Summary of Toxicology Studies with Butachlor

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DESCRIPTION OF THE TEST CHEMICAL

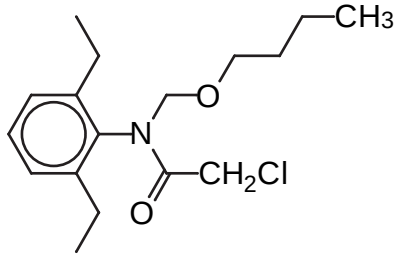
Butachlor was developed by Monsanto Company (USA) and introduced in 1968 for the pre-emergence control of undesirable grasses and broadleaf weeds in transplanted, direct seeded rice and barley fields. Butachlor was first introduced in Japan in 1973 and has been used for the control of grass weeds and broadleaf weeds in transplanted rice paddies.

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

Common name: Butachlor

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

Chemical structure:



Molecular formula: $C_{17}H_{26}NO_2Cl$

Molecular weight: 311.9

Physical state: clear amber liquid@room temperature

Melting point: 4-5˚C

Vapor pressure: 1.8 x 10^{-6}mm Hg@25˚C

Decomposition temperature: 156˚C

Water solubility: 20ppm@25˚C

Butachlor is miscible with alcohol, ether, acetone and other organic solvents.

ACUTE TOXICITY STUDIES

1. Butachlor Technical Material

The results of acute toxicity studies with butachlor are shown in Table 1. In these tests, no distinctive signs of toxicity were observed following exposure *via* the oral, dermal and inhalation routes.

Table 1 Results of acute toxicity tests with butachlor.

Route of administration	Species	Sex	Median lethal dose
Oral	Rat[a)]	♂	2620mg/kg
		♀	3050mg/kg
	Mouse[b)]	♂	4140mg/kg
		♀	5030mg/kg
Dermal	Rabbit[c)]	♂♀	13,000mg/kg
Inhalation	Rat[d)]	♂♀	>5.3mg/*l*

[a)] Institute of Environmental Toxicology, 1980. [b)] Institute of Environmental Toxicology, 1976. [c)] Bio/dynamics Inc., 1979. [d)] Monsanto Safety Evaluation-Newstead, 1998.

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

Route of administration	Species	Sex	Median lethal dose
Oral	Rat[a)]	♂♀	2800mg/kg
	Mouse[b)]	♂	4604mg/kg
		♀	5675mg/kg
Dermal	Rabbit[a)]	♂♀	>2000mg/kg
Inhalation	Rat[c)]	♂♀	1.63mg/*l*

[a)] Bio/dynamics Inc., 1979: Study conducted with butachlor 60% EC. [b)] Bozo Research Center, Inc., 1993: Study conducted with butachlor 32% EC. [c)] Bio/dynamics Inc., 1981: Study conducted with butachlor 60% EC.

2. Machete®EC Herbicide

Machete® EC Herbicide is an organic solvent-based liquid formulation containing approximately 32% or 60% butachlor. The results of acute toxicity studies with the formulated product are shown in Table 2. In these tests, no distinctive signs of toxicity were observed following exposure *via* the oral, dermal and inhalation routes.

IRRITATION STUDIES

1. Primary Dermal Irritation

Butachlor was applied topically to the shaven backs of 6 New Zealand white rabbits (2 sites per rabbit). Continuous 24hrs exposure resulted in slight erythema and edema. The primary irritation index was calculated to be 3.5 on a scale of 8.0.

Butachlor was moderately irritating to the rabbit skin. (Bio/dynamics Inc., 1979)

2. Primary Ocular Irritation

Butachlor was placed into the conjunctival sac of the right eye of 6 New Zealand white rabbits. The contralateral eye served as the control. Only a slight degree of irritation of the conjunctivae was seen in 2 of 6 rabbits. No effect on the cornea or iris was observed. Eyes regained their normal appearance by 3 days after exposure. Butachlor was slightly irritating to the eyes of rabbits. (Bio/dynamics Inc., 1979)

