life for alachlor in soil is commonly cited to be in the range of ten to 21 days (Ahrens, 1994; Barrett, 1996; Gish et al., 1991; Paterson and Schnoor, 1992; Wauchope et al., 1992). The half-life of alachlor in soil is dependent on a number of factors, including the moisture of the soil, which affects volatility, formulation, level of organic matter, soil composition, temperature and the concentration of alachlor applied to the soil.

The effect of soil moisture on alachlor degradation was determined in sandy loam soil plots held at 25°C (Walker and Brown, 1985). The half-life of alachlor decreased as soil moisture increased [t 1/2 = 23 days (6% moisture w/w); 8.3 days (9% w/w); 7.4 days (12% w/w); 5.7 days (15% w/w)].

While microbial action is the major route of alachlor degradation in soil, alachlor is lost to some extent by volatilization. Volatilization rates are highest when soil surfaces are moist, and are reduced when soil surface is dry (Glotfelty et al., 1989). Mean volatilization rates for alachlor are much higher (mean loss per day = 10 grams/ha) compared to other corn herbicides such as atrazine (mean loss per day = 1.9 grams/ha). Different formulations also affect persistence. The dissipation half-life of conventional commercial formulation of alachlor was found to range from four to 20 days, while a starch-encapsulated form was more persistent with a half-life ranging from eight to 41 days (Gish et al., 1994).

Soil type, and organic matter content may also affect the half-life of alachlor in soil. In soils with high organic matter content, the persistence of alachlor in soil is greatly affected by concentration. It should be noted that groundwater contamination with alachlor has been attributed to both normal agricultural use and to point-source contamination where the concentration far exceeds levels used in normal agricultural applications. Therefore, dissipation behavior under conditions of high concentrations of alachlor is important to determine. A study was conducted to determine the persistence of alachlor at 10, 100, 1,000 and 10,000 mg per kg soil (based on oven dry weight of soil) in clay loam and sandy loam. Regardless of soil type, at the highest concentration of 10,000 mg/kg soil, alachlor was extremely persistent with a half-life ranging from 12.6 to 13.5 yrs (Gan et al., 1995). In contrast, alachlor was readily degraded at the lowest application rate. The half-life with an application rate of 10 mg/kg soil was of 8.7 weeks in clay loam and 2.8 weeks in sandy loam. When application rates exceeded 1,000 mg/kg, alachlor became very persistent with a half life ranging from 88 to 97 weeks. The authors suggested that the lower rate of degradation with the higher concentrations of alachlor are due to the limited availability of microorganisms to degrade alachlor and because so little of the alachlor would be in solution at the high application rates.

Controlling loss of alachlor via leaching to susceptible groundwater sources, and preventing volatilization of alachlor after application, has resulted in research on different ways to formulate alachlor-containing products. Researchers have compared soil residues

Table 2. Transformation products of alachlor detected in soil from microbial breakdown

Chemical name:	Common abbreviation	Reference
N-demethoxymethyl alachlor		
2-chloro-2',6'-diethylacetanilide		b, d
2-chloro-*N*-methoxymethyl)-[2-ethyl-6-(1-hydroxyethyl phenyl]acetamide		c
2,6-diethyl-*N*-methoxy-methyloxanilic acid		a
2,'6'-diethyloxanilic acid	OA	e
2,6-diethyl-(methoxymethyl)aniline		e
2,6-diethyl-*N*-(methoxymethyl)acetanilide		b, d
2',6'-diethyl-*N*-methoxymethyl-2-sulfoacetanilide	ESA	a
2,6-diethethylaniline		b, d
2',6'-diethethylacetanilide		b
2',6'-diethyl-2-sulfoacetanilide		a
2',6'-diethyl-2-hydroxy-*N*-methoxymethyl-acetanilide		a, b
2'6'-1-diethyl-*N*-methoxymethyl-2-methylsulfinyl acetanilide		a
1-chloroacetyl-2,3-dihydro 7-ethylindole		b, d
bis-2-thio-2,6'-diethyl-N-(methoxymethyl)acetanilide		d
8-ethyl-2-hydroxy-1-methoxymethyl-1,2,3,4-tetrahydroquinoline		b
7-ethyl 1-hydroyacetyl-2,3-dihydroindole		b
9-ethyl-1,5-dihydro-1-(methyoxymethyl)-5-methyl-4,1-benzoxazepin-2-(3*H*)-one		b
3-dihydro-1-formyl-7-ethylindole		b

a (Aherns, 1994)
b (Chesters et al., 1989)
c (Clay et al., 1997)
e (Stamper and Tuovinen, 1998)

and determined dissipation half-lives of starch-encapsulated (SE) alachlor vs. a commercial formulation (CF) of alachlor under tillage and no tillage conditions (Gish et al., 1994). Surface and 1.1 meter core samples were taken immediately after application, and seven times over two years. Dissipation half-lives ranged from eight to 41 days for SE-alachlor and from four to 20 days for CF-alachlor, indicating that the starch encapsulated formulation increased the persistency of alachlor. Temperature affected persistency, since the half-life of SE alachlor was 15 days during warmer temperatures when core samples were taken in 1991, compared to 40 days in 1990 when temperatures were colder. Tillage practices did not appreciably affect the persistence of either formulation.

Though studies determining the half-life of alachlor degradation products was not found in the literature, estimates have been calculated. The estimated half-life is 90 days for alachlor-sulfinyl acetic acid, and 150 days for alachlor OA (Barrett, 1996).

Over a dozen alachlor transformation products have been identified in ground and/or surface water. This is largely based on the work of Potter and Carpenter (1995). A table with the chemical names of these degradative products has been compiled below, adapted from a table found in Stamper and Tuovinen (1998) (Table 3). Three of these alachlor transformation products, ESA, OA and 2,6-diethylaniline, are the most common degradative products monitored in studies that have evaluated levels of alachlor transformation products in ground and surface water. These studies are discussed in subsequent sub-sections of this Critical Evaluation.

Table 3. Transformation products of alachlor detected in groundwater and/or surface water

Chemical name:	Common abbreviation	Reference
N-(2',6'-diethylphenyl)methyleneamine		d, e
2,6-diethylaniline		c, d, e
2',6'-diethyloxanilic acid	OA	a
7-ethylindoline		d, e
2',6'-diethylformanilide		d, e
2',6'-diethylacetanilide		d, e
alpha-*N*-[(2',6'-diethylphenyl)amino]ethanol		d, e
2'-acetyl-6'-ethylacetanilide		d, e
N-(2',6'-diethylphenyl)-*N*-(methoxymethyl)acetamide		d, e
2-hydroxy-2',6'-diethyl-*N*-methylacetanilide		d, e
2'-acetyl-6'-ethyl-*N*-(methoxymethyl)acetanilide		d, e
2-hydroxy-2',6'-diethyl-*N*-(methoxymethyl)acetanilide		d, e
2-methylsulfinyl-*N*-(2'6'-diethyl)acetanilide		d, e
2-chloro-2'acetyl-6'-ethyl-*N*-(methoxymethyl)acetanilide		d, e
2-methylsulfonyl-(2',6'-diethyl)acetanilide		d, e
2-sulfonyl-2',6'-diethyl-*N*-(methoxymethyl)acetanilide	ESA	e, b, f
other names include:		
N-methoxymethyl-2',6'-diethyl-2-sulfoacetanilide	ESA	
2',6'-diethyl-*N*-methoxymethyl-2-sulfoacetanilide	ESA	

a (Barett, 1996)
b (Goolsby et al., 1995a)
c (Koplin and Carpenter, 1996)
d (Potter and Carpenter, 1995)
e (Stamper and Tuovinen, 1998)
f (Thurman et al., 1996)

2. Groundwater

a. Groundwater Levels of Alachlor:

The occurrence and persistence of alachlor has been determined in several national surveys of pesticides in groundwater, as well as in regional studies conducted in the US. These studies, including the minimum detection limits (MDL) used, are summarized in Table 4.

One of the first nation-wide studies to determine the level of pesticides in groundwater was the National Pesticide Survey conducted by EPA (USEPA, 1990a). The purpose of this study was to survey the frequency and levels of pesticides in drinking water wells. The survey's authors estimated that less than 0.1% of rural and domestic wells would be expected to have detectable levels of alachlor. It was estimated that this was equivalent to 3,140 wells with alachlor detection (95% CI 1 to 101,000 wells).

During the same time, another large-scale survey was conducted by the Monsanto Co. and the Research Triangle Institute to determine the frequency of detection of alachlor in private, rural domestic wells for all counties in the US that sold alachlor in 1986 (Holden et al., 1992). Out of the six million existing wells in the targeted areas, a probability-based selection procedure was used to select 1,430 wells to monitor alachlor in 89 counties. The sampling methodology included over-sampling wells from areas of higher alachlor use and where there was higher vulnerability of wells to be contaminated. Water samples were collected from June of 1988

to May of 1989. Levels of other herbicides, including atrazine and metolachlor, were also monitored. The estimated percent of wells with detectable alachlor was 0.78%, compared to 11.68% for atrazine and 1.02% for metolachlor. It was also predicted that only 0.09% of the wells would have alachlor levels above 0.5 µg/L. It should be noted that this study did find a high frequency of detection for alachlor (12%) in a small percentage of wells located within a half-mile of pesticide dealers, formulators or applicators. The levels of alachlor in these wells was not provided in the report.

A third national study was conducted as a part of the National Water Quality Assessment program (NAWQA) by the US Geological Survey (USGS) (Kolpin et al., 1998). Groundwater levels of pesticides were determined in 20 major hydrologic basins in the US. The samples were collected from 1993-1995 from 1,012 wells and 22 springs. This study related the frequency of detection data to patterns of land use in rural and urban areas. Alachlor was detected in 2.4% of the water samples. The range and median concentrations of alachlor were not provided. The maximum concentration of alachlor reported was 0.55 µg/L. Most of the detections were found in land use settings that grew corn, soybeans, peanuts or wheat; no alachlor detections were reported in urban areas. This is consistent with the major uses of alachlor in weed control on corn, soybeans, peanuts and wheat crops.

