Summary of Toxicology Studies with Alachlor

William F. HEYDENS
Toxicology Division, Monsanto Company
(Received July 8, 1998 ; Accepted November 20, 1998)

DESCRIPTION OF THE TEST CHEMICAL

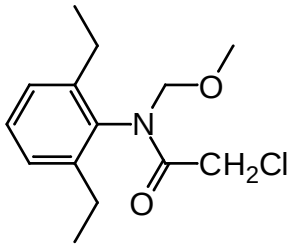
Alachlor was developed by Monsanto Company (USA) and introduced in 1967 for pre-emergence or pre-plant control of a broad spectrum of grass, sedge, and broadleaf weeds in corn, soybeans, dry beans, cotton, grain sorghum, sunflowers, peanuts, and other crops. In Japan, alachlor was first introduced in 1970 and has been used for the control of annual grass weeds in upland crops including corn, soybean, peanut, kidney bean, vegetables and strawberry.

Alachlor is a member of the chloroacetanilide class of chemistry and is the herbicidal active ingredient in LASSO® EC herbicide. This report summarizes the results of laboratory toxicology studies conducted with alachlor. The physical and chemical properties of alachlor are given below:

Common name: Alachlor

Chemical name: 2-chloro-2',6'-diethyl-*N*-(methoxymethyl)acetanilide

Chemical structure:



Molecular formula: $C_{14}H_{20}ClNO_2$

Molecular weight: 269.8

Physical state: solid at room temperature

Melting point: approximately 38˚C

Vapor pressure: 1.6×10^{-5} mmHg at 25˚C

Decomposition temperature: 105˚C

Water solubility: 242 ppm at 25˚C

ACUTE TOXICITY STUDIES

1. Alachlor Technical Material

The results of acute toxicity studies with alachlor are displayed in Table 1. In these tests, no distinctive signs of toxicity were observed following exposure via the different routes.

Table 1 Results of acute toxicity tests with alachlor.

Route of administration	Species	Median lethal dose
Oral	Rat[a]	l 350 mg/kg
	Mouse[b]	1100 mg/kg
	Rabbit[c]	1740 mg/kg
Dermal	Rabbit[d]	13300 mg/kg
Inhalation	Rat[e]	> 5.1 mg/*l*[f]

[a] Bio/dynamics Inc., 1985. [b] Tokyo Dental College, 1969.
[c] Younger Laboratories, 1969. [d] Bio/dynamics Inc., 1979.
[e] Monsanto Environmental Health Laboratory, 1982.
[f] Represents maximum attainable atmospheric concentration.

Table 2 Results of acute toxicity tests with Lasso® EC herbicide.

Route of administration	Species	Median lethal dose
Oral	Rat[a]	1000 mg/kg
	Mouse[b]	1480 mg/kg
Dermal	Rabbit[a]	8000 mg/kg
Inhalation	Rat[a]	> 6.5 mg/*l*[d]

[a] Bio/dynamics Inc., 1977. [a] Tokyo Dental College, 1979.
[c] Bio/dynamics Inc., 1982. [d] Represents maximum attainable atmospheric concentration.

2. Lasso® EC Herbicide

The results of acute toxicity studies with the formulated product, Lasso® EC Herbicide, are shown in Table 2. Lasso EC® is an organic solvent-based liquid formulation containing approximately 43% alachlor.

IRRITATION STUDIES

1. Primary Dermal Irritation

Alachlor was topically applied to the shaved backs of 6 New Zealand white rabbits (2 sites per rabbit). Continuous 24 hr exposure resulted in very slight to slight erythema and edema. The primary irritation index was calculated to be I .9 on a scale of 8.0. Alachlor was slightly irritating to the rabbit skin. (Bio/dynamics Inc., 1979)

2. Primary Ocular Irritation

Alachlor was placed into the conjunctival sac of the right eye of 6 New Zealand white rabbits. The collateral eye served as the control. Treated eyes were rinsed 24 hr after application. There were no signs of significant irritation in any of the rabbits

tested (irritation index of 0.4 on a scale of 1 10). The eyes of 3 rabbits had no signs of irritation on the day following treatment, and the eyes of the remaining 3 rabbits were clear of irritation by day 3. Alachlor was nonirritating to the eyes of rabbits.

(Bio/dynamics Inc., 1979)

DERMAL SENSITIZATION STUDIES

The potential of alachlor to induce delayed dermal hypersensitivity was evaluated in a modified Buehler assay.[1,2)] Induction involved topical exposure of 5 male and 5 female Hartley albino guinea pigs to undiluted alachlor for 6 hr, 3 days per week for three weeks. Treated and naive guinea pigs (3 per sex) were challenged with undiluted alachlor 2 weeks after the last induction dose. Alachlor produced essentially no irritation following initial application; erythema, edema and/or necrosis were observed in some animals during subsequent repeated exposures in the induction phase. On challenge, 8 out of 10 animals exhibited a positive skin response. Alachlor is considered to be a dermal sensitizer in the guinea pig. (Bio/dynamics Inc., 1983)