DERMAL SENSITIZATION STUDIES

The potential of butachlor to induce dermal hypersensitivity was evaluated in a modified Buehler assay.[1,2)] Induction involved topical exposure of 5 male and female Hartley albino guinea-pigs each, to undiluted butachlor for 6 hours, 3 days per week for 3 weeks. Treated and naive guinea-pigs (3 per sex) were challenged with 50 % butachlor in acetone 2 weeks after the last induction dose. Butachlor produced slight irritation following initial application; moderate irritation was observed after the second application; and moderate to severe erythema with edema was observed in all animals after the third application. On challenge, 5 out of 10 animals exhibited a positive skin response. After the second challenge which was conducted to confirm the response, all animals exhibited a positive skin response. Butachlor is considered to be a dermal sensitizer in the guinea-pig. (Bio/dynamics Inc., 1983)

SUBCHRONIC TOXICITY STUDIES

1. Ninety-Day Feeding Study in Rats

Butachlor was administered in the diet to groups of 12 male and female Fischer-344 (F-344) rats each, at concentrations of 0, 300, 1000, 3000 and 5000ppm for 90 days. The animals were housed individually in stainless steel cages. Decreased body weight gain was observed in both sexes at the 1000ppm dietary level and above. Mild anemia was observed in both sexes at 5000ppm and in females at 3000ppm. Evidence of liver toxicity (*e.g.* increased γ-glutamyl transpeptidase, alanine aminotransferase, cholesterol and liver weights as well as diffuse hepatocellular swelling) was observed in males only at the 1000ppm dietary level and above, and in females only at the 3000ppm dietary level and above. Early signs of glomerulonephropathy were apparent in males at the 1000ppm dietary level and above. Epithelial hyperplasia of the urinary bladder was apparent in males at the 5000ppm and in females at the 1000ppm dietary level and above. The No Observed Effect Level (NOEL) was considered to be 300ppm (17.5mg/kg/day for males and 19.0mg/kg/day for females). (Institute of Environmental Toxicology, 1987)

2. Ninety-Day Feeding Study in Wistar Rats

Butachlor was administered in the diet to groups of 15 male and female Wistar Imamichi rats each, at concentrations of 0, 500, 1000, 2500, 5000, 10,000, 20,000 and 40,000ppm for 90 days. Reduced body weight gains were observed in males and females at the 5000ppm dietary level and above. Statistically significant decreases in feed consumption were found in male and female rats at 10,000ppm. There was mortality at the 10,000ppm dietary level or above but not at the 5000ppm or lower. At the dietary levels of 2500ppm and higher, hemoglobin concentration, hematocrit value and RBC were decreased, suggesting possible anemia.

Decreased RBC CHE values in males at the 2500ppm dietary level and above, and females at 10,000ppm were noted. Plasma CHE was decreased in males at 10,000ppm but in females no decrease was found for plasma or brain CHE values.

Statistically significant differences were found in brain, pituitary, ovary, uterus and heart weights, but there were no histopathological changes, and therefore, these organ weight changes were not considered biologically significant. Decreased kidney weights were observed in females at the 5000ppm dietary level and in males at the 10,000ppm dietary level. Spleen weight was reduced in both males and females at dietary levels of 2500ppm and higher. Histopathologically, dose-related changes were found in the spleen, kidney and liver of animals at dietary levels of 2500ppm and higher. Atrophy was observed in the ovaries and testes at 40,000ppm; these findings may have been related to malnutrition at this very high dose level. The NOEL for this study was considered to be 1000ppm (68.0mg/kg/day for males and 72.2mg/kg/day for females).

(Nihon University of Veterinary Medicine and Zootechnology, 1971)

3. Ninety-Day Feeding Study in Sprague-Dawley Rats

Butachlor was administered in the diet to groups of 20 male and female Sprague-Dawley (S-D) rats each, at concentrations of 0, 1000, 5000, 7500 and 15,000ppm. Animals were housed individually in stainless steel cages. Physical signs of toxicity (hair loss and emaciation) and mortality (one female) were noted only at the 15,000ppm dietary level. These animals also exhibited markedly decreased body weights, decreased food consumption, changes in hematology, serum biochemistry and urinalysis parameters, liver and thyroid organ weight changes, histopathological evidence of liver damage, and gross and histopathological evidence of kidney damage. Most of these effects were also noted at the 5000 and 7500ppm dietary levels. The animals at the 1000ppm dietary level exhibited increased liver and thyroid weights. Because of the lack of corresponding histopathological effects, these organ weight changes (liver and thyroid) were considered equivocal evidence of toxicity and the NOEL of this study was therefore determined as 1000ppm (67.9mg/kg/day for males and 97.7mg/kg/day for females).