The most recent national database on major herbicides in groundwater has been assembled by the USGS (Barbash et al., 1999). This report was based on the results from the USGS's National Water-Quality Assessment (NAWQA) Program which sampled 2,227 sites in 20 major hydrologic basins in the US from 1993-1995, and the Midwest Pesticide Study (MWPS) which sampled 303 wells in a 12-state area between 1991 and 1994. The major purpose of the MWPS was to investigate the groundwater concentrations of the principle herbicides used in corn and soybean production. In the NAQWA study, alachlor detections that were at or above 0.05 µg/L were detected in less than one percent of the samples in shallow groundwater in agricultural areas and mixed land use areas, and none in urban sites. Comparatively, in the MWPS, approximately 3.3% of the 94 sites sampled in 1992 had alachlor levels that were at or above 0.05 µg/L (Barbash et al., 1999).

Other studies have determined the level and frequency of detection of alachlor in specific geographical regions of the US. Several of these studies are presented below. Over 90% of rural areas of Nebraska depend on groundwater for drinking water. A study conducted in Nebraska in the late 1980s (when alachlor use was still high) monitored 2,263 well water samples for a variety of pesticides (Spalding et al., 1989). Over 80% of the samples were taken from rural household drinking wells. Alachlor was detected in 0.77% of the wells, compared with 13.5% detection rate for atrazine. At the time of this study, alachlor was the second highest used herbicide (atrazine being first) in Nebraska. The maximum concentration of alachlor detected was 20.7 µg/L, while the mean levels were 1.66 µg/L and median levels were 0.09 µg/L.

A study conducted by the USGS determined the frequency of detection of herbicides in 303 wells located in twelve midwestern states (Burkhart and Kolpin, 1993). Regions monitored had a high potential for herbicides and fertilizers to contaminate waterways, and had at least 25% of their crop land in soybean or corn production. Alachlor was detected in 1.7% of the samples. The most frequently detected herbicide was atrazine (24% detection). Actual levels of the herbicides were not given.

The USGS, in conjunction with the Iowa Groundwater Monitoring Program (IGMP), determined temporal trends in the frequency of occurrence of pesticides found in Iowa groundwater samples collected from 1982 through 1995 (Kolpin et al., 1997a). The detection frequencies were related to trends in the overall annual usage and application rates of the pesticides. While application rates of alachlor did not differ substantially from 1982-95 (range 2.26 to 2.54 kg/ha) the average usage in the state of Iowa declined dramatically from the early 1980s to the mid 1990s. Average use of alachlor was 6.2 million kg/yr during 1982-1986; declined to 3.7 million kg/yr during 1987-91, and further declined to 2.35 million kg/yr during 1992-95. In contrast, the frequency of detection of alachlor in groundwater samples gradually increased from 1982 to 1995. Median frequency of detection was zero from 1982-86; increased to 2.2% during 1987-91; and further increased to 3.3% during 1992-95. The overall frequency of detection was 3.0%; the maximum concentration detected was 14 µg/L, and the frequency of exceeding alachlor's MCL of 2.0 µg/L was 0.4%. The authors offered several explanations for the increased percent detections over time when state usage levels of alachlor were dramatically declining. Most of the wells (92%) did not have alachlor concentrations above the detection limit of 0.10 µg/L. Hence, the sample size for wells with positive detections of alachlor was small. Secondly, though there was a sharp decline in alachlor usage in the state of Iowa, it may take additional time for these changes to be reflected in alachlor concentrations found in groundwater. One factor not considered by the authors is the relative high mobility of alachlor. Wells with alachlor detections may have been distant from the point of use.

The mobility and persistence of alachlor is supported by the findings of a study conducted in North Carolina (Maas et al., 1995). Water samples were collected from 171 domestic rural wells located in Eastern North Carolina. Samples were collected from 1989-93. Alachlor was detected in 8.8% of the samples (n = 171) collected from 1989-92. The relationship of detection frequency to distance of the well from mixing, storage, or loading area (MSL)

for pesticides was determined for well water samples collected from 1989-93. Alachlor was detected in 8.8% of the samples 0-32.8 meters from the MSL areas, in 4.3% of the wells greater than 32.8 meters from the MSL areas, and in 11.4% of the wells not located near a farm. None of these differences were statistically significant. Detection frequency was determined according to the distance from the well to the nearest crop. Alachlor was detected in 3.5% of the wells 0-16.4 meters from a crop, 6.6% detection 16.5-49.2 meters from a crop, and was detected in 17.7% of the wells greater than 49.2 meters from a crop. The authors offered no explanation for the higher percent detections of alachlor in areas distant from a crop, other than these data suggest that alachlor can travel long distances in the aquifer.

Alachlor was monitored in 30 wells located in Merced County, California by the Environmental Monitoring and Pest Management Branch of the California Dept. of Food and Agriculture (CADFDAG, 1990). The well water samples were obtained in an area of corn and bean production in February and May of 1987. In this study there were no confirmed detections of alachlor, metolachlor, or atrazine in any of the monitored wells.

Researchers have investigated whether tillage affects detection of alachlor in groundwater (Buhler et al., 1993). Alachlor levels in water samples collected from subsurface drainage of Webster clay soil was determined in fields that had alachlor applied from 1974-1991. Alachlor was detected in 4.6% of the samples collected from 1985-1990, while atrazine, another herbicide used on the same field, was detected in 97% of the samples. Tillage systems had little effect on tile drainage and detection of either herbicide. Ritter et al. (1994) monitored groundwater contamination by herbicides applied to irrigated field plots that used no-tillage and conventional tillage treatments. Plots were monitored from 1984-1986. Alachlor was detected at concentrations ranging from 0.2 to 2 ppb in all of the nine monitoring wells 24 days after alachlor was applied in 1984 (alachlor was not used in 1985 and 1986). The highest alachlor concentration observed 59 days after application was 15 ppb. The researchers found levels of alachlor in monitoring wells ranged from <0.1 to 3 ppb 216 days after application.

Once alachlor leaches into groundwater, it is relatively stable. This stablity is probably due to the low level of microbial activity in groundwater. This has been illustrated in a study where ground water samples were fortified with alachlor, and concentrations of alachlor degradation products were monitoried over the next 18 months (Cavalier et al., 1991). When the water samples were fortified with 1 μg alachlor per L, there was little breakdown of alachlor during the first 14 months of the study, with some degradation (0 to 60%) by 18 months. At a higher fortification level (5 μg/L), there was little or no degradation of alachlor during the first eight months, and by 18 months degradation varied considerably from 0 to 75% degradation.

While the frequency of detections of alachlor reported in groundwater samples collected in the early to mid 1990s appears to be low, earlier reports from studies in the 1980s suggest that higher levels of alachlor were detected during the 1980s. In a review by Ritter (1990), concentrations of alachlor in groundwater were reported as high as 16.6 μg/L in the Big Spring Basin of Iowa, and up to 15 μg/L in samples obtained in Delaware.

The USGS has monitored pesticides in the groundwater of NYS. Three well networks (16 agricultural wells, 26 urban/residential wells, and 49 domestic wells) were monitored in 1994 (Wall et al., 1998). Alachlor was not detected in any of the groundwater samples.

During May through August of 1998, the USGS monitored levels of alachlor and two of its degradative products, alachlor ESA and alachlor OA, in wells in Suffolk County, Long Island, NY (Phillips et al., 1999). Because of the sand and gravel water-table aquifer in this county, groundwater is highly susceptible to contamination from pesticide usage on land surfaces. Alachlor was detected in 10% of the wells sampled, but levels were all below NYS and EPA MCLs, with the maximum level detected at approximately 0.2 μg/L. While there are no EPA MCLs set for alachlor degradation products, the NYS MCL is set at the default level of 50 μg/L. In contrast to alachlor, alachlor ESA was detected in 8% of the samples ranging from 0.3 to 40 μg /L, while alachlor OA was detected in 2% of the samples (n = 1) at 25 μg/L. Another alachlor degradation product, 2,6-diethylanaline, was only detected in 2% of the samples (n = 1) at 0.01 μg/L.

The Suffolk County Department of Health Services (SCDHS), in collaboration with the Nassau County Health Department and Department of Public Works conducted a 19 month study from October 1997 to March 1999, to obtain information on the water quality and levels of pesticide residues in the ground waters of Nassau and Suffolk Counties in NYS (SCDHS, 1999). Water samples were obtained across all geographical areas and municipal and political boundaries, and included monitoring wells, private wells, community wells, and non-community wells. None of the 405 samples obtained from wells in Nassau County had detectable levels of alachlor (MDL = 0.2 μg/L; MCL = 2.0 μg/L). In Suffolk County 21 of the 1,539 well water samples had detectable levels of alachlor, and ten of these samples exceeded the MCL for alachlor. The maximum level of alachlor detected was 8.3 μg/L. Degradation products of alachlor such as ESA were not monitored in this study. Community supply wells found to have alachlor residues that exceeded the MCL were either taken out of service, or fitted with a granular activated carbon filtration system to reduce

contaminants to acceptable levels. The report stated that the hydrology of Long Island counties can contribute to the ability of pesticides to leach, including this areas' coarse sandy soils, acidic groundwater and high water table.