SUBCHRONIC TOXICITY STUDIES

1. Six-Month Oral Study in Dogs

Alachlor was initially administered orally *via* gelatin capsules to groups of 6 male and 6 female beagle dogs at dose levels of 0, 25, 50, and 100 mg/kg/day. The animals were housed individually in suspended stainless steel cages. The high dose level was reduced to 75 mg/ kg/day after 3 weeks because of severe toxicity. Additional groups of 6 males and 6 females were added to the study at dose levels of 0 and 5 mg/kg/day when it appeared that a lower dose level may be needed to establish a NOEL (no observed effect level). All animals were dosed for approximately 6 months (27 weeks). Mortality was observed at and above 25 mg/kg/day. Kidney weights were increased at 50 and 75 mg/kg/day, but the toxicological significance of this finding is doubtful in the absence of any correlative histologic lesion. Liver toxicity was noted in dogs given doses of 25 mg/ kg/day and higher as manifested by increased serum enzyme levels and the occurrences of microscopic lesions. Indications of anemia were also noted in dogs given doses of 25 mg/kg/day and above. An apparent increase in liver weight in male dogs given the 5 mg/kg/ day dose was not considered toxicologically significant because there was no indicator of hepatotoxicity (e.g. increased enzymes or microscopic lesions) and no evidence of liver toxicity in the subsequent I year dog study at l0 mg/kg/day. Thus, the NOEL for subchronic toxicity in dogs is considered to be 5 mg/kg/day. (Pharmacopathics Research Laboratories, 1981)

2. Ninety-Day Feeding Study in Rats

Alachlor was administered in the diet to groups of 10 male and 10 female Sprague-Dawley rats at concentrations of O, 100, 200, 400, and 800 ppm. The animals were

housed individually in aluminum rat cages. Several hematologic and biochemical changes were observed in treated animals when compared with controls, how-ever, these changes were either not dose related, not consistent across sexes and/or not supported by pathology findings. None of these changes were considered to be related to alachlor administration. No effects were observed in any of the other parameters examined. The NOEL is considered to be 800 ppm (approximately 40 mg/kg/day).

(Tokyo Dental University, 1972)

3. Ninety-Day Feeding Study in Mice

Alachlor was administered in the diet to groups of 10 male and 10 female ICR-JCL mice at concentrations of 0, 100, 200, 400, and 800 ppm. The animals were housed in synthetic resin cages, five mice per cage. Increases in the absolute and relative liver and kidney weights of treated females and decreases in the absolute and relative spleen weights of males from the 100, 400, and 800 ppm groups were observed. However, these changes were not considered to be related to alachlor administration. No effects were observed on mortality, body weights, food consumption, hematology, biochemistry, and gross or microscopic pathology. The NOEL is considered to be 800 ppm (approximately 40 mg/kg/day). (Tokyo Dental University, 1972)

4. Thirteen- Week Feeding Study in Mice

Alachlor was administered in the diet to groups of l0 male and 10 female CD-1 mice at concentrations of 0, 1000, 1500, 2000, and 25,000 ppm for 13 weeks. The animals were housed individually in stainless steel cages with wire mesh bottoms suspended over paper bedding. The only definitive treatment-related effects were in-creased relative and/or absolute liver weights at 1 500, 2000, and 2500 ppm. Increased relative kidney weights at 2000 and 2500 ppm were considered to be possibly related to treatment. However, there were no functional or pathological effects in either the liver or kidneys. The NOEL is considered to be 1000 ppm (154 and 235 mg/kg/day for males and females, respectively).

(Monsanto Environmental Health Laboratory, 1992)

CHRONIC TOXICITY AND ONCOGENICITY STUDIES

1. Twelve-Month Oral Study in Dogs

Alachlor was administered orally via gelatin capsules to groups of 6 male and 6 female beagle dogs at dose levels of 0, l, 3, and 10 mg/kg/day for 52 weeks. The animals were housed individually in stainless steel cages. There was no effect on survival in treated dogs. The body weight of high dose males was reduced at the end of the study. Evidence of minor gastrointestinal distress was noted at 10, and possibly, 3 mg/kg/day. Anemia occurred in high dose group males, and to a very minor degree, in mid dose (3 mg/kg/day) males. Increased liver weights and bromosulphalein (BSP)

were observed in high dose animals. Histologic examination revealed hemosiderin deposition in renal tubular epithelium, hepatocytes, and splenic phagocytes of high dose males. Very slight hemosiderin deposits were found in the kidney of one mid dose male and the spleen of another. The hemosiderin deposition correlates well with the hematology findings and is indicative of hemolytic anemia. The chronic NOEL in dogs is considered to be l mg/kg/day. (Pharmacopathics Research Laboratories, 1984)

2. Two-Year Chronic Toxicity / Oncogenicity Studies in Rats

1) Alachlor was administered to groups of 50 male and 50 female Long-Evans rats in the diet at dose levels equivalent to 0, 14, 42, and 126 mg/kg/day. The animals were housed individually in elevated stainless steel cages. Male and female rats were sacrificed after 1 16 weeks (27 months) and 106 weeks (24 months), respectively.