(Bio/dynamics, Inc./Institute of Environmental Toxicology, 1980)

4. Ninety-Day Feeding Study in DDY Mice

Butachlor was administered in the diet to groups of 7–8 male and female DDY mice each, at concentrations of 0, 500 (females only), 1000, 2500, 5000, 10,000 and 20,000ppm. No mortality was found in mice at the 5000ppm dietary level or lower. At the 10,000ppm dietary level, 60% of the mice died within 3 weeks on study; at the 20,000ppm dietary level all mice died within 1 week on study. All of these deaths were considered to be treatment related. Slightly decreased feed consumption was noted in both sexes at the 2500ppm and 5000ppm dietary levels. At the 10,000ppm dietary level, decreased feed consumption was noted in females. Decreased mean body weights and mean body weight gains were noted in females at the 2500ppm dietary level, and in both sexes at the 5000ppm and 10,000ppm dietary levels. A slight but statistically significant increase in serum glutamic-pyruvic transaminase values was observed in males at the 5000ppm and 10,000ppm dietary levels; because there were no histopathological findings observed in the liver corresponding to these clinical changes it was concluded that these results were not treatment related. RBC CHE activity was reduced in females from the 10,000ppm group, but not in males at the same dose.

Slight to moderate decreases in spleen weight were observed in females at the 1000, 5000 and 10,000ppm dietary levels. There were also sporadic changes in other organ weights (brain, pituitary, gonads, uterus, liver, adrenals, kidneys, heart). Since none of these findings exhibited a dose-related response and were not accompanied by corresponding histopathology changes, these findings were not considered to be treatment-related. Except for an increase in brown pigmentation of the splenic medulla, there were no treatment related histopathological changes observed. The NOEL for this study was considered to be 1000ppm (123.3mg/kg/day) for males and 2500ppm (278.1mg/kg/day) for females.

(Nihon University of Veterinary Medicine and Zootechnology, 1971)

5. Ninety-Day Feeding Study in CD-1 Mice

Butachlor was administered in the diet to groups of 30 male and female CD-1 mice each, at concentrations of 0, 1000, 3000, and 6000ppm for at least 91 days. The animals were housed individually in suspended stainless steel cages. The animals at the 6000ppm dietary level exhibited decreased body weights, hematological changes, decreased blood urea nitrogen levels, decreased brain cholinesterase activity, organ weight changes (brain, liver, and ovaries) and histopathological evidence of liver and kidney damage. Decreased body weights, and increased blood urea nitrogen, platelet levels, and liver weights were noted in the animals at 3000ppm dietary level. The animals from the 1000ppm group exhibited increased liver weights, but no corresponding histopathological changes and is therefore, considered to be equivocal evidence of toxicity. The NOAEL for this study was considered to be 1000ppm

(213.9mg/kg/day for males and 247.9mg/kg/day for females).

(Bio/dynamics, Inc., 1980)

6. Twenty-one Day Dermal Toxicity Study in Rabbits

Butachlor was administered dermally to groups of 10 male and female New Zealand White rabbits each, 5 days/week for 3 consecutive weeks at dose levels of 0, 100, 500 and 2500mg/kg/day. The animals were housed individually in suspended stainless steel cages. The only significant finding was an increase in glucose values in the females at the 2500mg/kg dose level. These changes in glucose were not considered biologically relevant or an indication of toxicity. Butachlor produced no other systemic, clinical chemistry or pathological changes. Local skin changes consisting of mild dermatitis were observed at lower dose levels while severe effects were seen at 2500mg/kg/day, the highest level tested. The NOEL for systemic toxicity from repeated dermal application of butachlor was considered to be 2500mg/kg/day.