The USGS also conducted a study of 32 Community Supply Wells throughout NYS. Alachlor as the parent compound was not detected in any well, while alachlor OA was detected in one well at 0.31 μg/L (frequency of detection, 3.1%) , and alachlor ESA was detected in three wells (frequency of detection, 9.4%) ranging from 0.44 to 1.4 μg/L (personal communication with David Eckhart, USGS, 2/28/00).

b. Groundwater Levels of Alachlor Degradation Products:
Many of the national groundwater monitoring surveys conducted in the early to mid-1990s, including the EPA's National Pesticide Survey and the National Well Water Study, did not determine levels of alachlor metabolites, particularly the ESA, in groundwater. The discovery that alachlor metabolites, including ESA, were widely detected in groundwater at levels far exceeding the level of alachlor observed, was first noted by researchers who were validating immunoassay screens originally designed to detect alachlor levels in water samples (Baker et al., 1993). Baker et al. (1993) reported that the high level of false positives detected by the alachlor immuoassays was due to cross-reactivity of the polyclonal antibody with the ESA metabolite of alachlor. The 157 water samples that had originally tested positive for alachlor by immunoassay were retested to determine the levels of alachlor by gas chromatography (GC) and immunoassay. In these samples, 136 samples again tested positive for alachlor by immunoassay, but 76 of these samples (103/136) were not found to contain any alachlor by GC. Twenty-four of the false positive samples were analyzed further, and the presence of ESA was confirmed in all samples by LC/MS. Further experiments to quantify the actual levels of ESA in rural well water lead to the conclusion that ESA was found in relatively high concentrations in private wells with median values of 14 μg/L (range = 1.2 to 74 μg/L). Since detections were also observed in the winter and early spring pre-planting seasons, it was suggested that ESA is relatively mobile and persistent contaminate in rural wells. Others have published sensitive methods for the detection of alachlor metabolites, including the use of solid-phase extraction, followed by LC/MS (Vargo, 1998). This method has the advantage of being able to distinguish between the ESA metabolites of alachlor, metolachlor and acetoachlor.

Subsequent studies have confirmed the presence of alachlor degradation products in the groundwater in Iowa (Kolpin et al., 1997b; Kolpin et al., 1997a), several other Midwestern states (Kolpin et al., 1996) and in Massachusetts (Potter and Carpenter, 1995).

As a part of the IGMP, the levels of herbicides and their degradation products were determined in samples of groundwater taken from 106 municipal wells (Kolpin et al., 1997b). ESA was the most frequently detected compound (65.1%), followed by atrazine (40.6%), deethylatrazine (34%) and cyanazine amide (19.8%). In contrast, alachlor was only detected in 7.5% of the samples. The maximum concentration of alachlor reported was 0.63 μg/L, while the maximum concentration reported for ESA was 23-fold higher at 14.8 μg/L. The authors suggested that the prevalence and preferential increased transport of ESA to groundwater may be due to the greater water solubility and stability of ESA compared to alachlor. Trends in pesticide levels monitored in Iowa's groundwater from 1982-1995 were determined by USGS (Kolpin et al., 1997a). These data were a part of the IGMP which monitored untreated water from 1,019 municipal wells for agricultural chemicals. The use of alachlor decreased 60% during the course of the study, from 6.2 million kg AI/yr in 1982-86 to 2.35 million kg AI/yr from 1992-95. The median frequency of detections remained relatively low, though increased with time, with 0% detections during 1982-86; 2.2% in 1987-91, and 3.3% in 1992-1995. The authors noted that 92% of the wells did not have alachlor levels over the limits of detection, and that levels of alachlor ESA were not available for the entire sampling period. Researchers were not able to determine if there was a trend in usage of alachlor and what were the levels of its more persistent degradation products.

In a study of pesticides and their degradation products in 837 water samples from 303 wells in twelve midwestern states, ESA was detected nearly 10 times more frequently (46% detection; MDL = 0.10 μg/L) than alachlor (3.3% detection; MDL = 0.05 μg/L) (Kolpin et al., 1996). Another alachlor metabolite, 2,6-diethylanaline, was detected in 16% of the samples (MDL = 0.003 μg/L). The maximum concentration of ESA (8.63 μg/L) in this study was higher than that reported for alachlor (4.27 μg/L) while the maximum concentration reported for 2,6-diethylanaline was much lower, at 0.02 μg/L. The samples for this study were collected from 1991-1994. The ESA metabolite of alachlor appeared to be persistent, since 90% of the wells that had levels of ESA higher than 0.10 μg/L remained above that level during all subsequent samples taken at one-year intervals.

In addition to alachlor, 20 alachlor degradation products were identified in groundwater samples collected in four monitoring wells placed beneath a Massachusetts corn field. (Potter and Carpenter, 1995). Alachlor had last been applied to the field in the spring of 1987, and water samples were collected in September of 1990. In all samples, the total concentration of the degradation products exceeded the concentration of alachlor by two-fold. The range of concentrations of the alachlor degradation products ranged from four to 570 ng/L.

Table 4. Detection frequencies and levels of alachlor and its degradation products in groundwater

(Author, Year) Location	Alachlor MDL (µg/L)	Alachlor Detection Frequency (%)	Alachlor Mean (Median) Level (µg/L)	Alachlor Maximum Level Detected (µg/L)	Alachlor ESA MDL (µg/L)	Alachlor ESA Detection Frequency (%)	Alachlor Mean (Median) ESA Level (µg/L)	Alachlor ESA Maximum Detected (µg/L)
(USEPA, 1990a) EPA, Drinking Water Wells	0.50	<0.1 %						
(Holden et al., 1992) Monsanto, Well Water Survey	0.03	0.78 %						
(Kolpin et al., 1998) USGS NAWQA	0.002	2.40 %		0.55				
(Barbash et al., 1999)								
USGS NAQWA	0.05	<1.00 %						
USGS, MWPS Midwest	0.05	3.30 %			0.10	45.8%		
Kolpin, unpublished	0.20	1.1%			0.20	50.0%		
(Spaulding et al., 1989) Nebraska	0.50	0.77 %	1.66 (0.09)	20.7				
(Burkart and Kolpin, 1993) USDA and USGS, Midwest	0.05	1.7%						
(Kolpin et al., 1997a) USGS, Iowa	0.10	3.0%		14.0				
(Maas et al., 1995) Eastern North Carolina	0.13	8.8%						
(CADFDAG, 1990) California, Merced County		0						
(Buhler et al., 1993) Minnesota	0.10	4.6 %	0.96					
(Ritter et al., 1994) Delaware	0.10		range <0.1 to 15	15.0				
(Ritter et al., 1990) Big Spring Basin, Iowa				16.6				
(Wall et al., 1998) USGS, Hudson River Basin; NY and Adjacent States	0.002	0%						
(Phillips et al., 1999) USGS, Suffolk County, NY	0.002	10.0%		0.2	0.2	8.0%		40
(Baker et al., 1993) Indiana, Kentucky and Ohio							(14)	74.0
(Kolpin et al., 1997b) Iowa	0.05	7.5%		0.63	0.10	65.1 %		14.8
(Kolpin et al., 1996) Midwest	0.05	3.3%		4.3	0.10	45.8 _		8.6
(Potter and Carpenter, 1995) Massachusetts				0.07				
(SCDHS, 1990) Suffolk County Dept. of Health Services, Long Island, NY								
Suffolk County	0.20	1.4%		8.3				
Nassau County	0.20	0%						
(Eckart, 2000) USGS, Unpublished data, NY		0%				9.4%		1.4
(Macomber et al., 1992) Ohio					0.5		33.6	74
(Kalkhoff et al., 1998) USGS, Iowa	0.05	<1%		0.4	0.05	50%		8

Abbreviations: MDL = minimum detection limit; ESA = ethanesulfonic acid; OA = oxanilic acid

Table 4. Detection frequencies and levels of alachlor and its degradation products in groundwater (continued)

(Author, Year) Location	Alachlor OA MDL (µg/L)	Alachlor OA Detection Frequency (%)	Alachlor OA Mean Level (µg/L)	Alachlor OA Maximum Detected (µg/L)	2,6 Diethyl-aniline MDL (µg/L)	2,6 Diethyl-aniline Detection Frequency (%)	2,6 Diethyl-aniline Mean Level (µg/L)	2,6 Diethyl-aniline Maximum Detected (µg/L)
(USEPA, 1990a) EPA, Drinking Water Wells								
(Holden et al., 1992) Monsanto, Well Water Survey								
(Kolpin et al., 1988) USGS NAWQA								
(Barbash et al., 1999)								
USGS NAQWA					0.003	1.0 %		
USGS, MWPS Midwest					0.003	16%		
Kolpin, unpublished	0.20	21.6%						
(Spaulding et al., 1989) Nebraska								
(Burkart and Kolpin, 1993) USDA and USGS, Midwest								
(Kolpin et al., 1997a) USGS, Iowa								
(Maas et al., 1995) Eastern North Carolina								
(CADFDAG, 1990) California, Merced County								
(Buhler et al., 1993) Minnesota								
(Ritter et al., 1994) Delaware								
(Ritter et al., 1990) Big Spring Basin, Iowa								
(Wall et al., 1998) USGS, Hudson River Basin; New York and Adjacent States					0.001	0 5		
(Phillips et al., 1999) USGS, Suffolk County, NY	0.20	2.0%		25	0.003	2.0%		0.01
(Baker et al., 1993) Indiana, Kentucky and Ohio								
(Kolpin et al., 1997b) Iowa								
(Kolpin et al., 1996) Midwest					0.003	16%		0.02
(Potter and Carpenter, 1995) Massachusetts								0.02
(SCDHS, 1990) Suffolk County Dept. of Health Services, Long Island, NY								
Suffolk County								
Nassau County								
(Eckart, 2000) USGS, Unpublished data, NY		3.1%						
(Macomber et al., 1992) Ohio								
(Kalkhoff et al., 1998) USGS, Iowa	0.05	22%		32				

Abbreviations: MDL = minimum detection limit; ESA = ethanesulfonic acid; OA = oxanilic acid

The levels of DEA and alachlor ESA were monitored by the USGS in the NAWQA and MWPS (Barbash et al., 1999). The MWPS sampled 303 wells in a twelve-state area in the Midwest from 1991 to 1994. The frequency of detection of sites that had levels of 2,6-diethylanaline at or above 0.003 µg/L was 1.0% in shallow groundwater samples from agricultural areas in the NAQWA study, while 16% of the samples were at or above 0.003 µg 2,6-dietyhlanaline per L in the MWPS. Alachlor ESA was not monitored in the NAQWA study, but 45.8% of the samples in the MWPS exceeded 0.10 µg/L for this alachlor degradation product. This is in comparison to no more than 3.3% of the samples with a detection of alachlor at or above 0.5 µg/L in groundwater from either the NAQWA or the MWPS. The USGS report also presented unpublished data submitted by Kolpin to the USGS on the frequency of alachlor metabolites detected during a 1996 statewide survey of 88 municipal wells in Iowa (Table 12, in Barbash, 1999). Frequency of detection of water samples that had alachlor degradates exceeding 0.20 µg/ L were: 1.1% for alachlor, 50.0% for ESA, and 21.6% for alachlor OA. Although the frequency of detections of alachlor as the parent compound are relatively low, even in high use states such as Iowa, the frequency of detections of alachlor degradative products exceeded that of the parent compound by many fold.