At the end of the study, mean body weights were reduced in males at 42 mg/kg/day and in both sexes (1 6-20%) at 126 mg/kg/day. The mean survival time of high dose animals was also significantly reduced. Inlife eye examinations revealed the presence of a progressive uveal degeneration syndrome which was characterized by free floating iridial pigment in the ocular chambers and pigment deposition on the cornea and lens; degeneration/cataract formation also occurred in the rats most severely affected. At termination, the syndrome was present in all surviving high-dose rats, approximately 67% of the mid dose rats, and none of the surviving low dose rats. At the week 88 examination, 2-of-43 low dose rats may have exhibited the mildest form of the syndrome unilaterally, but they died prior to subsequent examinations. However, no additional low dose rats',developed ocular abnormalities even though several mid dose rats acquired the syndrome during this period. The uveal degeneration syndrome observed in the Long-Evans rat is considered to be a peculiar response in this strain because the response has not been observed in mice, dogs, or other strains of rats treated with alachlor.

Liver weights were elevated in high dose males and females. Microscopic evidence of hepatocellular toxicity (e.g. central lobular necrosis) was seen primarily in 42 and 126 mg/kg/day dose group animals but also in some animals at 14 mg/kg/day. Neoplastic lesions were observed in the nasal turbinate mucosa, glandular stomach mucosa, and thyroid follicular epithelium (Figs. 1 a and 1b). Significant increases in stomach and thyroid (males only) tumor incidences were limited to the highest dose tested, a dose which exceeded the Maximum Tolerated Dose (MTD). Because of non-neoplastic toxicity in the liver, and possibly eyes, of low dose animals, no NOEL was established for chronic effects in this study. (Bio/dynamics Inc., 1981)

2) A second chronic study was conducted with Long-Evans rats to follow up on effects seen in the previous study and to establish a definitive NOEL. Alachlor was administered to groups of 50 male and 50 female rats in the diet at dosages of 0, 0.5, 2.5, and 15 mg/kg/day for approximately 25 months. The animals were housed

individually in stainless steel mesh cages suspended over paper bedding. The nasal tumors noted in the previous study were also observed in several animals from the 15 mg/kg/day group and in a single rat dosed at 2.5 mg/kg/day. No tumors were observed in the stomach or thyroid. There were no indications of hepatotoxicity or ocular lesions in animals treated with alachlor at any dose level. The only non-neoplastic effect was nasal inflammation and submucosa gland hyperplasia in animals from the 1 5 mg/kg/day group. These findings are considered to be unrelated to the neoplastic response. Base on the results of additional studies to investigate the mechanisms of tumor formations observed with alachlor in rats, it was concluded that non-genotoxic, threshold -based mechanisms are operative for formation of these tumors in nasal, stomach and thyroid.[6-16] The NOEL for all non-neoplastic and neoplastic chronic effects in this study is considered to be 0.5 mg/kg/day.

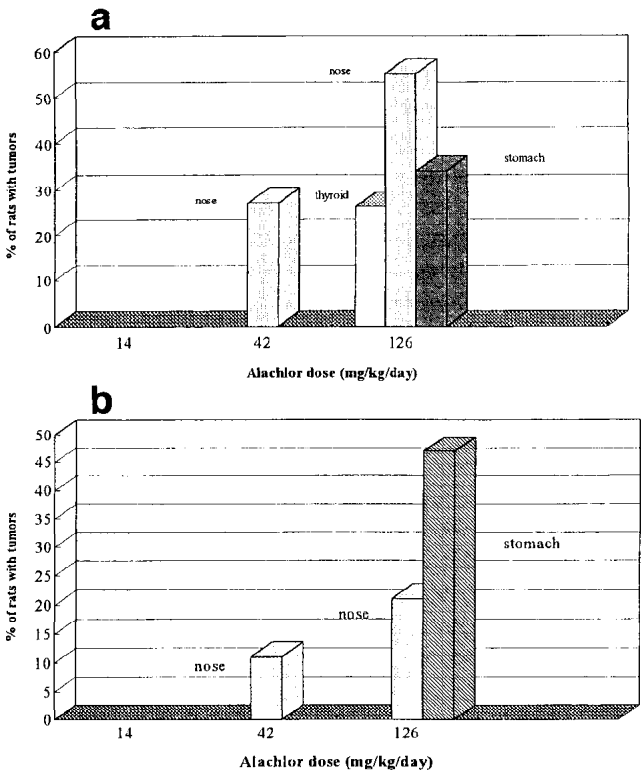


Fig. 1 Incidences of nasal, thyroid, and stomach tumors in Long-Evans rats from the first oncogenicity bioassay. a, males; b, females

(Monsanto Environmental Health Laboratory, 1984)