(International Research and Development Corporation, 1982)

CHRONIC TOXICITY AND ONCOGENICITY STUDIES

1. Twelve-Month Oral Study in Dogs

Butachlor was administered orally *via* gelatin capsules to groups of 6 male and female beagle dogs each, at dose levels of 0, 1, 5, and 25mg/kg/day for 12 months. The animals were housed individually in stainless steel cages. No treatment-related effects of butachlor were observed on survival, body weights, hematology, urinalysis or gross pathology. Target organ effects (alkaline phosphatase, organ weight increases and histopathology findings) were observed in the liver and pancreas in dogs administered 25mg/kg/day butachlor. The NOEL for this study was considered to be 5mg/kg/day. (Institute of Environmental Toxicology, 1987)

2. Two-Year Chronic Toxicity/Oncogenicity Study in Fischer-344 Rats

Butachlor was administered in the diet to groups of 90 male and female Fischer-344 (F-344) rats each, at concentrations of 10, 100 and 1000ppm for 24 months. Treatment-related signs of toxicity at the 1000ppm dietary level included an approx. 9% reduction in body weight gain, slight increases in liver, kidney, adrenal and thyroid weights, plasma chemistry and histopathological evidence of liver, kidney, bladder, and ocular toxicity. A maximum-tolerated dose (MTD) was achieved at the 1000ppm dietary level. Butachlor was not oncogenic in the F-344 rat. The chronic NOEL in F-344 rats was considered to be 100ppm (3.6mg/kg/day for males and 4.33mg/kg/day for females). (Institute of Environmental Toxicology, 1990)

3. Two-Year Chronic Toxicity/Oncogenicity Studies in Sprague-Dawley Rats

(1) Butachlor was administered in the diet to groups of 80 male and female

Sprague-Dawley (S-D) rats each, at concentrations of 0, 100, 1000 and 3000ppm for 26 months. The animals were housed individually in stainless steel cages. Increased deaths were observed in both sexes at the 1000ppm and 3000ppm dietary level during the last few months of the study. Reduced body weight was observed in males from the 1000ppm and 3000ppm dietary levels and in females from the 3000ppm dietary level. Food consumption was increased at these same dietary levels. Cholesterol levels were increased in males treated at the 3000ppm dietary level throughout the study and in males at the 1000ppm dietary level during the latter portion of the study. No other treatment-related biochemical alterations were observed. Increased urinary protein levels were seen in male and female rats at the 3000ppm dietary level and were considered to be treatment-related.

At sacrifice (interim and final) increases in liver, kidney, and thyroid weights and decreases in adrenal and spleen weights were observed only at the 3000ppm dietary level, however most of these changes were not statistically significant. Neoplastic changes were observed in the glandular stomach, nasal mucosa and thyroid gland, but only at very high toxic dose levels (*i.e.* >1000ppm). Other chronic, non-neoplastic changes were hepatocellular swelling and chronic nephropathy observed in animals from all treated levels. A NOEL for neoplasms was established at 100ppm. A definitive NOEL for non-oncogenic effects could not be established for this study.

(Bio/dynamics, Inc./Institute of Environmental Toxicology, 1983)

(2) A second chronic study was conducted with S-D rats to follow up on effects observed in the previous study and to establish a definitive NOEL for non-oncogenic effects. Butachlor was administered to groups of 80 male and female rats each, in the diet at concentrations of 0, 5, 20, and 100ppm for 24 months. The animals were housed individually in suspended stainless steel cages. The outcome of this second chronic rat study resulted in no observable adverse chronic effects in rats treated with butachlor at levels of 100ppm for 24 months. Specifically, there was no increase in the incidence, time of onset, or severity of kidney or liver pathology observed between untreated control rats and rats given 100ppm butachlor. No evidence of other chronic toxicity or oncogenicity related to butachlor treatment was observed. It was concluded that 100ppm produced no butachlor-related toxicity and was a definitive NOEL in this study (4.9mg/kg/day for males and 6.1mg/kg/day for females).

(Bio/dynamics, Inc./Institute of Environmental Toxicology, 1988)

(3) To determine the absolute NOEL for chronic toxicity in S-D rats, the effects observed in liver and kidney in two chronic studies were reviewed. The hepatocellular swelling observed in the first study at 100ppm was not considered to have been related to treatment since it was not repeated in the second study and the increased incidence in treated groups from the first study did not vary in response to dose. In fact, the lowest dose group in males from the first study had the highest incidence of this lesion, and the highest dose in females had the lowest incidence among the

treated groups. Therefore these changes were interpreted to have been spontaneous and not related to treatment.

