UNITED STATES ENVIRONMENTAL PROTECTION AGENCY
WASHINGTON, D.C. 20460

MAR 14 1996
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OFFICE OF
PREVENTION PESTICIDES AND
TOXIC SUBSTANCES

MEMORANDUM

SUBJECT: Carcinogenicity Peer Review of Alachlor - Third

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2/26/96

The Health Effects Division Carcinogenicity Peer Review Committee (HED/CPRC) met on September 27 and October 4, 1995 and January 3, 1996 to discuss and reevaluate the weight-of-the-evidence on Alachlor with particular reference to its carcinogenic potential, based on mechanistic and other data provided by the registrant. These data were not requested by the Agency but were provided by the registrant in support of their chemical. The classification of Alachlor at that time was a Group B2 - probable human carcinogen, with a recommendation that a low dose extrapolation model be applied to the animal data for the quantification of human risk (Q_1*).

New data provided by the registrant consisted of a new mouse carcinogenicity study, additional mutagenicity studies, mechanistic data, and additional metabolism studies and toxicology data from a related compound, Butachlor. Upon evaluation of all of the submitted data regarding the carcinogenicity potential of Alachlor and consideration of the full weight-of-the-evidence, the Health Effects Division Carcinogenicity Peer Review Committee could not reach a consensus as to the classification of Alachlor as a carcinogen. Therefore the CPRC recommended to defer the carcinogenicity classification of Alachlor and reconsider the classification in the near future using the new Cancer Assessment Guidelines when such guidelines are in effect. In addition, the CPRC recommended not to utilize the linear low dose approach, but to utilize the Margin of Exposure (MOE) methodology for the estimation of human risk. The CPRC concluded that the data in support of the mechanism for the nasal turbinates is indicative of a rat specific response. Although the rat and human were recognized to possess the same enzyme(s) involved in production of the putative toxic species from Alachlor, it was also recognized that the activity of these enzymes was substantially greater in the rat compared to the human. Thus, the model of rat nasal tumorigenesis may not be relevant for human cancer assessment. Thyroid tumors have been proposed to be the result of induction of hepatic glucuronyl transferase with subsequent decrease in circulating T3 and T4, a subsequent increase in TSH, and eventual hyperplastic response of the thyroid. The mechanistic data for thyroid tumor formation meet the criteria established by the Agency and the use of the MOE approach for human cancer assessment is consistent with Agency policy. The CPRC stated that the stomach tumor formation is a direct contact effect, non-genotoxic mechanism which parallels human pathological conditions. These tumors result from an indirect response to change in pH. The use of the MOE approach for human cancer assessment is consistent with Agency policy.

Summary of Carcinogenicity and Mechanistic Data

Administration of Alachlor in the diet of male Long-Evans rats at dose levels of 0, 14, 42, and 126 mg/kg/day resulted in: **significant increasing trends** and **significant pair wise increases** in thyroid follicular cell adenomas, carcinomas, and adenomas and/or carcinomas combined, stomach osteosarcomas, malignant mixed gastric tumors, and gastric adenocarcinomas and/or malignant mixed gastric tumors combined, and nasal respiratory epithelial adenomas and adenomas and/or adenocarcinomas combined; and **significant increasing trends** in stomach gastric adenocarcinomas.

For female rats, administration of Alachlor in the diet (at the same dose levels) resulted in: **significant increasing trends** and **significant pair-wise increases** in stomach malignant mixed gastric tumors, and gastric adenocarcinomas and/or malignant mixed-gastric tumors combined, and nasal respiratory epithelial adenomas and adenomas and/or adenocarcinomas combined; and **significant increasing trends** in thyroid follicular cell adenocarcinomas and adenomas and/or adenocarcinomas combined, and stomach osteosarcomas.

In a second rat study, administration of Alachlor in the diet of Long-Evans rats at dose levels of 0, 0.5, 2.5, and 15 mg/kg/day resulted in a **significant pair-wise difference** and a **significant positive trend** for the incidence of nasal respiratory epithelium adenomas at 15 mg/kg/day in male rats.

For female rats, administration of Alachlor in the diet (at the same dose levels) resulted in a **significant pair-wise difference** in the incidence of nasal respiratory epithelium adenomas and thymus malignant lymphosarcomas at 15 mg/kg/day. In addition, a **significant positive trend** was observed for nasal respiratory epithelium adenomas.

The lack of tumors (other than those of the nasal turbinates) observed in this second study as opposed to the first rat study is likely based on the use of lower dietary levels of Alachlor.

Administration of Alachlor in the diet at dose levels of 0, 26, 78, and 260 mg/kg/day to male CD-1 mice resulted in a **significant positive trend** for bronchioalveolar adenomas. There were **no** significant pair-wise differences with the control group.

In female mice (at the same dose levels), a **significant positive trend** as well as a **significant pair-wise difference** for the incidence of bronchioalveolar adenomas and adenomas and/or carcinomas combined at the 260 mg/kg/day dose level was observed.

In a second carcinogenicity study using CD-1 mice, male mice received dietary Alachlor at 0, 16.64, 65.42, or 262.40 mg/kg/day for 79 weeks. A **significant pair-wise difference** was observed for the incidence of bronchioalveolar adenomas and adenomas and/or carcinomas combined at all dose levels in comparison to control.

Female mice received dietary Alachlor at 0, 23,73, 90.34, or 399.22 mg/kg/day for 79 weeks. There were **no** significant compound-related tumors observed in female mice in this study.

Structure-Activity Relationships

Alachlor, a chloroacetanilide, is structurally related to several other chloroacetanilides, namely Acetochlor (Group B2 carcinogen), Butachlor (not classified), Metolachlor (Group C carcinogen), and SAN 582H (Group C carcinogen).

Mutagenicity

Alachlor was initially classified as weakly genotoxic by the CPRC based on previously submitted mutagenicity studies. Newly submitted studies showed weak positive responses of two Alachlor metabolites in the Ames salmonella assay, and a weak positive response of Alachlor in a UDS assay in Fischer-344 rat hepatocytes. The available data on mutagenicity and cell proliferation as a whole suggest that while genotoxic species of Alachlor may be produced, such genotoxic activity is observed only at doses of Alachlor in which GSH depletion and/or saturation of protein binding has occurred. This conclusion is supported from the submitted data in which GSH and protein reaction with Alachlor and/or Alachlor metabolites is observed at lower concentrations than the genotoxic activity.

The CPRC reviewed the data contained within the Peer Review Document with respect to the proposed mechanism(s) for induction of nasal, gastric, and thyroid tumors as presented. As previously noted, nasal tumors are believed to be the result of formation of the benzoquinone imine metabolite of Alachlor, binding of the imine to cellular protein, and subsequent cell damage with compensatory increase in cell turnover and eventual production of benign tumors. In comparison to other species, the Long-Evans rat has been demonstrated to be highly sensitive to induction of nasal tumors from Alachlor administration, based on the ability of this strain of rat to form the quinone imine metabolite of Alachlor and the localization of this metabolite to the nasal tissue of the rat.

Thyroid tumors have been proposed to be the result of induction of hepatic glucuronyl transferase with subsequent decrease in circulating T3 and T4, a subsequent increase in TSH, and eventual hyperplastic response of the thyroid.

Stomach tumors from Alachlor administration are proposed to result from mucosal toxicity leading to atrophy of the mucosa and parietal cells resulting in hypochlorhydria, increased stomach pH, and compensatory increase in gastrin levels. Gastrin is known to exert a trophic effect on fundic mucosal cells. Under these

conditions, a proliferative response is initiated, resulting in tumor formation. The effects noted are similar to human pathological conditions; chronic atrophic gastritis and Zollinger-Ellison syndrome.

The CPRC concluded that the data support the proposed mechanism(s) for tumor induction in the stomach and thyroid. The CPRC concluded that the data in support of the mechanism for tumor induction in the nasal turbinates is indicative of a rat specific response and may not be relevant for human cancer assessment, based on the available data.

A. Individuals in Attendance at one or both meetings:

1. Peer Review Committee: (Signature indicates concurrence with the peer review unless otherwise stated.)

Yiannakis M. Ioannou

Julie T. Du

Richard N. Hill

Yin tak Woo

Hugh Pettigrew

William T. Burnam

Kerry Dearfield — See attached memo for comments

Stephanie R. Irene

Karl P. Baetcke

Marion Copley

2. Reviewers: (Non-committee members responsible for data presentation; signature indicates technical accuracy of panel report.)

Stephen C. Dapson[1]

Timothy F. McMahon[1]

Yiannakis M. Ioannou[1]

Nancy E. McCarroll[1]

Lori Brunsman — for Lori Brunsman

Lukas Brennecke[2] (PAI/ORNL)

3. Other Attendees:
Bernice Fisher, Kathryn Boyle, Joycelyn Stewart, Mary Marion, Jerry Blondell, Jim Rowe

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[1]Also a member of the CPRC for this chemical; signature indicates concurrence with the peer review unless otherwise stated.

[2] Signature indicates concurrence with the pathology report.