3. Eighteen-Month Oncogenicity Studies in Mice

1) Alachlor was administered to groups of 50 male and 50 female CD-1 mice in the diet at levels of 0, 26, 78, and 260 mg/kg/day for 79 weeks. The animals were housed individually in elevated stainless steel cages. There was no treatment-related mortality in the study. Body weights of high dose females were reduced approximately 7% from week 50 through the end of the study. Increased liver weights were noted in males and females from the 78 and 260 mg/kg/day dose groups. Kidney weights were also elevated in males at these two highest dose levels. Therefore, the NOEL for chronic toxicity is considered to be 26 mg/kg/day. The incidence of benign pulmonary tumors in high dose females was slightly but statistically higher than the control value. Because this incidence was within the historical control range and there was no evidence of preneoplastic effects or other indicators of an oncogenic response (e.g. multiplicity or progression to malignancy), the tumors were considered to be unrelated to alachlor administration. (Bio/dynamics Inc., 1977)

2) As part of reregistration procedures in Europe, a second mouse bioassay was done using updated testing guidelines. This study was conducted at concentrations of 0, 100, 400, and 1600 ppm for 18 months. These concentrations corresponded to approximate dose levels of O, 20, 78, and 331 mg/kg/day. The animals were housed individually in stainless steel cages with wire mesh bottoms suspended over paper bedding. The 1600 ppm dose level produced: marginally reduced body weight gain; increases in liver weight, serum sodium/ chloride (females); and increased incidences of fibrous osteodystrophy of the bone (females), liver eosinophilic foci (males), and chronic nephritis (males), a common age-related degenerative disease. The incidence of liver hypertrophy was increased in males at the 400 and 1600 ppm dietary levels. The severity of the hypertrophy was generally minimal-to-slight and also slightly increased only at 400 and 1 600 ppm. This finding is considered to be a metabolic adaptive change rather than a toxic response. The low dose of 100 ppm (20 mg/kg/day for combined sexes) is considered to be the NOEL.

(Monsanto Environmental Health Laboratory, 1994)

In the second mouse bioassay, the incidences of bronchoalveolar neoplasms appeared to be higher in some groups of treated animals, although the incidences were not dose-related. Given the inconsistent/non-reproducible nature of the results within and between the two mouse bioassays, all slides and data were evaluated by a panel of expert pathologists to assess a possible relationship to treatment. The following additional factors were considered: lung tumors in treated mice were morphologically like age-associated tumors commonly seen spontaneously; there were no differences in preneoplastic changes, tumor multiplicity, progression to malignancy, or tumor latency between control and treated mice. Finally, it was noted that alachlor is non-toxic to the lung (as evidenced by the lack of tissue damage or cell proliferation in either study), and autoradiography data show no localization in the lung; these factors indicate that the lung is not a target tissue. Based on all these observations, the panel concluded that the weight of evidence indicates that alachlor administration does not cause an increase in lung tumors in mice. (Pathco., Inc., 1995)

GENOTOXICITY STUDIES

To support registrations worldwide, the genotoxic potential of alachlor has been evaluated in numerous assay systems using a variety of species, metabolic activation conditions, and endpoints.[3)] These assays tested alachlor produced by Monsanto in well-validated systems using established testing guidelines according to Good Laboratory Practice standards. Alachlor was tested at levels up to 5000 μg per plate in microbial assays with five *S. typhimurium* strains (TA98, TA100, TA1535, TA1537, and TA1538) and one strain of *E. coli* (WP2 *hcr*) with and without a rat liver metabolic activation system. The test material did not induce reverse gene mutations under any of the assay conditions. Alachlor was negative when tested up to cytotoxic

levels in a Chinese hamster ovary (CHO) mammalian cell gene mutation assay. Alachlor was not clastogenic to rat bone marrow cells in an *in vivo* cytogenetics assay when administered orally at dose levels up to 1000 mg/kg. Alachlor did not exhibit *in vivo* mammalian genotoxicity in rat and mouse micronucleus assays conducted at doses of 600 mg/kg (i.p.) and 1000 mg/kg (p.o.), respectively. Alachlor was negative in *in vivo/in vitro* rat UDS assays at dose levels below 1000 mg/kg; variable responses were observed at an oral dose of 1000 mg/kg. This dose is near the oral LD_{50} and has been shown to produce severe hepatotoxicity. Therefore, the biological relevance of the results at 1000 mg/kg is doubtful. The potential of alachlor to damage DNA was also evaluated in vitro in a bacterial recombination assay with *Bacillus subtilis* using rec^+ and rec^- strains. Alachlor was tested up to 2000 μg per disc, and no genetic damage was observed. Further studies have focused on nasal tissue, a target site for induction of tumors in the rat. Nasal turbinate S9 metabolic activation systems from rats, mice, and monkeys did not activate alachlor, a secondary sulfide metabolite of alachlor, or diethyl aniline to mutagens detectable in the Ames assay.

It is concluded that alachlor does not have significant genotoxic potential in mammals. This conclusion is supported by an analysis of the alachlor genotoxicity data using a scoring system developed by the International Commission for Protection against Environmental Carcinogens and Mutagens.[4,5)] This system generates an overall score using data from multiple test systems incorporating various weight of evidence considerations (*e.g*. doses used, species tested, *in vitro vs. in vivo*). Alachlor's score of - 9 clearly positions it well in the non-genotoxic range of the evaluation system and provides objective confirmation of the conclusions from careful expert evaluation that alachlor does not have general genotoxic potential. Furthermore, the alachlor genotoxicity results support the conclusion that the rat-specific oncogenic response does not result from genotoxicity but rather from alachlor's effects on non-genotoxic processes.