3. Surface Water

a. Surface Water Concentrations of Alachlor:

The frequency of detection and concentration of alachlor in surface water has been monitored in the midwestern and northeastern regions of the US and Canada. Some of these studies are summarized below.

In a review of the occurrence of herbicides in midwestern groundwater and surface water, the authors noted that alachlor is more frequently detected in surface water than groundwater in the Midwest (Goolsby et al., 1995a). The detection frequency and concentration of alachlor is affected by seasonal variability. Thurman et al. (1992) determined the levels of alachlor and other herbicides in 146 sites in 122 hydrologic basins in a ten state area in the Midwest, which is a part of the corn-soybean belt. While alachlor was detected in 18% of the water samples during the preplant period (median <0.05 µg/L; maximum 0.44 µg/L), alachlor was detected in 86% of the samples during the postplant period (median 0.92 µg/L; maximum 51 µg/L).

The relationship between the agricultural use of pesticides, including alachlor, and the flux of pesticides in the rivers feeding into the Mississippi basin were determined from May 1991 to the end of March 1992 (Larson et al., 1995). Flux of a pesticide is the mass transported past the sampling point during a specified period of time. The tributaries monitored included the Minnesota River, the Illinois River, the White River in Indiana, the Ohio River, the Platte River, the Missouri River and on the Mississippi River sampling sites included Clinton, Iowa; Thebes, Illinois; and Baton Rouge, Louisiana. Alachlor was detected at all sampling sites. Flux reported as a percent of use in the river basins ranged between 0.12% to 0.46%. Concentrations of alachlor were usually highest from May to June, which corresponds to when alachlor was applied and spring rains resulted in surface runoff into the rivers. In general, triazine herbicides (atrazine, simazine, and cyanazine) and acetanilides (alachlor and metolachlor) had higher percentages (>0.1% flux) reaching the rivers than other herbicides.

Levels of herbicides and pesticides were monitored in 128 surface water samples obtained from three agricultural basins in south-central Georgia as a part of the USGS NAQWA program during 1993 to 1995 (Hatzell, 1995). The primary crops grown in this region were peanuts and cotton, followed by corn, soybeans and wheat. Alachlor was detected in 10% of the samples, compared to 77% detection rate for metolachlor and a 95% detection rate for atrazine. Concentrations of alachlor were found up to 0.006 µg/L, while metolachlor levels were up to ten-fold higher, at 0.06 µg/L.

Levels of alachlor and other corn herbicides were monitored in the Blue Earth River area of Minnesota from April through the end of June, 1994 (Capel et al., 1995). Concentrations of alachlor ranged from 0.03 µg/L to 0.06 µg/L from late April through the third week in June. However, during the last week in June, levels of alachlor were as high as 1.2 µg/L. The previous week alachlor was detected in rain-water samples at 0.25 µg/L. It is not known if alachlor in the atmospheric, and subsequent deposition of alachlor in precipitation contributed to the levels of alachlor detected in river water samples, or if the levels in the river were primarily due to surface runoff of alachlor.

There are several reports of alachlor detections in streams and rivers of Northeastern states. Most of these detections were low, below the MCL for alachlor. In a study of herbicide concentrations in selected Vermont streams, 600 stream water samples were collected following rain events in 1992 and 1993 (Gruessner and Watzin, 1995). Alachlor concentrations were determined using GC/MS methods. Alachlor was detected only in Hungerford Brook. Alachlor was detected in 17.6% of the 17 samples, with mean concentrations of 0.13 µg /L and maximum concentrations were reported at 0.2 µg/L.

In one study conducted in Canada, the frequency of detection of alachlor at the mouths of Canadian rivers was extremely low in a survey conducted between 1986 to 1990 (Frank et al., 1991). Frequencies of detections for alachlor were 0.4%, 0%, and 1.4% at the mouths of the Grand, Saugeen and Thames Rivers, respectively. The authors stated that the low frequency of

detections may be due to the cessation of alachlor use in Canada after 1985.

A state wide survey of pesticide levels in NYS surface water was conducted by the USGS (Phillips et al., 1998). Samples from 64 rivers and streams were taken from June to July, 1997. Alachlor was detected in 50% of the samples. Levels ranged from 0.001 to 0.15 μg/L. None of the samples exceed EPA or NYS water quality standards. Levels of alachlor degradation products were not monitored. Other USGS studies that have monitored both alachlor and its degradation products in NYS surface waters are summarized at the end of the next section.

b. Surface Water Concentrations of Alachlor Degradation Products:

During the 1990s, several studies determined the levels of alachlor degradative products in surface waters in the Midwest and NYS.

Levels of alachlor ESA in Ohio surface water were reported by Macomber et al. (1992). This study was the first to validate an HPLC method of analysis for alachlor-ESA. In the five surface water samples analyzed, ESA was not detected in two of the samples, and levels of ESA ranged from 0.6 to 2 μg/L in other samples. As mentioned previously, ESA levels in the six groundwater samples analyzed in this study were much higher, ranging from four to 74 μg/L.

Using a GS/ion-trap/MS method, Pereria and Hostettler (1993) determined the levels of alachlor and metolachlor degradation products in surface water collected at stations along the entire length of the Mississippi River from St. Anthony Falls, MN to Belle Chasse, LA. Water samples were collected during three sampling cruises in July-August 1991, October-November 1991, and April-May 1992. Frequency of detection and levels were higher for alachlor than its degradation products. Alachlor was detected in 96.1% of the sampling sites, compared to 31% detection for 2-chloro-2',6'-diethylacetanilide, and 73% for 2-hydroxy -2',6'-diethylacetanilide. Pesticide concentrations ranged from 0.01 to 0.56 μg/L for alachlor, from 0.005 to 0.035 μg/L for 2-chloro-2',6'-diethylacetanilide, and 0.01 to 0.085 μg/L for 2-hydroxy -2',6'-diethylacetanilide. None of the samples exceeded the MCL for alachlor. Levels of alachlor and its degradation products showed seasonal and geographic variations, with higher concentrations observed in samples collected from July-August 1991 and April-May 1992, compared to samples collected from October-November 1991. The highest concentrations of alachlor were found near where the Iowa, Des Moines, Missouri, Illinois and Ohio Rivers fed into the Mississippi River. This study also estimated the mean mass transport of alachlor and other pesticides into the Gulf of Mexico. Approximately 18 metric tons of alachlor were carried from the Mississippi River to the Gulf of Mexico in 1991. The mass transport of atrazine was higher (160 metric tons) even though the amounts of atrazine and alachlor used in the 14-state area that drains into the Mississippi River were comparable in 1991. This may reflect the shorter half-life of alachlor compared to atrazine.

An extensive study of the occurrence of alachlor and alachlor ESA in rivers and reservoirs of the Midwest was conducted by the USGS from 1991-1993 (Thurman et al., 1996). In contrast to the study of Pereria and Hostetter, the concentrations of ESA exceeded the levels of alachlor for surface water samples taken along the length of the Mississippi River. The samples had progressively higher concentrations of ESA closer to the mouth of the Mississippi with levels ranging from 0.20 μg/l to 0.9 μg/L. In contrast, levels of alachlor were all below 0.1 μg/L along the entire length of the Mississippi. Levels of ESA were higher than the levels of alachlor in samples taken from 76 midwestern reservoirs monitored in this study. While median concentrations of alachlor were at or below 0.08 μg/L, median levels for ESA ranged from 0.38 to 0.68 μg/L, with 90th percentile levels as high as 5.4 μg/L. These results suggest that ESA is more stable than alachlor and is found at higher levels than the parent compound in midwestern rivers and reservoirs.

Higher levels of ESA compared to the parent compound, alachlor, were also reported in recent study of surface and groundwater levels of these compounds in Iowa. Researchers from the USGS determined levels of alachlor and ESA in 88 municipal wells and 12 streams sampled in 1995 (Kalkhoff et al., 1998). The frequency of detection for ESA was nearly 100% while less than 1% of the samples had detectable levels of alachlor. The median level for ESA reported was 1.6 μg/L, with maximum levels as high as 4 μg/L. In contrast, none of the samples had alachlor levels higher than 0.2 μg/L.