B. Material Reviewed

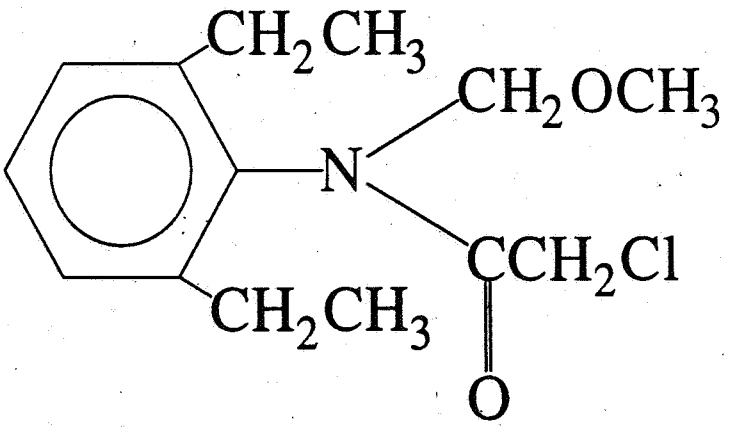
The material available for review consisted of previous peer reviews and related data and new data provided by the registrant: a new mouse carcinogenicity study; additional mutagenicity studies; mechanistic data including metabolism studies; toxicology data from a related compound, Butachlor. The material reviewed is attached to the file copy of this report.

C. Background Information

Alachlor, or 2-chloro-2'6'-diethyl-N-(methoxymethyl)-acetanilide, is a selective preemergence herbicide for the control of many preemergent broadleaf weeds and grasses on corn (all types), soybeans, peanuts, dry beans, potatoes, cotton, and certain woody ornamentals. Alachlor is marketed under the trade names Alanex®, CP 50144®, Lasso®, and Lazo®. In previous meetings of the Health Effects Division Carcinogenicity Peer Review Committee (March 25, 1986 and April 15, 1987), it was determined that Alachlor met all but one of the criteria for classification as a B2 carcinogen. That is, Alachlor produced an increased incidence in malignant, or combined malignant and benign, nasal turbinate tumors (and other tumor types) in Long-Evans rats in three different experiments at more than one dose level via dietary administration. Alachlor also produced a statistically significant increase in lung tumors in female CD-1 mice at 2 dose levels. In a second study with Long-Evans rats, nasal turbinate tumors occurred after only 5-6 months of exposure. The tumor incidence was as high as 50% and tumor site was unusual; i.e., not an increase of a normal high background tumor type. Additionally, a metabolite of Alachlor was mutagenic in the Ames Test at 6 concentration levels.

The above conclusions of the Peer Review Committee were supported by the Scientific Advisory Panel meeting of November 19, 1986.

The chemical structure of Alachlor is shown below:



ALACHLOR

The present peer review document is the result of the September 27, 1995, October 4, 1995, and January 3, 1996 meetings of the Health Effects Division Carcinogenicity Peer Review Committee, which convened to evaluate additional data submitted by the registrant in support of a request for the cancer reclassification of Alachlor. The additional data focused on the mechanism(s) of tumor formation in the stomach, nasal epithelium, and thyroid resulting from Alachlor administration. Studies were submitted to address the registrant's position of a non-genotoxic mechanism for induction of tumors induced by Alachlor. In addition to evaluating these data, the tumors observed in previously submitted carcinogenicity studies with Alachlor were reevaluated by the Science Analysis Branch, Health Effects Division and the tables representing these tumor types are presented in the present document.

The data and documents presented in this document were reviewed by Dr. Stephen C. Dapson and Dr. Timothy F. McMahon and secondarily reviewed by Dr. Yiannakis M. Ioannou. The tumor data from previously submitted studies were statistically reanalyzed and the newly submitted data were statistically analyzed by Lori Brunsman.

D. Carcinogenicity Studies with Alachlor

1. Chronic Feeding/Carcinogenicity Study of Alachlor in Rats:

A chronic feeding study in Long-Evans rats was conducted by Bio/dynamics Inc., Division of Biology and Safety Evaluation, East Millstone, New Jersey, for Monsanto Agricultural Products Company, St. Louis, Missouri, and issued November 13, 1981 (Project No. 77-2065; Study No. BD-77-421).

The study design allocated groups of 50 rats per sex to dose levels of 0, 100, 300, or 1000 ppm of Alachlor (Lasso Technical; 0, 14, 42, or 126 mg/kg/day, respectively) for 117 weeks in males (812 to 813 days) and 106 weeks in females (741 to 744 days). The test substance used for the first 11 months of the study was stabilized with 0.5% epichlorohydrin. The test substance used for the remaining 16 months of the study was stabilized with [redacted]. [Epichlorohydrin is carcinogenic for male Wistar rats and Sprague-Dawley rats: When given in drinking water it causes forestomach tumors (squamous cell papillomas and carcinomas) in male Wistar rats (Konishi et al. Gann 71:922-923, 1980); by inhalation it causes squamous carcinomas of the nasal cavity (Laskin, et al. J. Natl. Cancer Inst. 65:751-755, 1980). The effect of epichlorohydrin on tumor formation in this study is not known.]

The statistical evaluation of mortality of the 1981 Long-Evans rat study indicated a **significant increasing trend** with increasing doses of Alachlor in male rats (Table 1). Female rats had a **significant increasing trend** for mortality with increasing doses of Alachlor (Table 2).

Table 1. Alachlor - 1981 Long-Evans Rat Study
Male Mortality Rates[+] and Cox or Generalized K/W Test Results

	Weeks				
Dose (mg/kg/day)	1-26	27-52	53-78	79-117[f]	Total
0	0/50	0/50	1/50	23/49	24/50(48)*
14	0/49[a]	0/49	3/49	29/46	32/49(65)
42	0/50	0/50	2/50	30/48	32/50(64)
126	0/50	4/50	3/46	24/43	31/50(62)*

[+]Number of animals that died during interval/Number of animals alive at the beginning of the interval. ( )Percent.
[f]Final sacrifice at week 117; [a]One accidental death at week 6, dose 14 mg/kg/day.
Note: Time intervals were selected for display purposes only.
Significance of trend denoted at control.
Significance of pair-wise comparison with control denoted at dose level.
If *, then $p < 0.05$. If **, then $p < 0.01$.

Table 2. Alachlor - 1981 Long-Evans Rat Study
Female Mortality Rates[+] and Cox or Generalized K/W Test Results

	Weeks				
Dose (mg/kg/day)	1-26	27-52	53-78	79-117[f]	Total
0	0/50	0/50	3/50	14/47	17/50(34)**
14	0/50	1/50	5/49	15/42[a]	21/48(44)
42	1/50	1/49	3/47[b]	12/44	17/49(35)
126	1/50	0/49	8/49	20/40[c]	29/49(59)*

[+]Number of animals that died during interval/Number of animals alive at the beginning of the interval. ( )Percent.
[f]Final sacrifice at week 106; [a]Two accidental deaths, one each at weeks 79 and 105, dose 14 mg/kg/day; [b]One accidental death at week 61, dose 42 mg/kg/day.
[c]One accidental death at week 105, dose 126 mg/kg/day.
Note: Time intervals were selected for display purposes only.
Significance of trend denoted at control.
Significance of pair-wise comparison with control denoted at dose level.
If *, then $p < 0.05$. If **, then $p < 0.01$.

The results of the **first** Peer Review Committee meeting (on

March 25, 1986) stated that "the lowest dose of Alachlor tested in this study probably exceeded the Maximum Tolerated Dose as evidenced by high mortality, compared to controls. Increases in organ weights (liver, kidney, spleen, etc.) were also noted, as were gross findings, at all dose levels, indicative of a compound related effect."

However, this point was reconsidered in a **second** Peer Review Committee meeting on April 15, 1987, and as a result of the **second** meeting, it was felt that the 42 mg/kg/day dose level best approximated a "Maximum Tolerated Dose" for Alachlor, based on increased mortality in male rats at the 42 mg/kg/day dose level.

2. Chronic Feeding/Carcinogenicity Study of Alachlor in Rats:

A chronic feeding study in Long-Evans rats was conducted by Monsanto Environmental Health Laboratory, St. Louis, Missouri, for Monsanto Agricultural Products Company, St. Louis, Missouri, and issued February 12, 1983 (Project No. ML-80-186; Study No. EHL 800218; Report No. MSL-3282).

The study design allocated groups of 50 rats per sex to dose levels of 0, 0.5, 2.5, or 15 mg/kg/day of Alachlor (technical, 94.13%) for 110 weeks (25 to 26 months). The test substance was stabilized with [redacted] Epichlorohydrin was **not** used as a stabilizer.

The statistical evaluation of mortality of the 1983 Long-Evans rat study indicated no significant incremental changes with increasing doses of Alachlor in male rats (Table 3). Female rats had a **significant increasing trend** for mortality with increasing doses of Alachlor (Table 4).

Table 3. Alachlor - 1983 Long-Evans Rat Study
Male Mortality Rates[+] and Cox or Generalized K/W Test Results

	Weeks				
Dose(mg/kg/day)	1-26	27-52	53-78	79-111[f]	Total
0	0/50	1/50	4/49	28/45	33/50(66)
0.5	1/50	0/49	2/49	18/47	21/50(42)*[n]
2.5	0/50	2/50	6/48	13/42	21/50(42)[n]
15.0	1/50	1/49	3/48	22/45	27/50(54)[h]

[+]Number of animals that died during interval/Number of animals alive at the beginning of the interval. ( )Percent.
[f]Final sacrifice at week 110; [n]Negative change from control.
Note: Time intervals were selected for display purposes only.
Significance of trend denoted at control.
Significance of pair-wise comparison with control denoted at dose level.
If *, then $p < 0.05$. If **, then $p < 0.01$.

Table 4. Alachlor - 1983 Long-Evans Rat Study
Female Mortality Rates[+] and Cox or Generalized K/W Test Results

	Weeks				
Dose(mg/kg/day)	1-26	27-52	53-78	79-111[f]	Total
0	0/50	2/50	4/48	22/44	28/50(56)**
0.5	1/50	2/49	3/47	18/44	24/50(48)[n]
2.5	0/50	0/50	7/50	20/43	27/50(54)[n]
15.0	0/50	1/50	5/49	30/44	36/50(72)

[+]Number of animals that died during interval/Number of animals alive at the beginning of the interval. ( )Percent.
[f]Final sacrifice at week 110; [n]Negative change from control.
Note: Time intervals were selected for display purposes only.
Significance of trend denoted at control.
Significance of pair-wise comparison with control denoted at dose level.
If *, then $p < 0.05$. If **, then $p < 0.01$.