An apparent increase in chronic nephropathy was observed in all treatment groups in the first study, however there was no increase in severity with respect to controls. In a repeat study there was no evidence of increased chronic nephropathy in any treatment group. The NOEL for butachlor chronic toxicity in S-D rats was considered to be 20ppm (1.0mg/kg/day for males and 1.2mg/kg/day for females).

4. Twenty-Four-Month Oncogenicity Study in CD-1 Mice

Butachlor was administered to groups of 100 male and female CD-1 mice each, in the diet at concentrations of 0, 50, 500, and 2000ppm for 24 months. The animals were housed individually in suspended stainless steel cages. No effects on survival were observed. Mean body weight was decreased in both sexes at the 2000ppm dietary level and males at the 500ppm dietary level. Mean food consumption was increased at the 2000ppm dietary level relative to control. Serum alkaline phosphatase, glutamic oxaloacetic transaminase and glutamic-pyruvic transaminase values observed in females at the 2000ppm dietary level were statistically greater than control values. After 54 weeks of treatment, elevated total cholesterol values were observed in both sexes at the 2000ppm dietary level. Organ weight changes (decreased brain, increased liver and kidney) were observed in animals at the 500 and 2000ppm dietary levels.

Evidence of microcytic anemia was evident in both sexes at the 2000ppm dietary level. While decreased hematologic parameters were noted clinically, no corresponding effect on tissues capable of hemopoietic element production was observed macroscopically. At week 53, an increased incidence of retinochoroidal degeneration was noted in males from the treated groups, but this effect did not persist at subsequent evaluation intervals. At week 104, the incidence of cataracts was increased in animals from the 500 and 2000ppm dietary levels. The cataracts appeared to be similar pathologically to spontaneously occurring lesions and as is often seen at similar or even higher incidences in aged mice than those reported in this study.

Necropsy findings in animals were limited to the kidneys and lungs and correlated with the histopathologic findings reported below. Microscopic examination revealed effects in the kidneys (nephrosis), eyes (cataracts), gallbladder (hyperplasia in high dose females only), and lung (alveolar/bronchiolar epithelial hyperplasia; increased macrophages) of both sexes at the 500ppm and 2000ppm dietary levels. No effects were observed in the gastrointestinal tract or the nasal region. There was no statistically significant increase in either pulmonary adenomas or carcinomas in mice at the terminal sacrifice at any dose level. The small increase in the number of multiple adenomas/carcinomas observed in males from all 3 treatment groups can be attributed to an unexpectedly lower incidence found in the control male group, when compared to historical control values with this strain of mouse at the testing laboratory. The

NOEL for this study is considered to be 50ppm (7.13mg/kg/day for males and 8.56mg/kg/day for females).

(Hazleton Laboratories/Institute of Environmental Toxicology, 1985)

EVALUATION OF THE ONCOGENIC POTENTIAL OF BUTACHLOR

As noted above, neoplastic responses attributable to butachlor administration were observed in the stomach, thyroid, and nasal tissue of rats. A significant increase in stomach tumors was restricted to the female rat at the highest dose tested (*i.e.* 3000ppm). The incidence of thyroid and nasal tumors increased significantly in the females from the 3000ppm and 1000ppm groups but in males at 3000ppm only. The highest dose, 3000ppm exceeded the MTD as evidenced by excessive body weight loss (>30%), hepatocellular necrosis, and decreased survival. The MTD was shown to be 1000ppm. The production of tumors only under these extreme conditions of exposure in rats is of doubtful relevance for humans. Also, a weight-of-evidence evaluation of data obtained from extensive mutagenicity testing reveals that butachlor has no significant genotoxic activity in mammalian systems. These data indicate that the tumors observed in the rat arise 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 mechanistic investigations were undertaken with butachlor, as well as with alachlor, a close structural analog of butachlor which produces the same neoplastic response. These additional studies have been communicated elsewhere,[3] and the results are summarized below.