The levels of alachlor and an alachlor degradation product, 2,6-diethylanaline, were determined in surface waters samples of the Hudson River Basin in a series of studies conducted by the USGS in the mid-1990s. Approximately 80% of those that live in the Hudson River Basin used public water supplies obtained from surface water sources (Wall et al., 1998). In one study, samples were obtained from three watersheds with different land uses (Wall and Phillips, 1997a). Alachlor was detected in 20% of the samples obtained during May through August from the Mohawk River at Cohoes which drains from a combination of agricultural, urban and forested land. Median levels of alachlor were 0.011 μg/L, and maximum levels were 0.021 μg/L, which are well below the MCL for alachlor of 2.0 μg/L. About 12% of the samples had detectable levels of 2,6-diethylanaline, with median and maximum levels at 0.003 μg/L, which was the minimum detection level. Surprisingly, the site that drains from a primarily agricultural area, Canajoharie Creek, had no alachlor detection in samples obtained

Table 5. Detection frequencies and levels of alachlor and its degradation products in surface water

(Author, Year) Location	Alachlor MDL (µg/L)	Alachlor Detection Frequency (%)	Alachlor Mean (Median) Level (µg/L)	Alachlor Maximum Level Detected (µg/L)	Alachlor ESA MDL (µg/L)	Alachlor ESA Detection Frequency (%)	Alachlor ESA Mean (Median) Level (µg/L)	Alachlor ESA Maximum Detected (µg/L)
(Thurman et al., 1992) Midwest Pre-plant period	0.05	18%	(<0.05)	0.44				
Post-plant period	0.05	86%	0.92	51.0				
(Larson et al., 1995) Mississippi Basin Rivers Flux as % of use reported; no data on actual concentrations	0.002							
(Hatzell et al., 1995) South-central Georgia	0.002	10%		0.006				
(Capel et al., 1995) Blue Earth River, Minnesota	0.01			1.20				
(Gruessner and Watzin, 1995) Hungerford Brook, Vermont	0.01	17.6%	0.13	0.20				
(Frank et al., 1991) Ontario, Canada	<0.02		range 0 -1.4%					
(Phillips et al., 1998) New York State	0.002	50%		0.15				
(Macomber et al., 1992) Ohio					0.5			2.0
(Pereria and Hostettler, 1993) Mississippi River Basin	0.005			0.56				
(Thurman et al., 1996) Midwestern Rivers& Reserviors	0.05		(<0.05)	0.64	0.10		(0.48)	5.4
(Kalkhoff et al., 1998) Iowa	0.05	< 1.0%		0.4	0.05	99%	(1.6)	4.0
(Wall and Phillips, 1997a) Hudson River Basin-Mohawk River Subbasin at Cohoes, NY	0.002	20%	(0.11)	0.021				
(Wall and Phillips, 1997b) Hudson River Basin, NY and PA	0.002	9%	(0.013)	0.022				

Abbreviations: MDL = minimum detection limit; ESA = ethanesulfonic acid; OA = oxanilic acid

Table 5. Detection frequencies and levels of alachlor and its degradation products in surface water (continued)

(Author, Year) Location	Alachlor OA MDL (µg/L)	Alachlor OA Detection Frequency (%)	Alachlor OA Mean Level (µg/L)	Alachlor OA Maximum Detected (µg/L)	2,6 Diethyl-aniline MDL (µg/L)	2,6 Diethyl-aniline Detection Frequency (%)	2,6 Diethyl-aniline (Median) Level (µg/L)	2,6 Diethyl-aniline Maximum Detected (µg/L)
(Thurman et al., 1992) Midwest Pre plant period								
(Thurman et al., 1992) Midwest Post plant period								
(Larson et al., 1995) Rivers, Mississippi Basin Flux as % of use reported; no data on actual concentrations								
(Hatzell et al., 1995) South-central Georgia								
(Capek et al., 1995) Blue Earth River, Minnesota								
(Gruessner and Watzin, 1995) Hungerford Brook, Vermont								
(Frank et al., 1991) Ontario, Canada								
(Phillips et al., 1998) New York State								
(Macomber et al., 1992) Ohio								
(Pereria and Hostettler, 1993) Mississippi River Basin								
(Thurman et al., 1996) Midwestern Rivers & Reserviors								
(Kalkhoff et al., 1998) Iowa	0.05	25%		0.8				
(Wall and Phillips, 1997a) Hudson River Basin-Mohawk River Subbasin NYS					0.003	12%	(0.003)	0.003
(Wall and Phillips, 1997b) Hudson River Basin, NYS and PA					0.003	2%	(0.003)	0.003

Abbreviations: MDL = minimum detection limit; ESA = ethanesulfonic acid; OA = oxanilic acid

from May to August. In samples obtained from September through April, 8% of the samples had detectable levels of alachlor, with median and maximum levels at 0.002 μg/L of alachlor; no 2,6-diethylanaline was detected in samples from Canajoharie Creek. Usage of alachlor in these areas was not reported, and it is possible that other corn herbicides were used instead of alachlor. This is suggested by the nearly 100% frequency of detection of two other corn herbicides, metolachlor and atrazine.

As a part of the NAQWA program, the USGS determined levels of pesticides in 46 sites from 42 streams and rivers located in the Hudson River basin (Wall and Phillips, 1997b). Of the 46 sites sampled, only four had detectable levels of alachlor (9 % frequency of detection, with concentrations ranging from 0.006 to 0.022 μg/L; median = 0.013 μg/L. The alachlor degradation product 2,6-diethylanaline, was only detected at one site (2% frequency of detection), at a concentration of 0.003 μg/L.

It appears that levels of alachlor are low in NYS surface waters. Future studies will also monitor other alachlor degradation products, such as ESA (personal communication, David Eckhardt, USGS), since some midwestern studies have found that levels of this degradation product frequently exceed levels of alachlor, sometimes by more than 10 fold.

4. Tap Water:

We have noted that in many areas of the Midwest rural wells are frequently the primary source of drinking water. We have noted when studies were sampling drinking water wells in the groundwater section of this report.

Because communities located in agricultural watersheds in Ontario, Canada, access surface river water as a drinking water source, a study was undertaken to monitor raw river water and paired samples following treatment (chlorination from 1981 to 1985; chlorination plus charcoal treatment from 1985 to 1987) for triazine and chloroacetamide herbicide residues (Frank et al., 1990). Samples of raw water from the Sydenham River and paired drinking water samples from the town of Dresden were obtained 30 to 50 times a year between 1981 and 1987. Atrazine and its degradation product deethylatrazine were detected in 89 to 100% of the samples during this seven year collection period, while alachlor was detected only in the years 1982, 1984 and 1985 in two to 17% of the raw river water samples. In 1982 alachlor levels were 0.9 $\pm$ 1.0 μg/L (mean $\pm$ standard deviation) in raw river water and 0.09 $\pm$ 1.0 μg/L in drinking water. In 1984, levels of alachlor in raw river water were slightly higher (3.4 $\pm$ 5.6 μg/L) than levels found in drinking water (2.2 $\pm$ 2.7 μg/L). During 1984, the highest levels of alachlor detected in raw river water (16 μg/L) and drinking water (7.0 μg/L) exceeded Canada's Interim Maximum Acceptable Concentration of 5 μg/L for alachlor. During 1981 to 1984, treatment of raw river water consisted of chlorination only. In 1985, alachlor levels in raw water were lower that previous years, but raw river levels were higher (1.4 $\pm$ 1.4 μg/L) than alachlor levels detected in chlorinated and charcoal treated drinking water (<0.05 μg/L, minimum detection level for alachlor). The charcoal treatment appeared to be an effective means of reducing alachlor levels found in raw river water. Alachlor registration was canceled in Canada in 1985, and subsequently no alachlor residues were found in raw samples obtained during 1986 and 1987.

The concentration of alachlor in tap water can be affected by the type of treatment used at the water treatment plant. A study of Ohio (OH) surface water and finished tap water found that concentrations of alachlor during the spring when alachlor is applied to fields were as high as 4.5 μg/L in untreated surface water from the Sandusky River (Baker, 1983). However, after activated charcoal treatment at the Fremont, OH water treatment facility, levels of alachlor were reduced to less than 0.5 μg/L. This is in contrast to other communities, such as Tiflin, OH, that drew water from the Sandusky River, but used conventional water treatments. In Tiflin, OH levels of alachlor in Sandusky River samples were as high as 4.5 μg/L during the spring planting season, while the finished water levels of alachlor were in the range of 2 to 3 μg/L during the same time period. This suggests that activated charcoal is an effective means of alachlor removal during the seasons when it is mostly likely to contaminate surface water by run-off after spring application.

5. Precipitation:

Majewski and Capel (1995a) have reviewed atmospheric and precipitation pesticide monitoring studies conducted up to the early 1990s in the US and Canada. In general, studies have shown that the detection of alachlor in precipitation is seasonal, and coincided with spring/summer application times, and rainfall events. Low or no alachlor detections were reported in the fall and winter. This is probably because alachlor is used primarily as a herbicide on corn and soybean crops, and alachlor is applied on these crops primarily during the late spring/early summer. Crop usage also affected the geographic distribution of alachlor. Alachlor in the atmosphere and precipitation has been detected most frequently in the corn belt of midwestern states (Majewski and Capel, 1995b). Discussion of individual studies that have monitored alachlor in precipitation follows.

Alachlor has been monitored in the snow and rainfall in midwestern and northeastern sites (n = 86 sites) by the USGS during March 1990 through September 1991. Samples were analyzed for pesticide residue levels by enzyme-linked immunosorbent assay, and samples with positive detections were confirmed by GS/MS techniques (Goolsby et al., 1995b). The herbicides detected most frequently were atrazine (30.2%), alachlor (19.2%), the atrazine

degradation products deethylatrazine (17.4%) and metolachlor (13.3%). For alachlor, the maximum concentration detected was 3.2 μg/L. The majority of the alachlor detections were observed from April, peaking in June and July, and by late August detections were found in less than 10% of the sites. Unfortunately, though the raw data is available in this report, a geographic plot of the data was not available, so we can not comment on the geographic frequency of distribution of alachlor detections in the midwestern and northeastern states monitored.

As was previously mentioned in the section on surface water levels of alachlor, a study conducted in the Blue Earth River area of MN determined the levels of alachlor in river samples and rain samples during the spring of 1994 (Capel et al., 1995). With the exception of one time point, the levels of alachlor in the rain water usually exceeded the level of alachlor in the river water samples for the same sampling period. During the last week in April, the alachlor level in rain water was 0.58 μg/L, compared to 0.15 μg/L in river water. Rain water samples obtained from May through the third week in June were also slightly higher (range 0.05 to 0.25 μg/L) than levels found in surface water (range 0.03 to 0.06 μg/L). The volatilization of alachlor into the atmosphere may affect its levels in rain water.