The Peer Review Document from the **first** meeting stated that "The highest dose tested probably exceeded the MTD in female rats, as evidenced by a 16% increase in mortality over that in the control. (In male rats, high mortality in the corresponding control group may have obscured an increased mortality in high dose males.)"

3. Rat Tumor Reevaluation

A) 1981 chronic feeding study in Long-Evans rats (Bio/dynamics Inc., November 13, 1981, Project No. 77-2065; Study No. BD-77-421).

The following observations were noted in male rats in this study:

i) There were **significant pair-wise increases** in nasal respiratory epithelium adenomas and adenomas and/or adenocarcinomas combined were observed at 42 and 126 mg/kg/day Alachlor vs control ($p < 0.01$) as well as **significant trends** for these tumor types.

ii) There were **significant pair-wise increases** in malignant mixed gastric tumors and gastric adenocarcinomas and/or malignant mixed gastric tumors combined were observed at 126 mg/kg Alachlor vs control ($p < 0.01$) as well as **significant trends** for these tumor types.

iii) There were **significant pair wise increases** in thyroid follicular cell adenomas and adenomas and/or carcinomas combined were observed at 126 mg/kg Alachlor ($p < 0.01$) as well as **significant trends** for these tumor types.

iv) There were **significant differences in the pair-wise** comparisons of the 126 mg/kg/day dose group with the controls for stomach osteosarcomas, and thyroid follicular cell carcinomas, both at $p < 0.05$.

v) There were **significant trends** observed for brain oligodendrogliomas of the hypothalamus, stomach osteosarcomas, and thyroid follicular cell carcinomas, all at $p < 0.01$. There were also **significant increasing trends** in brain ependymomas and ependymomas and/or malignant ependymomas combined, and stomach gastric adenocarcinomas, all at $p < 0.05$.

For female rats in this study, the following tumor data were reported:

i) A **significant pair-wise difference** in the incidence of nasal turbinate adenomas and adenomas and/or adenocarcinomas combined was observed at 42 ($p < 0.05$) and 126 ($p < 0.01$) mg/kg/day Alachlor as well as **significant trends** for these tumor types.

ii) A **significant pair-wise difference** in the incidence of stomach malignant mixed gastric tumors and gastric adenocarcinomas and/or malignant mixed gastric tumors combined ($p < 0.01$) was observed at 126 mg/kg/day Alachlor, as well as **significant trends** for these tumor types.

iii) A **significant pair-wise difference** vs control was observed at 126 mg/kg/day Alachlor in the incidence of mammary gland adenofibromas, adenofibromas and/or fibroadenomas combined, and adenofibromas, fibroadenomas, and papillary adenocarcinomas combined ($p < 0.05$).

iv) A **significant pair-wise difference** was observed at 14 mg/kg/day in the incidence of mammary gland adenofibromas and/or fibroadenomas combined ($p < 0.05$).

v) There were **significant increasing trends** in liver adenomas, stomach osteosarcomas, and thyroid follicular cell adenomas and/or adenocarcinomas combined, all at $p < 0.01$.

Of all the tumors listed above, the increasing trend observed in brain oligodendrogliomas of the hypothalamus, and the significant trend in brain ependymomas and ependymomas and/or malignant ependymomas combined in **male** rats and the significant pair-wise comparisons for mammary gland adenofibromas, adenofibromas and/or fibroadenomas combined, and adenofibromas, fibroadenomas, and papillary adenocarcinomas combined and liver adenomas in **female** rats **were considered to have occurred at excessively toxic doses** and were not discussed further.

B) 1983 Chronic Feeding Study in Long-Evans Rats (Monsanto Environmental Health Laboratory, February 12, 1983, Project No. ML-80-186; Study No. EHL 800218; Report No. MSL-3282).

In this study, male rats were observed with the following tumor types:

i) A **significant pair-wise difference** in the incidence of nasal respiratory epithelium adenomas was observed at 15 mg/kg/day Alachlor vs control ($p < 0.01$) as well as a **significant trend** for this tumor type.

Female rats in this study were observed with the following tumor types:

i) A **significant pair-wise difference** in the incidence of adrenal benign pheochromocytomas and nasal respiratory epithelium adenomas at the 15 mg/kg/day dose level ($p < 0.05$ and $p < 0.01$, respectively) as well as **significant trends** for these tumor types.

ii) A **significant pair-wise difference** in the incidence of thymus malignant lymphosarcomas at the 15 mg/kg/day dose level vs control ($p < 0.05$).

Of all the tumor types observed in the two rat carcinogenicity studies, the tumors of the nasal epithelium, stomach, and thyroid formed the primary basis for classification of Alachlor as a Group B2 carcinogen. The registrant recently submitted additional data addressing the relevance of the Group B2 classification. These additional data suggested a non-genotoxic, threshold-based mechanism of action for induction of tumors in the nasal epithelium, thyroid, and stomach. For each of these tumor types (nasal, stomach, thyroid), the data from the registrant are summarized with respect to the hypothesized mechanism(s) of action through which these tumors are believed to arise. The Peer Review Committee's position regarding these data is also stated.

a. Thyroid Tumors

In the 1981 chronic feeding study in Long-Evans rats, male rats at the 126 mg/kg/day dose level were observed with a significantly increased incidence of thyroid follicular cell adenomas and adenomas and/or carcinomas combined, at $p < 0.01$. There were significant increasing trends in thyroid follicular cell carcinomas at $p < 0.01$. There were significant differences in the pair-wise comparisons of the 126 mg/kg/day dose group with the controls for thyroid follicular cell carcinomas, at $p < 0.05$. Female rats had significant increasing trends in thyroid follicular cell adenomas and/or adenocarcinomas combined, at $p < 0.01$. There was a significant increasing trend in thyroid follicular cell adenocarcinomas at $p < 0.05$. Data for thyroid tumors are presented in Tables 5 and 6.

It is noted that the thyroid tumors were observed in significant incidence only at the 126 mg/kg/day dose, which (as previously noted; see p. 10) was considered an excessively toxic dose by the Carcinogenicity Peer Review Committee. The 1983 chronic feeding study in Long-Evans rats did not present with increases in thyroid tumors.

Table 5. Alachlor - 1981 Long-Evans Rat Study
Male Thyroid Follicular Cell Tumor Rates[+] and
Peto's Prevalence Test Results (p values)

	Dose (mg/kg/day)			
	0	14	42	126
Adenoma	1/47	0/47	1/48	11[a]/45
(%)	(2)	(0)	(2)	(24)
p =	0.000**	-	0.396	0.001**
Carcinoma	0/42	0/40	0/42	2[b]/37
(%)	(0)	(0)	(0)	(5)
p =	0.003**	-	-	0.047*
Combined	1/47	0/47	1/48	13/45
(%)	(2)	(0)	(2)	(29)
p =	0.000**	-	0.396	0.000**

[+]Number of tumor bearing animals/Number of animals examined, excluding those that died before observation of the first tumor.
[a]First adenoma observed at week 76, dose 126 mg/kg/day
[b]First carcinoma observed at week 95, dose 126 mg/kg/day.
Note: Significance of trend denoted at control.
Significance of pair-wise comparison with control denoted at dose level.
If *, then $p < 0.05$. If **, then $p < 0.01$.

Table 6. Alachlor - 1981 Long-Evans Rat Study
Female Thyroid Follicular Cell Tumor Rates[+] and
Peto's Prevalence Test Results (p values)

	Dose (mg/kg/day)			
	0	14	42	126
Adenoma	0/49	0/40	2/42	2[a]/46
(%)	(0)	(0)	(5)	(4)
p =	0.067	-	0.064	0.165
Adenocarcinoma	0/45	0/38	0/40	2[b]/38
(%)	(0)	(0)	(0)	(5)
p =	0.027*	-	-	0.142
Combined	0/49	0/40	2/42	4/46
(%)	(0)	(0)	(5)	(9)
p =	0.009**	-	0.064	0.066

[+]Number of tumor bearing animals/Number of animals examined, excluding those that died before observation of the first tumor.
[a]First adenoma observed at week 72, dose 126 mg/kg/day; [b]First adenocarcinoma observed at week 84, dose 126 mg/kg/day.
Note: Significance of trend denoted at control.
Significance of pair-wise comparison with control denoted at dose level.
If *, then $p < 0.05$. If **, then $p < 0.01$.

Mechanistic data in support of the thyroid tumors consisted of two studies. In the first, dose levels of 0 and 126 mg/kg/day were used to measure indices of thyroid function (T3, T4, and TSH levels). While the results of this study showed no significant effect of Alachlor on T3, T4, or TSH levels, the results pertaining to TSH levels were considered invalid based on the use of human antibodies in the TSH assay.

In the second study [MRID# 42957201], Long-Evans rats were dosed with Alachlor for up to 120 days at dose levels of 0 and 126 mg/kg/day. Separate groups were exposed to control diet or Alachlor in the diet for 7, 14, 28, 60, or 120 days, with a separate group exposed to Alachlor for 60 days in the diet and then control diet for 60 days. The results of this study showed increased liver weights ($p < 0.05$ as compared to the control group) at all time points, increased activity of uridine 5'-di-phoso glucuronyl transferase (UDPGT), and increased thyroid weights from day 14 throughout the remainder of the study. TSH levels were statistically significantly increased from day 14 on, although the increase at day 120 was not significant. T3 levels were increased over control at 7, 14, 60, and 120 days; T4 levels were decreased at 7 and 28 days and increased at 14 days, returning towards normal at following time points. The dose group which received Alachlor for 60 days and then 60 days with control diet showed relatively unaffected T3, T4 and TSH levels. Thyroid follicular hypertrophy/hyperplasia was also noted in the treated animals mainly in the 28 and 60 day groups, with 1 animal in the 120 day group progressing to nodular hyperplasia.