EVALUATION OF THE ONCOGENIC POTENTIAL OF ALACHLOR

As noted above, neoplastic responses attributable to alachlor administration were observed in the stomach, thyroid, and nasal tissue of rats. Significant increases in stomach and thyroid tumors were restricted to the highest dose tested (*i.e.* 126 mg/kg/day). This high dose is considered to have exceeded a maximum tolerated dose (MTD) as evidenced by excessive body weight loss, hepatocellular necrosis, and decreased survival. The production of tumors only under these extreme conditions of exposure in rats is of doubtful relevance for humans. Although nasal tumors were produced at lower dose levels, a weight-of-evidence evaluation of data obtained from extensive mutagenicity testing reveals that alachlor has no significant genotoxic activity in mammalian systems. These data strongly suggest that tumors observed in the rat are arising through non-genotoxic, threshold-sensitive mechanisms. To better

understand the mechanisms involved in the production of these rodent tumors and their relevance to humans, a series of additional investigations were undertaken. These additional studies have been communicated in detail else-where, and the results are summarized below.

1. Rat Nasal Tumors

Extensive investigations have demonstrated significant species differences in the metabolism and pharmacokinetics of alachlor and have provided a mechanistic basis for the rat-specific production of nasal tumors as follows. Alachlor and its metabolites are converted to a diethyl quinoneimine metabolite, DEIQ, in rat nasal tissue.[6] This metabolite forms protein adducts,[7] which leads to cytotoxicity, prolonged cell proliferation and the eventual development of the predominantly benign, microscopic nasal tumors observed. This cell proliferation is considered to be a prerequisite for nasal tumor development, and its induction is threshold-sensitive, *i.e*., there is a dose below which cell proliferation and the ensuing tumor formation do not occur.

Critical differences in enterohepatic circulation and target tissue metabolism result in greater formation of DEIQ in rat nasal mucosa as compared to other species. Rat nasal tissue contains high levels of enzymes which metabolize alachlor to 4-amino-3,5-diethylphenol (DEA-phenol), the precursor to DEIQ. For example, the ability of rat nasal tissue to convert the secondary sulfide metabolite of alachlor to 2,6-DEA-phenol, the proximate metabolite of DEIQ, is more than 30 times greater than that of monkeys[8] and 751 times greater than that of human nasal tissue.[9] Species differences are even larger when the relative enzymatic rate of the initial metabolic step (alachlor conjugation to glutathione) is included in the overall rate of DEA-phenol formation from alachlor. When this is done, the overall ability of rats to convert alachlor to DEA-phenol is 3000- and 22,000-fold higher than that of humans when the initial glutathione conjugation occurs in the liver or nose, respectively.[9] These data indicate that the potential for the formation of the reactive DEIQ metabolite in human nasal tissue is negligible. The results further support the view that the rat is not an appropriate model for an assessment of alachlor's oncogenic risk to humans.

2. Rat Stomach Tumors

The majority of the work conducted to elucidate the mechanism by which chloroacetanilide herbicides induce stomach tumors in rats was conducted with butachlor, a close structural analog of alachlor which produces the same neoplastic response. A stomach tumor initiation-promotion study was conducted with butachlor as part of this research program.[10] Butachlor had no activity as an initiator, but did promote tumor formation following treatment with a known initiator. Thus, the results from this study have produced direct experimental evidence indicating a non-genotoxic mode of action for stomach tumorigenicity. A subsequent tumor promotion study

showed that alachlor produced stomach tumors *via* the same promotional activity.

Mechanistic studies with butachlor have shown that high dose exposure leads to mucosal atrophy with an accompanying profound loss of parietal cells as the initial lesion.[ll,12)] The resulting gastric hypochlorhydria and increased pH induces a compensatory production of gastrin. Together, the progressive mucosal atrophy and excessive gastrin secretion drive a proliferative response which culminates with tumor formation. It was subsequently demonstrated that long-term high dose alachlor exposure also produced mucosal atrophy, hypochlorhydria, and marked hypergastrinemia, all hallmarks of the unique oncogenic mechanism delineated for butachlor. Alachlor did not produce mucosal atrophy at a lower, non-oncogenic dose level. The results of these mechanistic investigations, in conjunction with the demonstrated promotional activity, provide strong evidence that alachlor produces stomach tumors in rats through the same non-genotoxic mechanism as butachlor.

This indirect mechanism of chloroacetanilide-induced stomach tumors does not present a human risk, since the conditions driving the requisite threshold-sensitive events are operative only in rats chronically exposed to very high dose levels. The prolonged exposure to excessively toxic doses required for tumorigenesis in rats could not occur in humans. Even if such exposure did occur, a study with rhesus monkeys showed that the key preneoplastic event (mucosal atrophy) does not occur in primates even at a dose that is twice that required to produce stomach tumors in rodents. Thus, there is compelling data supporting the conclusion that the stomach tumors are of no relevance to humans.