The levels of 20 different pesticides were determined in samples of Iowa rainwater collected from November, 1987 through September, 1990 (Nations and Hallberg, 1992). Samples were obtained from two rural, agricultural areas, the Big Spring Basin and the Bluegrass watershed, and from one urban area, Iowa City, Iowa. The four most commonly detected pesticides in rainwater from both the agricultural areas in the Big Spring Basin and the residential areas i n Iowa City were atrazine, cyanazine, alachlor and metolachlor, indicating that these corn herbicides were detected in the rain water of both rural and urban communities. Alachlor was detected in 28.7% (93/325) of the rain samples, with mean and median levels at 1.08 μg/L and 0.58 μg/L, respectively. The maximum level of alachlor detected in precipitation was 8.60 μg/L, which is over four times the MCL for alachlor. Detections of alachlor and other corn herbicides followed seasonal trends, with the highest concentrations observed during April, May and June, corresponding to the application of corn herbicides in Iowa. Sometimes detections in rainfall preceded application times for particular sites, and the authors suggested that herbicides in the rain of these samples was likely due to transport via volatilization from other sites where the herbicides had been applied. Volatilization was cited as the largest source of pesticides in the atmosphere (Nations and Hallberg, 1992).

B. Occupational Exposure:

Exposure to pesticide applicators during mixing, loading and application of alachlor is dependent on the use and types of protective equipment and clothing worn during handling of alachlor, duration of exposure and availability of water and soap for hand washing.

NIOSH conducted a biomonitoring study of the urinary metabolites of alachlor in commercial pesticide applicators (Sanderson et al., 1995a). The purpose of the study was to compare two different detection methods; an enzyme-linked immunoassay (ELISA) and a high-performance liquid chromatography technique (HPLC). The ELISA method used a polyclonal antibody to an alachlor-protein-thioether conjugate. It was assumed that this antibody would cross-react with mercaturic acid conjugates (thioether) which are major metabolites excreted into the urine of treated Rhesus monkeys (Sanderson et al., 1995a). For the HPLC method, first alachlor metabolites were converted to 2,6-diethylanaline, and 2,6-diethylanaline was extracted from the urine and analyzed by HPLC. Three urine spot samples were collected from 20 applicators, seven hauler-mixers, and eight controls (laboratory personnel) at the beginning and ending of the work shift and the next morning. While controls had alachlor metabolite levels near the limits of detection, haulers and applicators had elevated levels of alachlor metabolites which were similar for a given assay for all 27 workers, suggesting similar exposures in applicators and hauler mixers. However, metabolite levels measured were always several fold higher when measured by ELISA compared to HPLC. For instance, the average metabolite level in the post-shift urine was 6.5 μg/ml urine using ELISA, compared to 0.97 μg/ml using HPLC. The authors did not address fact that the two different methods were designed to detect different alachlor metabolites. Also, the authors did not have alachlor metabolites available to standardize the ELISA test, and instead inappropriately used alachlor to standardize the assay. Neither did they test for cross reactivity to other similar pesticides (such as metolachlor) or to the metabolites of metolachlor. It was stated that the participants in the study also used metolachlor during the course of the day, so it is possible that the ELISA may have reflected urinary levels of both alachlor and metolachlor metabolites. The concentrations of alachlor metabolites in the urine of the pesticide workers were not reflective of the amount of alachlor handled, the duration of exposure, or the number of acres sprayed, suggesting that certain work practices may play a more important role. However, the sample size was very small, and the duration of alachlor application was highly variable, ranging from 0.5 to 6.5 hrs. Although the types of protective clothing were recorded for each worker, there was not a correlation between protective measures or types of clothing and levels of urinary metabolites.

In the 1998 alachlor Reregistration Eligibility Decision (RED) document released by the EPA, information was given on the types of labeling changes the registrant must make to protect worker safety (USEPA, 1998b). The types of protective gear required to

be stated on the label depended on the formulation and on the method of application. Mixers, loaders, and persons cleaning equipment who are working with liquid formulations (such as emulsifiable concentrate) must wear a long-sleeved shirt, long pants, chemical-resistant gloves, and use a closed system for the transfer of the alachlor from the package to the mixing tank/ application apparatus. For workers applying alachlor by aerial spraying and by chemigation methods, the EPA will require Monsanto to develop water soluble packaging for the alachlor products. Also, after application, workers are required to wait for 12 hrs before reentry. Upon reentry into treated areas they are required to wear coveralls, chemical-resistant gloves, and shoes plus socks (USEPA, 1998b).

There have been very few reports of toxic effects from an occupationally related exposure to alachlor. One case of allergic contact dermatitis was reported in a female Korean farmer whose socks became soaked with alachlor during herbicide application. She wore the socks until the end of application, and a erythematious rash that lasted for 20 days appeared on her legs and feet (Won et al., 1993).

In a study of 16 operators during mixing and loading of closed system transfers of the emusifiable concentrate or the micro-encapsulated formulation of alachlor, dermal deposition of alachlor was very low during transfer of alachlor from bulk containers to mixing tanks (mean absorbed dose, 1.1 x 10^{-7} mg/kg bdwt/lb alachlor applied) (Cowell et al., 1987). Dermal absorption was estimated from the alachlor extracted from gauze patches pinned to clothing on the thigh, forehead (at cap), chest (outer shirt), chest under clothing (undershirt), thigh and forearm. Four of the operators where also monitored during soil application, and their mean absorbed dose from loading and application was 4.1 x 10^{-7} mg/kg bdwt/lb alachlor applied. Levels of alachlor or metabolites were undetectable in 12 hr urine composites collected for five days in 75% of the operators (12/16). Four of the operators did excrete the alachlor metabolite 2,6-diethylanaline in the range of 0.65 to 10.1 μg (total amount). It should be noted that the authors stated that all precautions regarding use of protective gear and clothing were strictly adhered to in this study.

Others have monitored commercial applicators (n = 20) of alachlor during mixing, loading and application, and have found that applicators have substantial exposure to alachlor and that exposure may be reduced by following certain precautions and by the use of "good work practices" (Sanderson et al., 1995b). In addition to monitoring exposure using gauze patches pinned to outer clothing, hand and glove washes were conducted by collecting ethanol (10%) washes of hands and the exterior and interior of the gloves at the end of the day. Surface wipes were taken at the steering wheel, gear shift knobs, and arm rests to determine if alachlor was being transferred to the interior of the application vehicles. The potential for exposure in these commercial applicators was high. Duration of the work day averaged 11.7 hrs, duration of alachlor application averaged 2.9 hrs, and the amount of alachlor handled averaged 436 lbs. The cabs of all vehicles were enclosed. The clothing patch measurements indicated that there was great variability among applicators, though the thighs received the greatest amount of alachlor deposition. Alachlor did penetrate shirts, while patches beneath shirt had 60% lower levels than patches on shirts indicating some protection. Only 24% of the participants wore shirts or jackets with long sleeves (product label recommends wearing long sleeves of tightly woven material). Hand and glove washes were only available for 12 subjects, but in general, the workers who wore no gloves had the greatest exposures, followed by workers who wore cotton-lined rubber gloves, and those that wore the unlined gloves had the lowest levels of exposure. However, washes of the interior of the gloves indicated that all gloves were contaminated, which may reflect the practice of removing gloves after mixing and loading, not wearing gloves in the cab during application, and redoning the gloves during clean-up operations. Whether subjects washed their hands during the work day was not controlled, and a single ethanol wash of hands and gloves is not adequate to properly monitor dermal hand contamination during the course of the day.

Other studies in Rheses monkeys have shown that washing hands with soap and water is an effective means of removal of alachlor from the hands (Wester et al., 1992). Up to 87.5% of the applied alachlor could be removed with three successive hand washings up to one hr after alachlor was applied. The level of decontamination was reduced at three hrs post application to 56% removal, while at 24 hrs only 28.7% of the applied alachlor was removed. Use of water alone was less effective than the use of soap and water.

These studies illustrate the need for the availability of hand washing stations in the field for those who are handling, mixing or applying alachlor to reduce dermal exposure. Education and training on the importance of wearing appropriate outer clothing is also needed to minimize unnecessary exposures to alachlor in pesticide mixers and applicators.

C. Alachlor Levels in Breast Milk and Cow's Milk:

We could not locate any published reports of alachlor in human breast milk. Because alachlor is rapidly metabolized, and is excreted primarily in the urine in primate species, it would not be suspected of being a breast milk contaminant in humans. There is one report of the detection of low levels of alachlor below tolerance levels in cow's milk (Pvlypiw and Hankin, 1991). Pooled raw milk samples (n = 78) from cows in Connecticut were found to have mean concentrations of 0.0067 ppm alachlor, with a range of

0.001 to 0.018 ppm. Some of the samples (10.3%) had no detectable levels of alachlor. None of the samples exceeded the 1990 EPA tolerance for alachlor in milk set at 0.02 ppm.

D. FDA's Food Pesticide Residue Monitoring Program:
As was discussed previously, one of the federal agencies responsible for monitoring the level of pesticide residues in food is the FDA. Alachlor is one of the pesticides monitored in this program. In their 1998 residue monitoring report, the FDA reported that alachlor was not detected in the 3,625 domestic and 4,969 imported foods analyzed for pesticide residues (FDA, 1999). The FDA also conducts "Total Diet Studies" where foods are purchased from supermarkets and grocery stores in four geographic regions of the US. These are also called Market Basket surveys. The foods are selected to reflect foods typically eaten by Americans. The foods are prepared, and then analyzed for pesticide residues. In a report issued by the FDA in 1999, a summary was compiled of residues found by pesticide for Total Diet Studies conducted between 1991 and 1997. The report stated that alachlor residues were not detected in any of these Total Diet Studies (FDA, 1999). This data indicates that alachlor is not detected on foods typically eaten by Americans.