The results of the above studies suggest that thyroid tumors (which only occur in the male rat), result from induction of hepatic UDPGT, with a consequent decrease in circulating T3 and T4 and a subsequent increase in TSH. This action is known to result in a hyperplastic response of the thyroid. The mechanism of thyroid tumorigenesis observed with Alachlor is consistent with the mechanism of thyroid tumorigenesis observed with other chemicals causing a disruption of thyroid hormone balance, and the **Health Effects Division Peer Review Committee concurred with this mechanism for Alachlor.**

The conclusions of two expert panels convened to consider the tumorigenicity of Alachlor are as follows with regard to the thyroid tumors related to Alachlor administration:

"The thyroid follicular cell tumors, which only occur in the male rat, result from induction of hepatic UDPGT resulting in increased thyroid hormone levels (i.e. TSH). The significant

increase in TSH at the dose level in excess of the MTD was time dependent and reversible. The role for this hormonally-mediated, threshold-dependent, non-genotoxic mechanism is well established and not considered relevant to humans." [Conclusions of an Expert Panel convened by Monsanto, September, 1995].

"For the thyroid tumors, the data demonstrated that Alachlor administration was accompanied by an increased elimination of thyroid hormone secondary to induction of hepatic UDPGT leading to an increase in TSH production which, when sustained, could lead to tumor formation."

"Mechanistic studies indicate that rat...thyroid tumors are induced through threshold-type mechanisms involving enhancement of cell proliferation and possibly confounded by toxicity at high doses." [Conclusions of the Scientific Panel Meeting on Alachlor conducted in Brussels June 6, 1994].

b. Gastric Tumors

The following observations were noted in male rats from the 1981 chronic feeding study with regard to gastric tumors:

There were **statistically significant increased** incidence of malignant mixed gastric tumors and gastric adenocarcinoma and/or mixed gastric tumors combined at the 126 mg/kg/day dose vs control ($p < 0.01$), as well as **significant trends** for these tumor types (Table 7).

The following observations were noted in female rats from the 1981 chronic feeding study with regard to gastric tumors:

There were **statistically significant increased** incidence in malignant mixed gastric tumor and gastric adenocarcinoma and/or mixed gastric tumor combined at the 126 mg/kg/day dose level ($p < 0.01$), in addition to **significant trends** for these tumor types (Table 8).

The 1983 chronic feeding study in Long-Evans rats did **not** present with an increase in stomach tumors in either sex at the dose levels tested (0, 0.5, 2.5, and 15 mg/kg/day).

**Table 7. Alachlor - 1981 Long-Evans Rat Study
Male Stomach Tumor Rates+ and Peto's
Prevalence Test Results (p values)**

	Dose (mg/kg/day)			
	0	14	42	126
Leiomyosarcoma				
	0/43	0/43	0/35	1[a]/44
(%)	(0)	(0)	(0)	(2)
Osteosarcoma	0/37	0/29	0/27	3[b]/29
(%)	(0)	(0)	(0)	(10)
p =	0.002**	-	-	0.028*
Gastric Adenocarcinoma				
	0/39	0/38	0/32	2[c]/37
(%)	(0)	(0)	(0)	(5)
p =	0.016*	-	-	0.098
Malignant Mixed Gastric Tumor				
	0/43	0/40	0/35	11[d]/41
(%)	(0)	(0)	(0)	(27)
p =	0.000**	-	-	0.000**
Gastric Adenocarcinomas and/or Malignant Mixed Gastric Tumors Combined				
	0/43	0/40	0/35	13/41
(%)	(0)	(0)	(0)	(32)
p =	0.000**	-	-	0.000**

+Number of tumor bearing animals/Number of animals examined, excluding those that died before observation of the first tumor.
[a]First leiomyosarcoma observed at week 52, dose 126 mg/kg/day.
[b]First osteosarcoma observed at week 101, dose 126 mg/kg/day.
[c]First gastric adenocarcinoma observed at week 91, 126 mg/kg/day.
[d]First malignant mixed gastric tumor observed at week 78, dose 126 mg/kg/day.
Note: Significance of trend denoted at control.
Significance of pair-wise comparison with control denoted at dose level.
If *, then $p < 0.05$. If **, then $p < 0.01$.

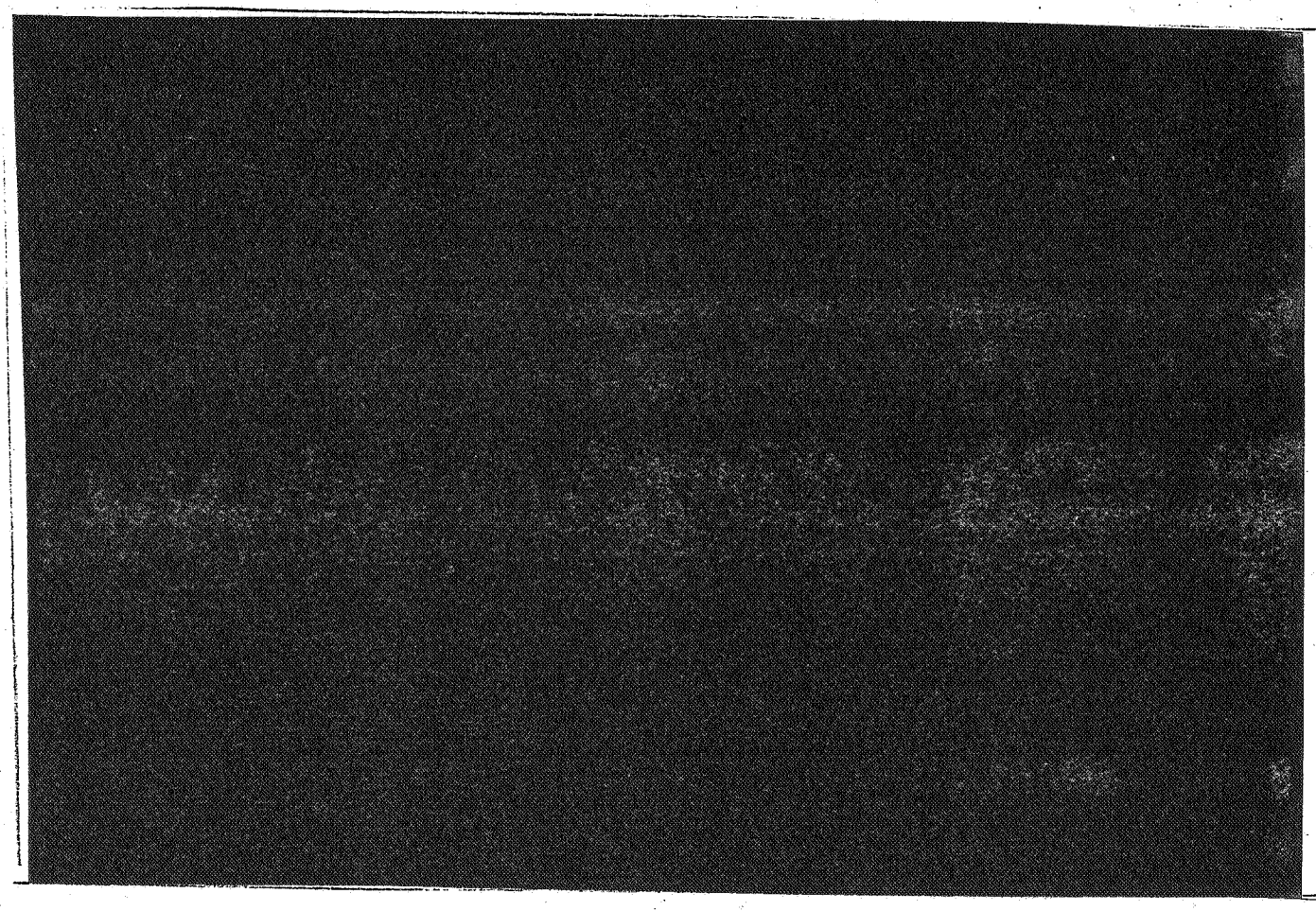
Table 8. Alachlor - 1981 Long-Evans Rat Study
Female Stomach Tumor Rates[+] and Peto's Prevalence Test Results (p values)

	Dose (mg/kg/day)			
	0	14	42	126
Leiomyosarcoma				
	0/34	0/29	0/36	1[a]/26
(%)	(0)	(0)	(0)	(4)
Osteosarcoma	0/41	0/35	0/40	4[b]/35
(%)	(0)	(0)	(0)	(11)
p =	0.008**	-	-	0.089
Gastric Adenocarcinoma				
	0/34	0/28	0/34	1[c]/22
(%)	(0)	(0)	(0)	(5)
Malignant Mixed Gastric Tumor				
	0/45	0/37	1/42	16[d]/41
(%)	(0)	(0)	(2)	(39)
p =	0.000**	-	0.155	0.000**
Gastric Adenocarcinoma and/or Malignant Mixed Gastric Tumors Combined				
	0/45	0/37	1/42	17/41
(%)	(0)	(0)	(2)	(41)
p =	0.000**	-	0.155	0.000**

[+]Number of tumor bearing animals/Number of animals examined, excluding those that died before observation of the first tumor.
[a]First leiomyosarcoma observed at week 102, dose 126 mg/kg/day.
[b]First osteosarcoma observed at week 89, dose 126 mg/kg/day.
[c]First gastric adenocarcinoma observed at week 105, 126 mg/kg/day.
[d]First malignant mixed gastric tumor observed at week 76, dose 126 mg/kg/day.
Note: Significance of trend denoted at control.
Significance of pair-wise comparison with control denoted at dose level.
If *, then $p < 0.05$. If **, then $p < 0.01$.