3. Rat Thyroid Tumors

Results from mechanistic work have shown that high dose alachlor exposure results in increased thyroid hormone elimination via induction of hepatic UDPGT activity, thereby causing a long-term, compensatory increase in circulating TSH Ievels.[13)] It is widely recognized that such prolonged stimulation of the thyroid gland via elevated TSH secretion leads to follicular cell hyperplasia and neoplasia in experimental animals.[14-16)] This hormone-mediated mechanism of carcinogenesis is considered to be a threshold phenomenon to which rats are particularly susceptible. The results with alachlor provide strong evidence that this non-genotoxic mechanism is operative in the production of alachlor-induced thyroid tumors in rats.

REPRODUCTION AND DEVELOPMENTAL TOXICITY STUDIES

1. Three-Generation Reproduction Study in Rats

Groups of 10-12 male and 17-24 female Sprague-Dawley rats were fed alachlor in the diet at dose levels of 0, 3, 10, and 30 mg/kg/day. Dosing was continuous throughout premating, mating, pregnancy, and lactation periods for 3 successive generations. Each parental generation was mated to produce two litters per generation. Offspring

from the second litters were selected to be parents for the subsequent generation. All animals were given a gross necropsy. Mating and fertility indices, as well as offspring weight/survival at birth and throughout lactation was evaluated for all litters. Histopathological evaluation was conducted on 10 male and 10 female control and high dose parents from all three generations as well as pups from the second litter of the last generation (F_3b). No adverse effects were observed on any reproductive parameter in any generation. Therefore, the NOEL for reproductive toxicity in rats is considered to be 30 mg/kg/day. (Bio/dynamics Inc., 1981)

2. Developmental Toxicity Study in Rats

Alachlor was administered in corn oil by gavage to four groups of mated Sprague-Dawley female rats at dosages of 0, 50, 150, and 400 mg/kg/day on days 6 through 19 of gestation. Fetuses were obtained on gestation day 20, examined externally, weighed, and processed for skeletal and soft tissue examinations. Maternal and fetal toxicity occurred in the high dose group as evidenced by maternal deaths, a slight decrease in fetal body weight, and a slight increase in postimplantation loss. No malformations were observed in treated offspring. The NOEL for both maternal and developmental toxicity in the rat is considered to 1 50 mg/ kg/day.

(International Research and Development Corp., 1 980)

3. Developmental Toxicity Study in Rabbits

Three groups of 1 8 mated female Dutch belted rabbits were administered alachlor by oral gavage in corn oil at dosages of 50, 100, and 150 mg/kg/day. An additional 20 mated females received only corn oil and served as controls. All animals were dosed daily from gestation day 7 through 19. All surviving females were sacrificed on gestation day 30 and routine uterine examinations were performed. All fetuses were weighed and examined for external malformations. Each fetus was then examined internally by dissection and then stained for skeletal evaluation.

The administration of alachlor resulted in maternal toxicity as indicated by maternal body weight loss at 150 mg/kg/day and decreased food consumption at levels of 100 and 150 mg/kg/day. There were no indications of embryotoxicity, fetal toxicity or malformations in any treated group. Therefore, the NOELS for maternal and developmental toxicity in the rabbit are considered to be 50 and 150 mg/kg/day, respectively. (Bio/dynamics Inc., 1988)

PHARMACOLOGY STUDIES

Alachlor was administered intraperitoneally to groups of 3 male and 3 female ICR mice at single doses of 0, 19.5, 78.1, 313, 1250, and 5000 mg/kg. All animals given the two highest dose levels died during the study. Male and female mice given alachlor at doses of 313 mg/kg and above exhibited abnormal signs of toxicity

characterized by excitation of the central nervous system and non-specific depression of activity. No signs of toxicity were observed in animals given alachlor at dose levels of 19.5 or 78.1 mg/kg. Hexobarbital sleeping time was prolonged in mice given doses of 78.1 and 313 mg/kg. The transport activity of the small intestine was inhibited at 313 mg/kg.

Acute oral administration of alachlor to 3 male albino rabbits at dose levels of 0, 78. l, 313, 1250, and 5000 mg/ kg resulted in deaths of the animals at the two highest dose levels tested. Prior to death, animals receiving these lethal doses also exhibited abnormal signs indicating non-specific depression in several aspects of behavioral, somatic, and autonomic functions. No abnormal clinical signs were noted in animals given doses of 78.l or 313 mg/kg. There were no distinct changes in EEG, respiratory rate, blood pressure, electrocardiogram, or heart rate at or below 313 mg/kg. A decrease in body temperature was noted only in animals given the 1250 mg/kg dose. Plasma hemoglobin and prothrombin times were increased in the 1250 mg/kg group. Activated partial thromboplastin time was not affected.

In conclusion, alachlor produced various clinical and pharmacological effects, primarily at acutely lethal dose levels. Effects generally disappeared within a few hours. The high dose levels required to produce severe effects confirm that the acute toxicity of alachlor is relatively low.