1. Rat Nasal Tumors

Previous studies 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. Butachlor is metabolized in a similar manner to alachlor. Additional studies conducted with butachlor have further demonstrated that a common mechanism of action is operative for the formation of rat nasal tumors with butachlor and alachlor. Alachlor and butachlor are metabolized by common metabolic processes leading to the formation in rat nasal tissue of the quinoneimine metabolite of diethylaniline (DEIQ). This metabolite has been shown to localize and accumulate selectively in rat nasal tissue but not in the nasal tissues of other species.[4] This metabolite forms protein adducts,[5] which leads to cytotoxicity, prolonged cell proliferation and the eventual development of the predominantly benign, microscopic nasal tumors observed. Increased 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 the rat nasal mucosa as compared to other species.

Rat nasal tissue contains high levels of enzymes which metabolize alachlor or butachlor 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[6] and 751 times greater than that of human nasal tissue.[7] 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.[7] These data indicate that the potential for the formation of the reactive DEIQ metabolite in human nasal tissue is negligible and demonstrate that with respect to alachlor as well as to butachlor, the rat is not an appropriate model for the assessment of nasal oncogenic hazard to humans.

2. Rat Stomach Tumors

An extensive research program was conducted to elucidate the mechanisms through which butachlor induced stomach tumors in rats. A stomach tumor initiation-promotion study was conducted with butachlor as part of this research program.[8] Butachlor had no activity as an initiator, but did promote tumor formation following treatment with a known initiator. Thus, the results from this study produced direct experimental evidence indicating a non-genotoxic mode of action for stomach tumorigenicity.

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.[9,10] The resulting gastric hypochlorhydria and increased pH induce a compensatory production of gastrin. Together, the progressive mucosal atrophy and excessive gastrin secretion drive a proliferative response which culminates in tumor formation. Butachlor did not produce mucosal atrophy at a lower, non-oncogenic dose level. The results of these mechanistic investigations provide strong evidence that butachlor produces stomach tumors in rats through a non‐genotoxic, threshold-based mechanism.

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 events (mucosal atrophy) do 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 butachlor exposure results in increased thyroid hormone clearance via induction of hepatic UDPGT activity, thereby causing a long-term, compensatory increase in circulating TSH levels.[11] 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.[12-14] This hormone-mediated mechanism of carcinogenesis is considered to be a threshold phenomenon to which rats are particularly susceptible. The results with butachlor provide strong evidence that this non-genotoxic mechanism is operative in the production of butachlor-induced thyroid tumors in rats.

GENOTOXICITY STUDIES

The genotoxic potential of butachlor has been evaluated in numerous assay systems using a variety of species, metabolic activation conditions and endpoints. These assays tested Monsanto-produced butachlor in well-validated systems using established testing guidelines according to Good Laboratory Practice standards. Butachlor produced no evidence of a mutagenic response in the *E. coli* wp2 reverse mutation assay, but gave weak and inconsistent positive responses in the Ames/*Salmonella* reverse mutation assay. Increased revertant colonies caused by high doses of butachlor (0.5 to 10mg/plate) were less than 3-fold above control values and occurred only in the TA-100 strain of *Salmonella*. In contrast, when tested in cultured Chinese hamster ovary (CHO) cells, no evidence of mutagenic activity was detected in the hypoxanthine-guanine phosphoribosyl transferase (HGPRT) forward gene mutation assay. The result of a CHO *in vitro* cytogenetics assay was also negative for clastogenic effects. Four *in vivo* mammalian tests have been conducted with butachlor. A bone marrow cytogenetics study with S-D rats at intraperitoneal (*i.p.*) dose levels of 75, 250 and 750mg/kg, and a micronucleus test with Swiss-Webster mice at *i.p.* dose levels of 250, 500 and 1000mg/kg/day both revealed signs of general toxicity, but no evidence of adverse chromosomal effects. Exposure of CD-1 male mice to butachlor for 7 weeks at dietary concentrations of 100, 1000 and 5000ppm revealed significant body weight depression, but no evidence of dominant lethal effects in male germ cells. An *in vivo/in vitro* DNA repair assay with F-344 rats at oral dose levels of 50, 200 and 1000mg/kg showed no increase in hepatocyte unscheduled DNA synthesis.