In response to scientific and regulatory questions raised in Japan, an extensive research program was undertaken to understand the mechanism by which chloroacetanilides induce stomach tumors in rats. The majority of this work was conducted with Butachlor in Sprague-Dawley rats. Since Butachlor is a close structural analog of Alachlor, and the two compounds produce the same glandular stomach tumors, extrapolation of the mechanistic information to Alachlor is scientifically justified. To further support this, some bridging data have been developed with Alachlor and were previously reported to the Agency. The purpose of these provided data are to: (a) report the results and conclusions of the mechanistic studies conducted with Butachlor; (b) integrate these results with those from the Alachlor work to show that the same mechanisms are operative for both herbicides.

The following are overviews of the data presented in the studies. It is noted that these data have not been formally reviewed by the Agency at the time of the preparation of this document.



ALACHLOR

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Pages _____ through _____ are not included.

The material not included contains the following type of information:

____ Identity of product inert ingredients.

____ Identity of product impurities.

____ Description of the product manufacturing process.

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In addition to the data on Butachlor, the registrant conducted an initiation-promotion study with Alachlor (*Gastric Tumor Promotion Study of Alachlor in Long-Evans Rats*, Monsanto Company, The Agricultural Group, Environmental Health Laboratory for Monsanto Company, Monsanto Study No. ML-93-137, Monsanto EHL Study# EHL 93049, February 3, 1995) as a followup to a stomach tumor initiation/promotion study with Butachlor. In this study, 100 male and 100 female Long-Evans rats obtained from Charles River Breeding Laboratory, Portage, MI, 6 weeks of age and weighing 168-219 g for males and 139-176 g for females were administered by oral gavage a single dose of 150 mg/kg of the known gastric tumor initiator N-methyl-N'-nitro-N-nitroso-guanidine (MNNG) to 4 groups of 20 animals per sex. One of these groups was not further treated. Another group received dietary administration of 8000 ppm catechol, and two groups received either 15 or 126 mg/kg/day of Alachlor in the diet for 1 year while another group (not MNNG treated) received a single oral dose of DMSO (5 ml/kg) followed by dietary administration of Alachlor at a level of 126 mg/kg/day (there was another group of 15 animals per sex obtained near the end of the study to serve as "control" animals for serum gastrin levels, gastric fluid amount, pH, and HCl concentration). The investigators determined, at the end of the study, stomach pH, gastric acid secretion over a 4 hour period in 5-6 of the control and DMSO/Alachlor treated animals. They also obtained blood from 9-10 control and DMSO/Alachlor treated animals for serum gastrin determinations. The stomachs of all animals were examined grossly and microscopically.

Alachlor was found to promote the development of glandular stomach tumors in females and to a lesser extent in males (see following table). No effect of treatment was noted in the MNNG alone treated animals (1 tumor). Alachlor alone produced no tumors in males and 4 tumors in females. MNNG/Alachlor treated animals produced tumors in 75% of treated females and 30% of treated males at 126 mg/kg/day. These tumors were neoplasms of the glandular stomach, mostly in the fundus region. In the 15 mg/kg/day Alachlor + MNNG no tumors were observed in the females. However several tumors were found in males at both doses and in females at the 126 mg/kg/day dose following MNNG. The investigators interpreted this as due to MNNG rather than Alachlor, since they occurred at equivalent frequency in the males at both doses, the lower of which had no promotional activity.

[redacted]

Alachlor administration was noted to produce atrophy of the fundic mucosa in almost every animal at 126 mg/kg/day both with and without the initiator, MNNG. No atrophy was noted in any animal at 15 mg/kg/day Alachlor for 1 year. High dose Alachlor

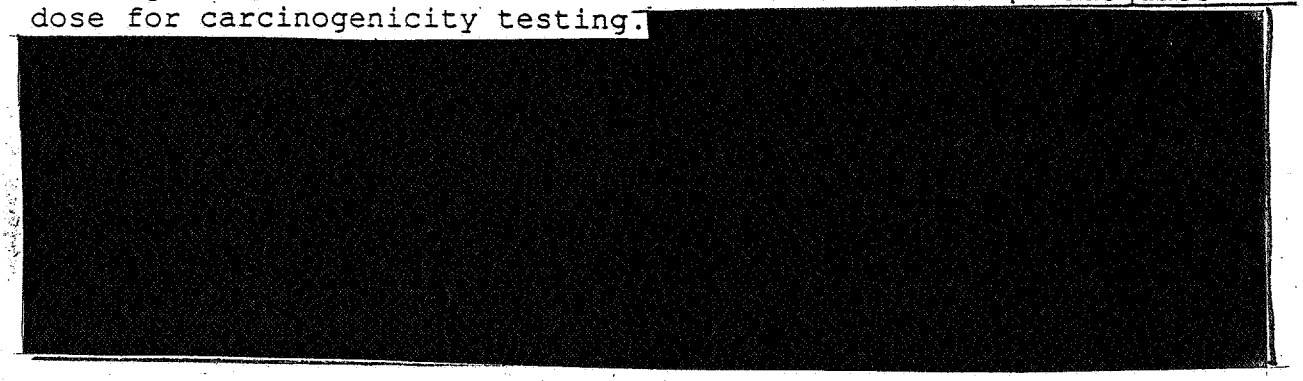
animals of both sexes had reduced amount of fluid in the stomachs. Stomach pH was numerically increased, and gastric hydrochloric acid secretion was decreased and serum gastrin levels were elevated in high dose animals. **The investigators believe that these data provide evidence that Alachlor produces glandular stomach tumors in rats through the same non-genotoxic, threshold sensitive mechanism as Butachlor and that this mechanism may be operative in humans under certain specific pathological states** (see Appendix I).

Summary of Pathology and Mechanistic Data

Treatment Groups Initiation: Promotion:	MNNG None (Control)	MNNG Catechol 8000 ppm	MNNG Alachlor 15m/k/d	MNNG Alachlor 126m/k/d	DMSO Alachlor 126m/k/d
MALES:					
Fundic tumors					
Adenoma/adenocarcinoma/undifferentiated carcinoma	1	0	0	6	0
Pyloric tumors					
Adenoma/carcinoma	0	16	3	3	0
Total animals with tumors					
Glandular stomach(fundic)	1	0	0	6*	0
Mucosal atrophy, fundus	0	1	0	20	19
Mechanistic evaluations[a]					
Serum gastrin (pg/ml)	51.20				361.143**
Gastric pH	2.24				2.56
Gastric fluid wt. (gm)	7.9612				1.70465**
HCl Secretion (~mole/hr)	125.9				17.0**
FEMALES:					
Fundic tumors					
Adenoma/adenocarcinoma/undifferentiated carcinoma	0	1	0	12	3
Mixed tumor	0	0	0	2	1
Fibroma	0	1	0	0	0
Squamous cell papilloma	0	0	0	1	0
Pyloric tumors					
Adenoma/carcinoma	0	13	0	4	0
Total animals with tumors					
Glandular stomach(fundic)	0	2	0	14**	4
Mucosal atrophy; fundus	0	0	0	17	20
Mechanistic evaluations[a]					
Serum gastrin (pg/ml)	38.45				678.279**
Gastric pH	2.87				4.50
Gastric fluid wt. (gm)	6.0690				1.43446**
HCl Secretion (µmole/hr)	66.4				16.8*

[a] Group means presented; controls were MNNG-naive retired breeders obtained at end of study.
* Significantly different from control at $p \leq 0.05$; two-sample t-test/one-tailed Fisher's Exact Test
** Significantly different from control at $p \leq 0.01$; two-sample t-test/one-tailed Fisher's Exact Test

Alachlor has been shown to produce glandular stomach tumors in Long-Evans rats at doses considered in excess of an adequate dose for carcinogenicity testing.



PENDING REGISTRATION INFORMATION IS NOT INCLUDED

Monsanto provided conclusions of two expert panels with regard to the gastric tumors observed in the 1981 rat chronic feeding study; the following are the conclusions of those panels and are the opinion of the registrant and not necessarily the Agency:

> The tumors result from mucosal atrophy including parietal cell depletion at high dose levels. The depletion of parietal cells results in hypochlorhydria, increased stomach pH, and compensatory increases in gastrin levels. Gastrin is known to exert a trophic effect on ECL and fundic mucosal cells. Therefore, the hypergastrinemia, along with mucosal atrophy, trigger a marked proliferative response which ultimately leads to tumor formation. Stomach tumor development involving gastrin is recognized as a secondary hormonal mechanism exhibiting a clear threshold. [Conclusions of an Expert Panel convened by Monsanto Company, September, 1995].

[**Reviewer's Note**: Such a mechanism of tumor formation may be operative in humans under certain pathological states (see Appendix I)].

> Based on studies with Butachlor, a structurally related acetanilide which produces the same tumor pattern, it was concluded that in the case of the stomach tumors, Alachlor administration would lead to mucosal toxicity, a reduction in parietal cells, increased pH and a marked increase in serum gastrin levels. The panel concluded that the marked elevation in gastrin levels played a key role in tumor development. Overall, the panel felt the ...stomach tumors were of limited significance due to

the fact that they were only seen at high doses which produced a sequelae of biochemical and histological changes that lead to tumor development. The panel concluded that the ...stomach tumors were produced via threshold-type mechanism. [Conclusions of the Scientific Panel Meeting on Alachlor conducted in Brussels June 6, 1994].