(The Institute of Environmental Toxicology, 1993)

EPIDEMIOLOGY STUDIES

Three epidemiology studies have been conducted on alachlor manufacturing workers which focus on the ocular and oncogenic effects seen in the rat bioassays. A group of the highest-exposed workers was examined for the presence of a specific eye abnormality, analogous to the initiating lesion in Long-Evans rats, called pigmentary dispersion syndrome (PDS).[17] None of the exposed workers had PDS, and prevalence rates for other eye abnormalities were similar in exposed and unexposed individuals. In another study, there was no indication of increased mortality rates from cancer or any other causes among workers with up to 25 years of followup.[18] Likewise, cancer incidence rates over a 24-year period were not elevated in the highest-exposed alachlor workers, nor were there any cases of tumors in the nose, stomach, or thyroid, the organs in which oncogenic effects were produced in rats.[18] These manufacturing workers had year-round exposure greatly exceeding agricultural exposure, which is limited to a two- or three week period prior to planting in the spring. In fact, manufacturing exposure was estimated to exceed yearly agricultural exposure by a factor of l0,000 or more.[19] Potential dietary exposures are even lower than agricultural exposure. Therefore, the absence of eye effects and increased mortality and cancer rates in manufacturing workers serve as an important indicator of the low potential for adverse effects among the general population, who are exposed to extremely low

levels of alachlor if at all.

CONCLUSIONS

The results of tests in laboratory animals indicate that alachlor has low mammalian toxicity following acute oral, dermal, and inhalation exposure. Alachlor has some potential to produce allergic skin reactions following repeated or prolonged exposure. Subchronic and chronic exposure produces primarily liver and kidney toxicity for which large margins of safety exist. Ocular effects were produced in Long-Evans rats but have not been observed in Sprague-Dawley rats, CD-1 mice, beagle dogs or humans. Alachlor does not have general genotoxic potential and does not interfere with normal reproductive and developmental processes. Alachlor is not oncogenic in the CD-1 mouse. Neoplastic lesions in the rat are observed in the nasal turbinate mucosa, stomach glandular mucosa, and thyroid follicular epithelium. Significant increases in the stomach and thyroid tumors occurred only at the highest dose tested which produced excessive toxicity.

A large body of data supports the conclusion that alachlor produces these tumors in rats through nongenotoxic mechanisms for which thresholds exist. The tumors are not accompanied by DNA damage as assessed in target tissue itself and in other standard *in vivo* mammalian assays up to the MTD. The conditions driving the requisite threshold-sensitive preneoplastic events occur only in experimental studies employing doses at and above the Maximum Tolerated Dose (MTD). Observed species differences in physiology and metabolism indicate that the rat is particularly susceptible to the induction of nasal, thyroid and stomach tumors following chronic exposure to this agent. Consequently, humans would be at significantly lower risk for development of these tumors. In fact, substantial margins of safety do exist, as human exposure is several orders of magnitude below the doses producing tumors in rats. Therefore, it is concluded that the alachlor-induced tumors in rats are not relevant to humans under actual conditions of exposure. This conclusion is supported by the lack of mortality and tumors in manufacturing workers who have the highest exposure to alachlor.

In summary, an extensive health and safety database has been developed to support the registration of alachlor and LASSO® products worldwide. A thorough evaluation of these data indicates that alachlor products are of generally low toxicity and present minimal opportunity for human exposure. When used in accordance with label directions, alachlor will not adversely affect human health.

Based on the review of the studies summarized above, the Japan Ministry of Health and Welfare (MHW) established an Acceptable Daily Intake (ADI) for alachlor as 0.005 mg/kg/day with the NOEL from the chronic rat study of 0.5 mg/kg/day and the safety factor of 100. This ADI supports the current withholding limits established by the Japan Environmental Agency (EA) of 0.05 ppm in grains (excluding rice), and beans, and 0.01 ppm in vegetables, potatoes, sugar beet, sugarcane and fruits.