The weight of evidence supports the conclusion that butachlor does not have significant genotoxic potential in mammals. This conclusion is supported by an analysis of the butachlor genotoxicity data using a scoring system developed by the International Commission for Protection against Environmental Carcinogens and Mutagens.[15,16] 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*). The score of -12 for butachlor 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 butachlor does not have general genotoxic potential. Furthermore, the butachlor genotoxicity results support the conclusion that the rat-specific oncogenic responses do not result from genotoxicity but rather from butachlor's effects on non-genotoxic processes.

REPRODUCTION AND DEVELOPMENTAL TOXICITY STUDIES

1. Two-Generation Reproduction Study in Rats

Groups of 25 male and female Sprague-Dawley rats each, were fed butachlor in the diet at concentrations of 0, 100, 1000, and 3000ppm. Treatment began 10 weeks prior to the first mating to insure that a complete spermatogenic maturation cycle had occurred during butachlor treatment and continued throughout mating, pregnancy, and lactation periods for 2 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 evaluations were conducted on both sexes in the control and 3000ppm dietary level of parents from both generations as well as 6 male and female pups from the second litter of the second generation (F_2b). No adverse effects were observed on any reproductive parameter in any generation. Reductions in pup weights occurred at the 1000ppm and 3000ppm dietary levels during lactation but maternal and/or parental toxicity was also observed at these dose levels. The NOEL for reproductive toxicity in rats was considered to be 3000ppm (198.10−283.33mg/kg/day for males and 245.65−319.73mg/kg/day for females). The NOEL for general toxicity in parents and pups was considered to be 100ppm (6.74−8.13mg/kg/day for males and 8.40−9.58mg/kg/day for female). (Bio/dynamics, Inc., 1984)

2. Developmental Toxicity Study in Rats

Butachlor was administered by gavage to 3 groups of 25 mated C-D female rats at dosages of 49, 147, and 490mg/kg/day on days 6 through 19 of gestation. An additional 25 mated females received distilled water and served as controls. On gestation day 20, fetuses were examined externally, weighed, and processed for skeletal and soft-tissue examinations. Maternal toxicity occurred in the high dose group as evidenced by a decrease in maternal body weight. No butachlor-related effects were observed on total implantations, post-implantation loss, viable fetuses or fetal sex distribution at any dosage level. No effects considered related to butachlor exposure were seen following external, soft-tissue or skeletal examination of fetuses from all 3 dose levels tested. The NOEL for maternal toxicity in the rat was considered to be

147mg/kg/day. Butachlor did not induce a teratogenic effect in rats.

(International Research and Development Corp., 1980)

3. Developmental Toxicity Study in Rabbits

Three groups of 16 mated female Dutch belted rabbits were administered butachlor by oral gavage at dosages of 49, 147, and 245mg/kg/day and an additional 16 mated females received distilled water and served as controls. All animals were dosed daily from gestation day 7 through 27. All surviving females were sacrificed on gestation day 28 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 butachlor resulted in significant maternal toxicity at the 147mg/kg/day and 245mg/kg/day dose levels. In addition to treatment-related deaths and abortions, mean maternal body weight losses, increases in post-implantation loss and decreases in fetal body weights were observed. The NOEL for maternal toxicity in the rabbit was considered to be 49mg/kg/day. Butachlor did not induce a teratogenic effect in rabbits. (International Research and Development Corporation, 1980)

PHARMACOLOGY STUDIES

Butachlor was administered intraperitoneally to groups of 5 male and female ICR mice each, at single doses of 0, 125, 215, 350, 600, and 1000mg/kg. All animals given the highest dose level died within 4 to 24 hrs after administration. Animal awareness, motor activity and autonomic nervous system responses were suppressed at levels of 600mg/kg or above. Male and female mice given butachlor at a dose of 1000mg/kg exhibited abnormal signs of toxicity characterized by excitation of the central nervous system, writhing, exophthalmos, hypothermia, lacrimation and soft stool. Most of the signs observed in the 600mg/kg group were diminished within 24 hrs after administration. No signs of toxicity were observed in animals given butachlor at dose levels of 350mg/kg or less.