The Health Effects Division Carcinogenicity Peer Review Committee evaluated the data submitted in support of the threshold-type mechanism for induction of gastric tumors by Alachlor and **concurred with the explanation put forth by the registrant.**

c. Nasal Tumors

The following observations were noted with respect to nasal tumor formation in male rats from the 1981 chronic feeding study (Table 9):

The were **significant pair-wise differences** noted in the incidence of nasal respiratory epithelium adenomas and adenomas and/or adenocarcinomas combined ($p < 0.01$) at 42 and 126 mg/kg/day vs control, in addition to **significant trends** for these tumor types.

The following observations were noted in female rats with respect to tumor formation from the 1981 study (Table 10):

There were **significant pair-wise differences** noted in the incidence of nasal turbinate adenomas, and adenomas and/or adenocarcinomas combined at 42 ($p < 0.05$) and 126 ($p < 0.01$) mg/kg/day vs control, in addition to **significant trends** for these tumor types.

The following observations were noted with respect to nasal tumor formation in male rats from the 1983 chronic feeding study (Table 11):

There were **significant pair-wise differences** noted in the incidence of nasal respiratory epithelium adenomas at 15 mg/kg/day vs control ($p < 0.01$), in addition to a **significant trend.**

The following observations were noted in female rats with respect to tumor formation from the 1983 study (Table 12):

There were **significant pair-wise differences** noted in the

incidence of nasal respiratory epithelium adenomas at 15 mg/kg/day vs control ($p < 0.01$), in addition to a **significant trend.**

Table 9. Alachlor - 1981 Long-Evans Rat Study
Male Nasal Respiratory Epithelium Tumor Rates[+] and Peto's Prevalence Test Results (p values)

	Dose (mg/kg/day) 0	14	42	126
Adenoma	0/46	0/47	10/41	23[a]/40
(%)	(0)	(0)	(24)	(58)
p =	0.000**	-	0.000**	0.000**
Adenocarcinoma	0/27	0/20	1[b]/21	0/19
(%)	(0)	(0)	(5)	(0)
Combined	0/46	0/47	11/41	23/40
(%)	(0)	(0)	(27)	(58)
p =	0.000**	-	0.000**	0.000**

[+]Number of tumor bearing animals/Number of animals examined, excluding those that died before observation of the first tumor.
[a]First adenoma observed at week 59, dose 126 mg/kg/day; [b]First adenocarcinoma observed at week 116, dose 42 mg/kg/day.
Note: Significance of trend denoted at control.
Significance of pair-wise comparison with control denoted at dose level.
If *, then $p < 0.05$. If **, then $p < 0.01$.

Table 10. Alachlor - 1981 Long-Evans Rat Study
Female Nasal Turbinates Tumor Rates[+] and Peto's Prevalence Test Results (p values)

	Dose (mg/kg/day) 0	14	42	126
Adenoma	0/47	0/41	4/41	10[a]/41
(%)	(0)	(0)	(10)	(24)
p =	0.000**	-	0.029*	0.001**
Adenocarcinoma	0/34	0/28	1[b]/34	0/22
(%)	(0)	(0)	(3)	(0)
Combined	0/47	0/41	5/41	10/41
(%)	(0)	(0)	(12)	(24)
p =	0.000**	-	0.020*	0.001**

[+]Number of tumor bearing animals/Number of animals examined, excluding those that died before observation of the first tumor.
[a]First adenoma observed at week 78, dose 126 mg/kg/day.
[b]First adenocarcinoma observed at week 103, dose 42 mg/kg/day.
Note: Significance of trend denoted at control.
Significance of pair-wise comparison with control denoted at dose level.
If *, then $p < 0.05$. If **, then $p < 0.01$.

**Table 11. Alachlor - 1983 Long-Evans Rat Study
Male Nasal Respiratory Epithelium Tumor Rates+ and Exact Trend Test and Fisher's Exact Test Results (p values)**

	Dose (mg/kg/day)			
	0	0.5	2.5	15.0
Adenoma	0/45	0/47	0/45	11[a]/45
(%)	(0)	(0)	(0)	(24)
p =	0.000**	1.000	1.000	0.000**

+Number of tumor bearing animals/Number of animals examined, excluding those that died before week 53.
[a]First adenoma observed at week 94, dose 15.0 mg/kg/day.
Note: Significance of trend denoted at control.
Significance of pair-wise comparison with control denoted at dose level.
If *, then p < 0.05. If **, then p < 0.01.

**Table 12. Alachlor - 1983 Long-Evans Rat Study
Female Nasal and Thymus Tumor Rates+ and Peto's Prevalence Test Results (p values)**

	Dose (mg/kg/day)			
	0	0.5	2.5	15.0
Nasal Respiratory Epithelium Adenoma				
	0/38	0/38	1/43	9[a]/34
(%)	(0)	(0)	(2)	(26)
p =	0.000**	-	0.164	0.000**
Thymus Malignant Lymphosarcoma				
	0/48	2[b]/50	2/48	3/43
(%)	(0)	(4)	(4)	(7)
p =	0.081	0.068	0.079	0.022*

+Number of tumor bearing animals/Number of animals examined, excluding those that died before observation of the first tumor.
[a]First nasal respiratory epithelium adenoma observed at week 92, dose 15 mg/kg/day.
[b]First thymus malignant lymphosarcoma observed at week 21, dose 0.5 mg/kg/day.
Note: Significance of trend denoted at control.
Significance of pair-wise comparison with control denoted at dose level.
If *, then p < 0.05. If **, then p < 0.01.

From these data, it is evident that the nasal turbinate tumors observed in rats are related to the administration of Alachlor as well as the dose administered. Since the original classification of Alachlor as a B2 carcinogen, the registrant (Monsanto Company) has conducted a series of special metabolism and disposition studies with Alachlor and several Alachlor metabolites in rats, mice, and monkeys in support of the argument

that Alachlor should be reclassified with respect to its potential to cause cancer in humans. This argument is based upon data in support of the hypothesis that metabolic activation of Alachlor is a prerequisite for nasal tumor formation and that these tumors, while observed in rats, are not relevant to humans, based on significant species differences in hepatic and nasal tissue metabolism. The argument for reclassification is also supported by data from the registrant demonstrating the lack of genotoxic potential for Alachlor.

The studies provided by the registrant (Monsanto) fell into the following general categories:

1) mutagenicity/cell proliferation studies on Alachlor and its metabolites;

2) autoradiography studies examining the localization and distribution of Alachlor and its metabolites in rats, mice, monkeys, and hamsters;

3) in vivo metabolism studies in rats and mice; and

4) in vitro metabolism studies using various tissues from rats, mice, and monkeys.

Category 1 - Mutagenicity/Cell Proliferation Studies

Additional mutagenicity studies have been conducted in various test systems. Ames Salmonella assays have tested the mutagenic activity of urine and bile from Alachlor dosed rats. Mutagenicity in the Ames Salmonella test was also assessed on Alachlor and two metabolites with and without metabolic activation by nasal turbinate homogenates. The potential for Alachlor to induce unscheduled DNA synthesis (UDS) and in vivo chromosomal effects was assessed in rat liver and in rat bone marrow (micronucleus assay), respectively. Finally, Alachlor was tested for its capacity to affect cell proliferation.

The mutagenic activity of urine from Alachlor treated rats has been tested in the Ames Salmonella assay and reported to EPA [MRID # 00155392]. The results of this study indicated a weak response in strain TA98 (without activation with ß-glucuronidase) and strain TA1537 (with activation and ß-glucuronidase). A follow-up study was performed with bile from Alachlor treated female Long-Evans rats [MRID # 00155393]. Bile used in this study produced no mutagenic response in Salmonella.

Alachlor and two of its metabolites were tested for mutagenic activity in the Ames Salmonella assay in the absence or presence of nasal turbinate homogenates (non-induced nasal S9 fractions) prepared from rats, mice, or monkeys. The metabolites tested were the secondary sulfide of Alachlor (2',6'-diethyl-2-methylthio-acetanilide or DMTA) and 2,6-diethylaniline (DEA). These two metabolites were selected for testing because the secondary sulfide is the precursor to DEA which in turn is the precursor to the postulated reactive electrophilic benzoiminoquinone. The following conclusions can be drawn from the results of this study:

- Alachlor was negative for mutagenicity.

- The secondary sulfide produced a weak positive response in TA 1535 but the response was not consistent. Metabolic activation did not appear to play a role in the response. The response was lower using monkey nasal tissue compared to that of the rat and mouse tissue.

- Marginal activity was seen in TA 100 and TA 1535 with DEA. There were no substantial differences apparent among the rat, mouse, or monkey nasal turbinate S9.

Overall, the DMTA data are not consistent with a mode of action involving mammalian nasal turbinate metabolic activation because the marginal activity was observed both in the absence and in the presence of exogenous activation. The DEA data, while consistent with published reports of very weak mutagenic activity of DEA, are not consistent with the observation that nasal tumors appear in rats treated with Alachlor but not in Alachlor treated mice. However, autoradiography data on Alachlor localization (see below) support the conclusion that mice would not develop nasal tumors as opposed to rats.

In an *in vivo/in vitro* rat hepatocyte UDS assay, Alachlor was shown to induce a weak UDS response at 1000 mg/kg, a dose level producing significant toxicity (approximately the LD_{50} for Alachlor) [MRID# 00141061]. Considerable inter-animal variability in the response was observed at this high-dose level. In a second *in vivo/in vitro* UDS study [MRID# 42651302], the ability of Alachlor to induce unscheduled DNA synthesis and S-phase synthesis was studied in male Fischer-344 rat hepatocytes prepared from animals treated (orally, once) with Alachlor with doses up to 1000 mg/kg at 2- or 12-hours prior to sacrifice.