REFERENCES

1) Buehler: *Arch. Dermatol*. **91**, 171 (1965)
2) H. Ritz & E. Buehler: "Current Concepts in Cutaneous Toxicity," ed. by V. Drill & P. Lazar, Academic Press, New York, pp. 25-40, 1980
3) L. Kier, W. Heydens, H. Lau, D. Thake, K. Asbury & A. Wilson: *Toxicologist* **30**, Part 2, Abstract I 182 (1996)
4) D. Brusick, J. Ashby, F. de Serres, P. Lohman, T. Matsushima, B. Matter, M. Mendelsohn, D. Moore II, S. Nesnow & M. Waters: *Mutat. Res*. **266**, I (1992)
5) P. Lohman, M. Mendelsohn, D. Moore II, M. Waters, D. Brusick, J. Ashby & W. Lohman: *Muta. Res*. **266**, 7 (1992)
6) P. Feng, A. Wilson, R. McClanahan, J. Patanella & S. Wratten: *Drug Metab. Dispos.* **18**, 373 (1990)
7) A. Wilson, ~l. Lau, K. Asbury, D. Thake & W. Heydens: Presented at International Congress of Toxicology-VII, Seattle, 1995
8) A. Li, K. Asbury, W. Hopkins, P. Feng & A. Wilson: *Drug Metab. Dispos.* **20**, 616 (1992)
9) A. Wilson, H. Lau, K. Asbury & W. Heydens: *Toxicologist* **15**, Abstract 1398 (1995)
10) A. Wilson, D. Branch, M. Shibata, D. Thake, T. Shirai & A. Hagiwara: Presented at Annual Conference oflnternational Federation of Societies of Toxicologic Pathologists, Tours, 1995
l l) G. Hard, M. Iatropoulos, D. Thake, D. Wheeler, M. Tatematsu, A. Hagiwara, G. Williams & A. Wilson: *Exp: Toxic. Pathol.* **47**, 95 (1995)
12) D. Thake, M. Iatropoulos, G. Hard, K. Hotz, C. Wang, G. Williams & A. Wilson: *Exp. Toxic. Pathol*. **47**, 107 (1995)
13) A. Wilson, D. Thake, W. Heydens, D. Brewster & K. Hotz: *Fundam. AppL Toxicol.* **33**, 16 (1996)
14) R. Hill, L. Erdreich, O. Paynter, P. Roberts, S. Rosentahl & C. Wilkinson: *Fund. Appl. Toxicol.* **12**, 629 (1989)
15) G. Thomas & E. Williams: *Mutat. Res.* **248**, 357 (1991)
16) G. Zbinden: *Arch. Toxicol*. Suppl **12**, 98 (1988)
17) B. Ireland, J. Acquavella, T. Farrell, M. Anne & T. Fuhremann: *J. Occup. Med.* **36**, 738 (1994)
18) J. Acquavella, S. Riordan, M. Anne, J. Collins, B. Ireland & W. Heydens: *Environ. Health Perspect.* **104** (7), 2 (1996)
19) J. Acquavella, B. Ireland, T. Leet, M. Anne, T. Farrell & M. Martens: In: Proceedings of the XII Joint CIGR, IAAMRH, IUFRO International Symposium: Health , Safety and Ergonomic Aspects in Use of Chemicals in Agriculture and Forestry, June, 1993, Kiev, Ukraine: Institute for Occupational Health 184, 1994

要　約

アラクロール毒性試験の概要

米国モンサント･カンパニー毒性部

アラクロールは，米国モンサント･カンパニーが開発した一年生イネ科雑草および一部の広葉雑草を対象とする畑作用除草剤である．動物実験の結果，水剤の哺乳動物に対する急性経口，経度，吸入毒性はいずれも軽微で，眼および皮膚一次刺激性は低い．モルモットを用いた皮膚感作性試験において陽性の反応が認められた．至急性および慢性毒性試験の結果，主として肝臓および腎臓に検体投与による影響が認められたが，これらの影響には閾値が存在した．ラットを用いた慢性毒性/発がん性併合試験において腺胃，鼻部および甲状腺の腫瘍が認められたが，腫瘍発生のメカニズムに関する試験研究の結果，これらの腫瘍は閾値の存在する非遺伝子傷害性の作用によって引き起こされていることが解明されている．これらの腫瘍の発生に結びつく前段階の症状には閾値があり，最大耐量以上の用量でアラクロールを投与した動物実験においてのみ認められた．また，鼻部の腫瘍の発生には，ラットにおいてのみ認められる種特異的代謝が関与していた．このようにラットにおいて観察された腫瘍をヒトに外挿することは妥当ではない．この結論は，アラクロールへの暴露量が最も高いと考えられるアラクロール製造工場の労働者を対象とした疫学調査の結果，死亡率および腫瘍発症率の増加が認められなかったことからも支持されている．水剤には，哺乳動物生体内における遺伝毒性は認められず，正常な繁殖や発生過程を阻害することもなかった．

ここに要約した毒性試験成績の評価に基づき，ラットにおける慢性毒性/発がん性併合試験における無毒性量0.5mg/kg/日および安全係数100を用い，0.005mg/kg/日のADIが設定されている．

アラクロールは，ラッソー乳剤として昭和45年3月登録を取得して以来，大豆，とうもろこし，落花生，野菜，果樹等において雑草発生前土壌処理剤として広く使用されている．登録保留基準は，麦･雑穀(小麦を除く)，大豆，大豆以外の豆類に0.05ppm，いも類，第二大粒果実類，小粒果実類，第一葉菜類，第二葉菜類，根･茎類，てんさい，さとうきびに0.0lppmと設定されている．

Contacts

Toxicology Division, Monsanto Company, 800 N.Lindbergh Blvd., St.Louis, MO 63167,U.S.A.

Regulatory Affairs Team, Agro-Scicnce Division, Monsanto Japan Limited, Nihonbashi Daini Bldg., 41-12 Nihonbashi Hakozakicho, Chuo-ku, Tokyo 103-0015, Japan

問合せ

日本モンサント株式会社アグロサイエンス事業部規制･環境部

〒103-0015東京都中央区日本橋箱崎町41-12　日本橋第二ビル