After acute oral administration of butachlor to 3 male and 3 female albino rabbits at dose levels of 0, 1000, 2300, and 5000mg/kg, no abnormalities were observed at any of the levels tested. Butachlor was administered intravenously to groups of 3 male rabbits at dose levels of 50 and 150mg/kg. In the 150mg/kg group, respiration was increased, and transient decreases in blood pressure, heart rate and blood flow were observed after administration. No abnormality was observed in the 50mg/kg group.

In further experiments to explore the effects on the autonomic nervous system, gastrointestinal tract, skeletal muscle, or blood, butachlor produced no significant effect. In conclusion, butachlor has little or no effect on standard physiological parameters and pharmacological end-points. The effects observed occurred primarily

at acutely lethal dose levels. The high dose levels required to produce severe effects confirm that the acute toxicity of butachlor is relatively low.

(Nippon Experimental Medical Research Institute Co., Ltd., 1993)

EPIDEMIOLOGY STUDIES

Three epidemiology studies have been conducted on the manufacturing workers at the Monsanto Muscatine plant in Iowa motivated primarily by the findings from the available toxicology studies with alachlor in which ocular and oncogenic effects were observed in the Long-Evans rat. The same types of tumors were also observed in the toxicology studies with butachlor in the Sprague-Dawley rat. The outcome of extensive research investigations has proven that the same mechanisms are operative for the formation of stomach, nasal and thyroid tumors in rats with these compounds. The history of the Muscatine plant indicates that there is a high correlation of alachlor and butachlor exposure among manufacturing workers, and therefore, the results from the alachlor epidemiology studies are also relevant for butachlor.

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 follow-up.[18] Likewise, cancer incidence rates over a 24-year period were not elevated in the highest-exposed alachlor/butachlor workers, nor were there any cases of tumors in the nose, stomach, or thyroid, the organs in which oncogenic effects were produced in rats.[19] These manufacturing workers had year-round exposure greatly exceeding agricultural exposure, which is limited to a two- or three week period of application. In fact, manufacturing exposure was estimated to exceed yearly agricultural exposure by a factor of 10,000 or more.[19] Potential dietary exposures are even lower than agricultural exposure. Therefore, the absence of eye effects or 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/butachlor if at all.

CONCLUSIONS

The results of tests in laboratory animals indicate that butachlor has low mammalian toxicity following acute oral, dermal, and inhalation exposure. Butachlor 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. Butachlor is not genotoxic and does not interfere with normal reproductive and developmental processes. Butachlor is not oncogenic

in the Fischer-344 rat and CD-1 mouse. Neoplastic lesions were observed in the Sprague-Dawley rat in the nasal turbinate mucosa, stomach glandular mucosa, and thyroid follicular epithelium. Significant increases in the stomach, thyroid and nasal tumors occurred only at or above the MTD dose which produced excessive toxicity.

A large body of data supports the conclusion that butachlor produces these tumors in rats through non-genotoxic mechanisms for which thresholds exist. The conditions driving the requisite threshold-sensitive preneoplastic events occur only in experimental studies employing doses at or above the 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 exist, as human exposure is several orders of magnitude below the doses producing tumors in rats. Therefore, it is concluded that the butachlor-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 butachlor.

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

Based on a review of the studies summarized above, the Japan Ministry of Health and Welfare (MHW) established an Acceptable Daily Intake (ADI) for butachlor of 0.01mg/kg/day employing the NOEL from the chronic rat study of 1mg/kg/day and a safety factor of 100. This ADI supports the current withholding limits established by the Japan Environmental Agency (EA) of 0.1ppm in rice.

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要　　約

ブタクロールの毒性試験の概要

米国モンサント・カンパニー

ブタクロールは，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稲作用除草剤である．動物実験の結果，本剤の哺乳動物に対する

急性経口，経皮，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．亜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ブタ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，ブタクロールへの暴露量が最も高いと考えられるブタクロールおよび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．本剤には，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1mg/kg/日および安全係数100を用い，0.01mg/kg/日のADIが設定されている．

ブタクロールは，マーシェット®粒剤として昭和48年3月登録を取得して以来，水田用の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，米に0.1ppmと設定されている．

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