VI. Recommendation for Breast Cancer Risk Classification

There is not sufficient data to rate alachlor as a human breast carcinogen. Alachlor is rated as a Class 3 Breast Cancer Carcinogen "not classifiable" as to it's breast cancer risk. This rating is based on the following evidence:

- Currently there are no epidemiological human case-control studies available that have examined the risk of breast cancer in alachlor exposed populations. While one study conducted in an alachlor manufacturing plant did not find an increased risk of breast cancer incidence or mortality in a small study of 225 female workers, the size of the cohort would need to be larger before a meaningful conclusion could be made about occupational exposures to alachlor in females and breast cancer risk. The results of a recent ecological study conducted in Minnesota suggested that geographical areas of the state with higher use of pesticides, including alachlor, did not have a higher breast cancer risk than urban areas of the state with lower levels of agricultural pesticides. However, actual exposure to alachlor was not determined in this study.
- The experimental animal cancer bioassays in rats and mice do not provide evidence that alachlor is a mammary carcinogen. Evidence for alachlor's ability to be an estrogen mimic in mammalian systems is very weak to negative. There is very limited evidence that it may be a weak estrogen mimic in reptiles, including the alligator. Studies conducted to date do not suggest that alachlor influences steroid hormonal pathways in a way that would have an effect on breast cancer risk.
- There is limited evidence that certain alachlor metabolites may be weakly mutagenic.
- Alachlor has been identified as a carcinogen at other sites (nasal turbine cancer, stomach, lung and thyroid cancer) in experimental animals. There is not always perfect concordance between the site of a cancer in experimental animals and a cancer site in humans. Therefore, a compound identified as a carcinogen in experimental animals must be viewed with caution as having the potential to induce cancer at other sites in humans.

VII. Research Gaps and Recommendations for Future Research

- Cohorts of manufacturing workers exposed to alachlor during its production should continue to be monitored for the incidence and mortality from cancer, including breast, lung, nasal and stomach/gastric cancers. Many of these cancers have long latency periods, and the cohort may not yet be old enough to observe the development of occupationally-induced cancers.
- The presence and the persistence of alachlor's degradative products, including 2,6-diethylanaline, ESA and OA should be monitored in groundwater, surface water and tap water in states and water supplies that use alachlor for crop production. Because of the mobility of these degradative products, water supplies both near and distant from the point of use need to be monitored.
- Since alachlor degradative products, including ESA and OA, were often found in ground and surface water supplies at levels that exceeded the level of alachlor, it will be important for maximum contaminant levels to be set for these compounds by EPA. Until these levels are set, then state agencies should set maximum contaminant levels. The degradative products of alachlor, rather than the parent compound, may be more important to monitor because of the prevalence, persistency, and mobility of alachlor degradative products.
- It should be determined if mammalian systems can metabolize ESA and/or OA to toxic benzoquinone metabolites. It is known that a variety of alachlor metabolites are weakly mutagenic, but it has not been established if alachlor soil degradative products such as OA and ESA are capable of being metabolized to mutagenic or clastogenic compounds.

VIII. Summary of Studies Currently Being Conducted

The following studies were abstracted from the Computer Retrieval of Information on Scientific Projects (CRISP) database, which lists research studies funded by federal agencies, or summaries were obtained through personal communications with the principal investigator (PI).

Agricultural Health Study
Joint intramural research, NCI and NIEHS
Dr. Michael Alavanja, Project Officer, National Cancer Institute (personal communication with Dr. Alavanja)

This 10-year prospective study, which is in its fifth year, will follow 90,000 farmers, commercial pesticide applicators, and spouses of farmers and applicators in Iowa and North Carolina. The survey will document pesticide usage by questionnaire, and in a sub-set of the population, actual pesticide exposures will be measured in the urine and blood using validated biomarkers. Information will also be gathered on home use of pesticides, as well as agricultural uses of pesticides. This study is unique, since it will include one of the largest cohorts of female pesticide applicators ever followed, as well as including the female spouses of farmers and pesticide applicators. Approximately 58,000 men and 32,000 women are enrolled in this study. Case-control breast cancer studies as well as other cancer case-control studies are planned.

Mechanism of Induction of Olfactory Tumors by Alachlor
Funded by the National Institute of Environmental Health Sciences
Dr. Mary B. Genter, PI, University of Cincinnati
(adapted from the abstract posted on the 1999 CRISP Database, https://www-commons.cit.nih.gov/crisp/index.html)

The purpose of this study is to determine the cell type and origin of alachlor-induced olfactory mucosal tumors and characterize cellular and molecular changes associated with the progression from preneoplastic lesions to adenomas to adenocarcinomas. They will determine the metabolic enzymes for conversion of alachlor to its mutagenic metabolites(s); this information will aid in cross-species extrapolation for the risk associated with the development of alachlor-induced nasal tumors. The researchers will also evaluate other chloracetanilide herbicides for potential mutagenicity in the olfactory mucosa in order to ensure a more complete hazard identification for this class of compounds. These studies will provide important new data on the site-specific mechanism of carcinogenesis and will aid in the refinement of risk assessment and risk management strategies for the chloracetanilide compounds.

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X. Appendix A. Common Abbreviations, Acronyms and Symbols

aER	alligator estrogen receptor
AI	Active Ingredient
BCERF	Cornell Program on Breast Cancer and Environmental Risk Factors in New York State
bdwt	body weight
CAS	Chemical Abstract Service
CDEPA	2-chloro-N-(2,6-diethylphenyl)acetamide
CF	Commercial Formulation
CfE	Cornell University Center for the Environment
CHO	Chinese hamster ovary cells
CI	Confidence Interval
CRISP	Computer Retrieval of Information on Scientific Projects; database of scientific intra and extramural projects supported by the Dept. of Health and Human Services (i.e., NIH, EPA, USDA)
DEA	2,6-diethylanaline, metabolite of alachlor
DEBQI	3,5 diethylbenzoquinoine-4-amine, metabolite of alachlor
DEN	diethylynitrosomine
DNA	deoxyribonucleic acid
E-SCREEN	screening assay for estrogenicity that measures proliferative response in estrogen-dependent breast tumor cells
ELISA	enzyme linked immunoassay
EPA	United States Environmental Protection Agency
ER	estrogen receptor
ERE	estrogen response elements
ESA	ethanesulfonic acid
FDA	Food and Drug Administration
FISH	fluorescence in situ hybridization
ha	hectare
HA	Health advisories are non-enforceable limits of the concentration of the chemical in the drinking water that is not expected to cause any adverse noncarcinogenic health effects when consumed for no more than the time period specified, with a margin of safety
HEEA	2,6-hydroxyethylethylaniline, urinary metabolite of alachlor
hER	human estrogen receptor
HPLC	high-performance liquid chromatography
IARC	International Agency for Research on Cancer, sponsored by the World Health Organization, Lyon, France
ICET	Cornell Institute for Comparative and Environmental Toxicology
i.p.	intraperitoneal
kg	kilogram
KLH	keyhole limpet hemocyanin
L	liter
LC	liquid chromatography
lb	pound
MCL	Maximum Contaminant Level
MCLG	Maximum Contaminant Level Goal
µg	microgram
mg	milligrams
MCF-7	Michigan Cancer Foundation; cells derived from human breast tumor
MDL	Minimum detection limit; lowest concentration of a pesticide residue detectable by a given method
MOE	Margin of Exposure; non-linear dose response with respect to tumor induction
MM	Multiple myeloma
MNNG	N-methyl-N'-nitro-N-nitroso-guanidine
MS	mass spectrophotometry
MWPS	Midwest Pesticide Study
n	number of subjects/animals in the group
NAWQA	National Water Quality Assessment program
NCI	National Cancer Institute
NHL	Non-Hodgkin's lymphoma
NIOSH	National Institute for Occupational Safety and Health
NK	natural killer cells
nM	nanomoles; nanomolar
NOEL	No Observable Effect Level
NTP	National Toxicology Program
NYS DEC	New York State Department of Environmental Conservation
NYSDOH	New York State Department of Health
NYS	New York State
OA	2-(2',6'-diethylphenyl)(methoxymethyl)amnio]-2-oxoacetic acid
OR	Odds Ratio
pM	picomoles; picomolar
ppb	parts per billion
ppm	parts per million
RACB	Reproductive Assessment by Continuous Breeding; National Toxicology Program Protocol
SCE	sister chromatid exchange
SCGE	single-cell gel electrophoresis
SE	starch encapsulated
SIR	Standard Incidence Ratio
SRR	Standardized Mortality Rate Ratio
T3	triiodothyronine
TSH	thyroid stimulating hormone
UDPGT	uridine 5'-diphosphate glycuronyl transferase
US	United States
USDA	United States Department of Agriculture
USGS	United States Geological Survey
yr	year

Symbols:

α	alpha
β	beta
γ	gamma
μ	micro
<	less than
>	greater than
≥	greater than or equal to
%	percent
p	p value
±	plus or minus
=	equal
®	registered trademark

XII. Appendix B. BCERF Critical Evaluations of Breast Cancer Risk

This includes an overview of the Critical Evaluations and explanation of the BCERF Breast Cancer Risk Classification Scheme (revised 10/98 sms).

The Process:
Starting Point - Existing Critical Evaluations on Evidence of Carcinogenicity
IARC Monographs (**I**nternational **A**gency for **R**esearch on **C**ancer)
NTP ARC (**N**ational **T**oxicology **P**rogram, **A**nnual **R**eport on **C**arcinogens)
ATDSR (**A**gency for **T**oxic **D**isease **S**ubstance **R**egistry)

Conduct **Literature Searches** using databases to obtain historical and the most recent information; i.e. Toxline, Medline, Biosis, Cancerlit
-Peer-reviewed scientific literature-available through Cornell libraries and interlibrary loans.
-**Technical Reports**–NTIS-National Technical Information Service
-**TOXNET databases**–USEPA's Integreated Risk Information System (IRIS) database source of oncogenicity and regulatory status information
-**Gray literature**–Studies submitted to US Environmental Protection Agency (EPA) that are not published–i.e.
-industry generated oncogenicity studies
-Some abstracts of cancer bioassays are on line (IRIS database)
-Request reports from industry
-Request reports from EPA through Freedom of Information Act

The Critical Evaluation includes some general background information, including: chemical name, chemical formula, Chemical Abstract Subject Registry no. (CAS #), chemical structure, trade name(s), trade names of mixtures, metabolites/degradation products, history of use, and current regulatory status.