The results of this assay were as follows:

- A positive UDS response was again observed at 1000 mg/kg at the 12 hour time point.
- S-phase synthesis was unaffected by treatment with Alachlor at either the 2- or 12-hour time point.
- UDS was unaffected at the 2-hour time point.

The potential for Alachlor to induce chromosomal effects was tested in a rat bone marrow micronucleus assay [MRID# 42651303]. The results of this study demonstrated that Alachlor does not exhibit *in vivo* mammalian genotoxicity in rat bone marrow cells based on the finding that no treatment-related increases in micronucleated polychromatic erythrocytes (PCEs) were noted up to 600 mg/kg.

The effects of Alachlor on cell proliferation in nasal mucosa, liver, thyroid, and glandular stomach were studied in the Long-Evans rat and in nasal mucosa of CD-1 mice [MRID# 42852102]. The following were concluded:

- Alachlor produced a dose-related increase in cell proliferation in the nasal mucosa of Long-Evans rats. An increase in cell proliferation was produced in the other tissues studied but only at the highest dose levels tested (up to 252 mg/kg/day).
- The effect on cell proliferation in the rat was reversible after cessation of Alachlor treatment.
- Alachlor did not increase cell proliferation in the nasal mucosa of CD-1 mice.

Category 2 - In Vivo Metabolism Studies

Based upon the available data for Alachlor, the following hypothesis has been proposed for the production of tumors in the nasal mucosa: Alachlor is metabolized in the rat to the glutathione (mercapturic acid) conjugate, which is excreted through the bile into the gut. In the gut, enteric bacteria metabolize the conjugate to the thiol conjugate, with subsequent S-methylation of the thiol. This product, the methyl sulfide, is reabsorbed into the systemic circulation where conversion to the

secondary sulfide occurs. Hydrolysis of the secondary sulfide by arylamidase produces the diethylaniline metabolite of Alachlor. Oxidation of the diethylaniline metabolite produces the putative toxic metabolite, diethylbenzoquinone imine (DEBQI). This metabolite binds to cellular protein, resulting in eventual cell death. Ensuing regenerative cell proliferation can then lead to neoplasia through "fixation" of spontaneous mutations. The following summaries of submitted studies (both *in vivo* and *in vitro*) are presented in support of this mechanism:

- There are significant interspecies differences in the toxicity of Alachlor which may be related to unique metabolism by the rat, especially the Long-Evans rat. The chronic toxicity/carcinogenicity studies on Alachlor were conducted in the Long-Evans rat; however, the metabolism study submitted to fulfill the FIFRA requirement was conducted in Sprague-Dawley rats. Therefore, another FIFRA rat metabolism study (Guideline #85-1) was conducted to examine the metabolism of Alachlor in Long-Evans rats. The results indicated the following:

- Alachlor is extensively metabolized in Long-Evans rats.

- The metabolism of Alachlor is similar regardless of administration by multiple or single dosing and by either oral or intravenous dosing.

- Alachlor is principally metabolized initially via GSH conjugation and to a lesser extent by hydroxylation (P-450 mediated).

An additional study was also conducted in Long-Evans rats to determine the effect of chronic dietary pretreatment (with Alachlor) and age on the metabolism of Alachlor [MRID# 42651307]. The results of this study were as follows:

- Excretion of Alachlor derived radioactivity in feces was significantly higher, and urinary excretion significantly lower in 18 month old rats compared with the young rats. There were no evident trends in half-life of elimination in urine and feces for the various dose groups.

- Young male rats excreted a higher proportion of hydroxylated metabolites than young female rats. The proportion of hydroxylated metabolites increased with increasing dietary dose levels (0.5 to 126 mg/kg/day).

•There was very little effect of chronic dosing, age, or sex on the nature of metabolites excreted in the feces.

•A higher concentration of the tert-amide disulfide conjugate was observed in the fecal samples of rats that were administered 126 mg/kg (an excessive dose) compared with those rats that were administered 0.5 mg/kg. This finding indicates possible saturation of the binding sites (free thiols) of the fecal material for the thio intermediate, the precursor for the formation of the disulfide conjugate.

To further investigate the interspecies differences, special metabolism studies [MRID#'s 42651305 and 42852106] were conducted in CD-1 mice to examine the metabolic fate of Alachlor in this species (this species was also used for carcinogenicity testing). The results of this study were as follows:

•Alachlor was rapidly metabolized in the mouse with the majority of the radioactivity being eliminated within 48 hours of dosing at doses of 890 mg/kg (males) and 819 mg/kg (females).

•The metabolism of Alachlor in mice appears to follow the same metabolic pathways as those observed in rats, that is, GSH conjugation and P-450 oxidation.

•Comparison of the metabolism of Alachlor in the CD-1 mouse with the Long-Evans rat indicates that some quantitative and qualitative differences in metabolites between the two species exist. For example, mouse urine contains greater amounts of glucuronic acid conjugates and cysteine conjugates than the rat, but lesser amounts of phenolic metabolites.

Alachlor methyl sulfide, a metabolite of Alachlor detected in feces and plasma of Long-Evans rats, is formed initially by conjugation of Alachlor with GSH in liver and is further metabolized and excreted via biliary/enterohepatic circulation. The present study examined the metabolism of alachlor methyl sulfide. This metabolite is presumably formed as a result of enterohepatic circulation. Previous work has demonstrated that administration of alachlor methyl sulfide resulted in localization of radioactivity to the nasal turbinates of rats. The results of the present study showed the following:

•Alachlor methyl sulfide is extensively metabolized in Long-Evans rats.

• The sulfone metabolite of alachlor was identified as the major urinary metabolite in urine.

• The 4-Amino-2,5-diethylphenyl sulfate metabolite was observed in the urine of all dosed rats.

•The sulfone metabolite was identified as N-[2-ethyl-6-(1 hydroxyethyl)phenyl]-2-(methylsulfonyl)acetamide .

Category 3 - In Vitro Metabolism Studies

In vitro studies conducted by the registrant demonstrated the presence of the reactions necessary for production of the DEBQI intermediate. These include glutathione conjugation of Alachlor, hydrolysis of the secondary sulfide by arylamidase, and hydroxylation of 2,6-diethylaniline. Further *in vitro* studies demonstrated significant species differences in the rates of these reactions. Comparative *in vitro* metabolism of Alachlor by several tissues in the Long-Evans rat [MRID# 42852110] showed the presence of arylamidase activity in liver and nasal tissue resulting in formation of the 2,6-diethylaniline metabolite. Oxidation of the 2,6-diethylaniline metabolite to 4-amino-3,5-diethylphenol was shown to be approximately 50 times greater in nasal microsomes than in liver microsomes. Rat and mouse liver and nasal tissues were compared for their ability to metabolize Alachlor to the proposed DEBQI intermediate [MRID# 42852111]. The velocity of the nasal aryl amidase reaction in rat nasal tissue towards the sec-amide metabolite of Alachlor was observed to be 14-20 times higher in rat than in mouse. The velocity of the nasal arylhydroxylase towards diethylaniline in rat nasal tissue was found to be approximately 2-fold higher than in mouse. This study demonstrated that certain key enzymes responsible for production of the proposed toxic intermediate of Alachlor are more active in rat nasal mucosa vs mouse nasal mucosa. Liver and nasal cytosolic or microsomal fractions were used from rat and monkey to study metabolism of Alachlor to the GSH conjugate, the hydrolysis of Alachlor secondary sulfide by arylamidase, and the hydroxylation of 2,6-diethylaniline [MRID # 42651314]. Velocity of rat liver glutathione-S-transferase (GST) was 3.9 times greater than monkey GST towards Alachlor. Velocity of rat nasal GST was 114.3 times greater than monkey GST towards Alachlor. Velocity of secondary sulfide hydrolysis was equivalent in rat and monkey liver preparations, but was 4 times greater in rat nasal tissue vs monkey nasal tissue. Velocity of DEA hydroxylation in rat liver was 3 times greater than in monkey liver, and 7.6 times greater in rat nasal tissue than in monkey nasal tissue. **Thus, the enzymes thought to be responsible for production of the toxic intermediate of Alachlor are more active in rat nasal tissue vs monkey nasal tissue.**

In MRID# 43482301, cytosolic and microsomal fractions from rat and human liver and nasal tissue were studied to determine the differential species capability to conjugate Alachlor with glutathione, to hydrolyze the secondary methyl sulfide (secondary sulfide), and to hydroxylate the 2,6-diethylaniline metabolite of Alachlor. Velocity of glutathione conjugation in rat liver and nasal tissue was 4.0 and 32.5 times greater than in human liver and nasal tissue, respectively. Velocity of hydrolysis of the secondary sulfide was 5.8 times greater in rat nasal tissue vs human. Velocity of DEA hydroxylation was 7.5 times greater in rat liver vs human, and 129.8 times greater in rat nasal tissue vs human.

Category 4- Autoradiography Studies

The distribution of Alachlor and its metabolites in the body may play an important role in the site- and species-sensitive tumorigenicity of Alachlor. Therefore, whole body autoradiography studies using 14C-Alachlor were conducted in Long-Evans rats, CD-1 mice, and squirrel monkeys [MRID# 42852103]. The following results were obtained:

- In general, radioactivity was found in liver, kidney, lung, nasal vibrissae, body hair, surfaces of mouth and tongue, nasal turbinates, surfaces and roots of teeth, and periorbital fat.

- Localization of labelled Alachlor was most evident in rat nasal turbinates, less evident in the mouse, and absent in the monkey.