Evidence of cancer in other (non-breast) organ systems is provided in synopsis form with some critical commentary, along with the current overall carcinogenicity classification by international (IARC) and US Federal agencies (NTP, USEPA).

Human epidemiological studies, animal studies, and other relevant studies on possible mechanisms of carcinogenesis are critically evaluated for evidence of exposure to agent and breast cancer risk based on "strength of evidence" approach, according to a modification of IARC criteria as listed in the IARC Preamble. (See attached sheets for a more detailed explanation of the BCERF Cancer Risk classification scheme)

The **emphasis of the document** is a critical evaluation of the evidence for breast cancer risk, classification of the agent's breast cancer risk, identification of research gaps, and recommendations for future studies. A section is devoted to brief summaries of new research studies that are in progress. A bibliography with all cited literature is included in each critical evaluation. Major international, federal and state agencies will be provided with copies of our report.

General Outline of BCERF Critical Evaluations-revised 12/99 sms

I. Chemical Information
II. History of Use, Usage
  A. History of Usage and Uses
  B. Current Usage (when applicable)
III. Current Regulatory Status
  A. Current Regulatory Status
  B. Drinking Water Standards and Health Advisories
  C. Food Residue Tolerances and Action Levels (when applicable)
  D. Workplace Regulations (when applicable)
IV. Summary of Evidence of Overall Carcinogenicity (non-breast sites)
  A. Human Studies
    1. Case-Studies
    2. Human Epidemiological Cohort Studies
    3. Human Epidemiological Case-Control Studies
  B. Experimental Animal Studies
  C. Current Classification of Carcinogenicity by other Agencies
    1. IARC (International Agency for Research on Cancer)
    2. NTP (National Toxicology Program)
    3. USEPA (Environmental Protection Agency)
V. Critical Evaluation of the Scientific Evidence for Breast Cancer Risk
  A. Humans Studies
    1. Case-Studies
    2. Human Epidemiological Cohort Studies
    3. Human Epidemiological Case-Control Studies
    4. When available will summarize information detection/accumulation in human tissues / and validation of biomarkers
  B. Experimental Animal Studies
  C. Other Relevant Information, including mechanisms by which exposure may affect breast cancer risk (examples: co-carcinogenicity, tumor promotion estrogenicity, endocrine disruption, reproductive toxicology, genotoxicity, cell proliferation, oncogene/tumor suppressor gene expression, immune function, etc.)
VI. Other Relevant Information
  A. Specific for the pesticide;
    (i.e. may include information on environmental fate, potential for human exposure, etc.)
VII. Summary, Conclusions, Recommendation for Breast Cancer Risk Classification
VIII. Identification of Research Gaps, and Other Recommendations
IX. Brief Summaries of New Human Studies Currently Being Conducted
X. Bibliography
XI. Appendix A. Common Abbreviations, Acronyms and Symbols
XII. Appendix B. BCERF Critical Evaluations of Breast Cancer Risk; Approach,
General Outline of Critical Evaluatons, BCERF and Risk Classification Scheme
XII. Appendix C. Trade Names

BCERF Breast Cancer Risk Classification Scheme (adapted from the IARC Preamble by S.M.Snedeker)

Group 1: **Human breast carcinogen;** *sufficient evidence* of carcinogenicity to humans is necessary. *Sufficient evidence* is considered to be evidence that a **causal** relationship has been established between exposure to the agent and human breast cancer.

Group 2A: **Probable breast carcinogen**; this category generally includes agents for which there is *1) limited evidence* of breast carcinogenicity in humans and *sufficient evidence* of mammary carcinogenicity in experimental animals. The classification may also be used when there is *2) limited evidence* of breast carcinogenicity in humans and strong supporting evidence from other relevant data, or when there is 3) *sufficient evidence* of mammary carcinogenicity in experimental animals and strong supporting evidence from other relevant data.

Group 2B: **Possible breast carcinogen;** this category generally includes agents for which there is 1) *limited evidence* in humans in the absence of *sufficient evidence* in experimental animals; 2) *inadequate evidence* of carcinogenicity in humans or when human data is nonexistent but there is *sufficient evidence* of carcinogenicity in experimental animals, 3) *inadequate evidence* or no data in humans but with *limited evidence* of carcinogenicity in experimental animals together with supporting evidence from other relevant data.

Group 2C: **Potential to affect breast cancer risk**; this category includes agents for which there is **inadequate or nonexistent human and animal data**, but there is **supporting evidence from other relevant data** that identifies a mechanism by which the agent may affect breast cancer risk. Examples are, but are not limited to: evidence of agent's estrogenicity, disruption of estrogen metabolism resulting in potential to affect exposure to estrogen; evidence of breast tumor promotion, progression or co-carcinogenicity; increased expression of proto-oncogenes or oncogenes; evidence of inactivation of tumor suppressor gene associated with breast cancer; evidence of adverse effect on immune function; or evidence of a structural similarity to a known breast carcinogen (structure-activity relationship).

Group 3: **Not classifiable** as to its breast carcinogenicity to humans. Agents are placed in this category when they do not fall into any other group.

Group 4: **Probably not a breast carcinogen in humans**: This category is used for agents for which there is evidence suggesting a lack of breast carcinogenicity in human studies and in animal studies, together with a lack of related evidence which may predict breast cancer risk. The absence of studies does **not** constitute evidence for a lack of breast carcinogenicity.

Brief Definitions of Sufficient, Limited, and Inadequate Evidence: (adapted for breast carcinogenicity from the IARC Preamble by S.M. Snedeker)

Human Studies

Sufficient evidence of carcinogenicity in humans: Must have established evidence between exposure to the agent and human breast cancer. Case-reports are given the least weight in considering carcinogenicity data in humans—they are suggestive of a relationship, but by themselves cannot demonstrate causality. Consistent, case-control studies which have controlled for confounding factors and have found high relative risks of developing breast cancer in relation to an identified exposure are given the most weight in determining a causal relationship.

Limited evidence of breast carcinogenicity in humans: A positive association has been observed between exposure to the agent and breast cancer, but chance, bias or confounding factors could not be ruled out.

Inadequate evidence of breast carcinogenicity in humans: The available studies are of insufficient quality, consistency or statistical power to permit a conclusion regarding the presence or absence of a causal association.

Experimental Animal Studies

Sufficient evidence of breast carcinogenicity in animals: Evidence of malignant tumors or combination of benign and malignant tumors in (a) two or more species of animals, (b) or two or more independent studies in one species carried out at different times or in different laboratories or under different protocols.

Limited evidence of breast carcinogenicity in animals: The studies suggest a carcinogenic effect, but are limited for making a definitive evaluation because: (a) the evidence of carcinogenicity is restricted to a single experiment; (b) there are unresolved questions regarding the adequacy of the design, conduct or interpretation of the study; or (c) the agent increases the incidence of only benign neoplasms of lesions of uncertain neoplastic potential, or of certain neoplasms which may occur spontaneously in high incidences in certain strains of animals.

Inadequate evidence of breast carcinogenicity in animals: The studies cannot be interpreted as showing either the presence or absence of a carcinogenic effect because of major qualitative or quantitative limitations.

XII. Appendix C: Trade Names of Alachlor Products* (Meister 1999)

Table 6. Trade names and producers of alachlor-containing products

Trade names	Producer/formulator name
Alagam®	Makhteshim-Agan
Alagan®	Makhteshim-Agan
Alanex®	Makhteshim-Agan
Alanox®	Crystal Chemical Inter-America and Dupocsa
Alapaz®	Pazchem, Ltd.
Chemiclor®	Chemiplant, S.A.
Chimiclor®	Diachem S.p.A.
Cropstar®	Monsanto Co.
Curfew®	Kilpest India, Ltd.
Dynachlor®	Ladda Co. Ltd.
Gramisso®	Insecticidas Internacionales, C.A.
Lasso®	Monsanto Co.
Lasso® II	Monsanto Co.
Micro-Tech®	Monsanto Co.
Partner®	Monsanto Co.
Propachlor-48®	Probelte, S.A.
Sanachlor®	Sanachem (Pty)
Sholay®	Rallis India Ltd.
Strike®	Luxan B.V.
Woprolach®	B.V. Industrie- & Handelsonderneming Simonis
Discontinued Trade Names	Producer/formulator name
Alatox® 480	Pyosa
Satochlor®	Chemol Trading Ltd. Co.

Table 7. Trade names of pre-mixes containing alachlor

Trade names	Other pesticides in pre-mix	Producer/formulator name
Agimix®	+ atrazine	Herbitecnica Industria De Defensivos S/A
Alazine®	+ atrazine	Makhteshim-Agan
Bronco®	+ glyphosate	Monsanto Co.
Bullet®x	+ atrazine	Monsanto Co.
Freedom®	+ trifluralin	Monsanto Co.
Lance®	+ trifluralin	Herbitecnica Industria De Defensivos S.A
Lariat®	+ atrazine	Monsanto Co.
Nudor Extra®	+ atrazine	not specified
Rastra®	+ atrazine	Pyosa, S.A. de C.V.
Discontinued trade names of pre-mixes	Other pesticides	Producer/formulator name
Cannon®	+ trifluralin	Monsanto Co.

**Note: Trade names are used herein for convenience and informational purposes only. No endorsement of products is intended and no criticism of unnamed products is implied. Trade names of alachlor and mixtures containing alachlor listed are those currently in use. Discontinued trade names are listed at the end of each table.*

XIII. Appendix D. Public Comments Received

After technical internal and external peer-review, the Critical Evaluation will be posted on the BCERF web site for 30 days. If any public comments are received, they will be scanned as submitted, and become a part of Appendix D.