- Markedly more radioactivity remained in the intestines of rats than mice or monkeys, suggesting slower elimination related to greater enterohepatic circulation in the rat.

A whole body autoradiography study was also performed using 14C-Alachlor-methylsulfide [2-(methylthio)-N-(2,6,-diethylphenyl)-N(methoxymethyl)acetamide] at doses of 0.7 and 7.0 mg/kg, a metabolite of Alachlor formed as a result of metabolism by gut microflora and by the liver and the biliary excretion process and a precursor of the proposed reactive intermediate [MRID# 42651304]. The results of this study demonstrate that a metabolite of Alachlor present in bile of rats can localize to nasal turbinate tissues following oral administration, and may be related to the mechanism of nasal tumorigenesis.

Another autoradiography study conducted was a comparative study of the distribution and localization of Alachlor, metolachlor, and MON 4601 (three structurally related chloracetanilides) in rats at doses of 7 and 700 mg/kg [MRID# 42852104]. This study demonstrated the following:

- The overall patterns of distribution were similar for all three chemicals, but alachlor showed faster clearance from the g.i. tract in comparison to metolachlor and MON 4601.

- Alachlor was observed to localize in nasal turbinates to a greater extent at the low dose in comparison to metolachlor and MON 4601. At the high dose, nasal turbinate localization appeared less for alachlor than for metolachlor or MON 4601.

A third autoradiography study conducted was a comparative study of the distribution and localization, particularly in the nasal tissue, of Alachlor in Sprague-Dawley, Fisher 344, and Long-Evans rats and Golden Syrian hamsters following oral doses of 7 and 70 mg/kg [MRID# 42852105]. The following results were obtained:

- No significant differences in tissue distribution were noted among the three strains of rats at both dose levels.

- In the hamster, the liver was the major area of localization for radioactivity at five days after dosing.

- Nasal tissue localization of Alachlor was higher in the Long-Evans rats in comparison to Sprague-Dawley and Fisher 344 rats, and was absent in hamsters.

Conclusions

The above data point to the significance of the metabolic activity in the nasal turbinates of Long-Evans rats and the impact of this activity on the biotransformation of Alachlor to a reactive intermediate which in turn results in tumor formation in this tissue. **Monsanto** believes that the sensitivity of the Long-Evans rat to nasal tumorigenesis is justified when the following findings are considered collectively:

- Alachlor induces nasal turbinate tumors in the Long-Evans rat, but not the CD-1 mouse.

•Alachlor localizes in the nasal tissue of Long-Evans rats to a greater extent than in CD-1 mice and is absent in squirrel monkey nasal tissue.

•Alachlor causes increased cell proliferation in the nasal tissue of Long-Evans rats but not CD-1 mice.

•The nasal tissue of Long-Evans rats contains low GSH levels resulting in more efficient oxidative capabilities of nasal tissue which in turn leads to the formation of the reactive product, 2,6-diethyl-benzoquinonimine (DEBQI).

•Although Long-Evans rats, CD-1 mice, and squirrel monkeys possess the enzymes necessary to convert Alachlor to DEBQI, the enzyme velocities in the nasal tissue of rats are many times greater than that of the mouse or monkey.

The registrant also provided information from a Scientific Panel Meeting on Alachlor (convened in Brussels June 6, 1994) to consider safety and exposure data on Monsanto's herbicide, Alachlor, presumably by the request of Monsanto. According to the report, the **panel** was convened to assess the available toxicity and mechanistic data and to determine if the data were adequate to support the safety of Alachlor in the context of its use and resultant human exposure. The **panel** evaluated the genotoxicity data and animal studies, the available mechanistic data, epidemiology and information on exposure to farmers and consumers. The **panel** also attempted to identify possible additional studies that could be conducted to clarify certain aspects of the existing data base.

The **panel** noted that the available genotoxicity data did not provide evidence that Alachlor or its principal metabolites are genotoxic in validated assay procedures. Included in the database were a few positive findings which were associated either with extremely high test concentrations or with Alachlor of uncertain origin and purity. Only low level binding of Alachlor to rat liver or nasal cavity DNA was suggested [MRID# 43369201], but the results of this study were inconclusive. When the genotoxicity data were evaluated using internationally developed criteria (e.g., ICPEMC) Alachlor clearly scored as negative. A similar conclusion was drawn using EPA scoring methods.

The **panel** focused considerable discussion on the significance of the rat nasal tumors induced by Alachlor. The panel noted several important points:

a) Tumors at this site were predominantly adenomas and the tumor incidence showed a steep dose-response relationship accompanied by a no-effect level.

b) Autoradiographic studies demonstrated localization of Alachlor (or a metabolite) in rat nasal turbinates but not in nasal turbinates of mice where no nasal tumors were seen or in primates.

c) Alachlor undergoes extensive metabolism and enterohepatic circulation in the rat, but not in other species, including humans, leading to a much longer half-life in the rat and generally more complex metabolism.

d) In vitro metabolic studies demonstrated the formation of a potentially reactive metabolite of diethylaniline which could form a highly reactive quinone imine that could bind to protein sulfhydryl groups.

e) Considering the extensive gastrointestinal metabolism of Alachlor and high rate of metabolism in the nasal turbinates of rats relative to other species, the rat would form considerably more (approximately 20,000 times) reactive metabolite than humans.

f) While the nasal turbinates of rats might be expected to produce a reactive metabolite, there is no evidence that such metabolites are genotoxic since neither Alachlor nor its secondary methylsulfide metabolite produced genotoxicity in an Ames assay using rat nasal turbinate metabolic activation.

g) Evidence suggests that the formation of nasal tumors with chloracetanilides and related compounds is specific to the rat, and possibly to certain strains of rats, since Butachlor and phenacetin produce nasal tumors in Sprague-Dawley rats but not in F-344 rats or in the mouse.

h) The available data strongly suggest a mechanism for rat nasal tumor development that is associated with cytotoxicity and cell proliferation. This is in keeping with the threshold in the dose-response curve for nasal tumor development. Such a mechanism is consistent with the available data on phenacetin.

Considering all the evidence, the **panel** concluded that the induction of nasal tumors in the rat by Alachlor was likely a rat-specific phenomenon of limited relevance to humans under actual conditions of exposure.

Overall the **panel** had the following conclusions on the Alachlor data for the nasal tumors:

1. The available genotoxicity data do not indicate that Alachlor or its principal metabolites are genotoxic nor is there any significant binding to hepatic or nasal turbinate DNA.

2. Mechanistic studies indicate that the rat nasal, thyroid and stomach tumors are induced through threshold-type mechanisms involving enhancement of cell proliferation and possibly confounded by toxicity at high doses. The data indicate that in the rat Alachlor administration could lead to the formation of a reactive quinone imine metabolite which is potentially cytotoxic. Metabolism and pathology in the rat nasal cavity is likely a species- and strain-specific response.

3. Given the lack of relevance of the experimental findings to actual human exposure conditions, the panel concluded that Alachlor as it is currently used in agriculture does not present a cancer risk to humans.

The mechanism of Alachlor induced nasal tumors is considered by the **registrant** as a non-genotoxic mechanism. This argument is largely based upon the mutagenicity database, in which the registrant argues that Alachlor has no significant genotoxic activity in mammalian systems. Studies examining the effect of Alachlor administration on tissue glutathione levels following *in vivo* administration of oral and intraperitoneal doses of Alachlor to Long-Evans rats as well as the effect of Alachlor on glutathione levels in cultured hepatocytes have been conducted [MRID#'s 42651318 and 43641603]. These studies showed depletion of hepatic glutathione followed by recovery after a single i.p. dose of 350 mg/kg or single oral doses of 126 and 350 mg/kg. In cultured hepatocytes, Alachlor was hepatotoxic at concentrations above 400µM, and significant glutathione depletion was also observed at concentrations above 300µM Alachlor. While no significant depletion of nasal glutathione levels were observed, the DNA damaging effect of Alachlor might be related to depletion

of glutathione and subsequent tissue toxicity, and not to a direct mode of action. It is noted that significant hepatotoxicity in the form of elevated serum ALT, AST, and LDH as well as centrilobular cytoplasmic eosinophilia, centrilobular inflammation, and centrilobular hepatocellular degeneration/necrosis was observed at a dose of Alachlor (1000 mg/kg) which also caused a weak UDS response. The registrant suggests these data are consistent with a non-genotoxic mode of action for Alachlor.

With regard to the nasal tissue, two studies addressed the mechanism of nasal turbinate induced tumors. In the first study [MRID# 43641604], female Long-Evans rats were fed 14-C labelled Alachlor in the diet at a targeted dose level of 126 mg/kg/day for a total of 13 days. On days 1, 3, 7, and 13, 3 rats were sacrificed and the covalent binding of Alachlor derived radioactivity to nasal protein was determined. The results of this study showed a direct correlation between the total level of Alachlor binding to rat nasal proteins and length of treatment. The major adduct was identified as the 3,5-diethylbenzoquinone-4-imime (DEBQI)-cysteine adduct. Formation of DEBQI in the rat nasal tissue is believed to be required for induction of nasal tumors. In the second study [MRID# 43641602], the *in vitro* cytotoxicity of Alachlor, DEA, secondary sulfide, and secondary amide were assessed in preparations of rat nasal turbinate as evidenced by leakage of the enzyme acid phosphatase into the culture medium. Concentrations of Alachlor and metabolites used were either 1 or 5mM. Alachlor at both 1 and 5 mM was shown to increase acid phosphatase levels in the culture medium. Neither the secondary sulfide nor the secondary amide caused an increase in acid phosphatase levels at 1 mM (5mM concentration not possible due to solubility limitations). DEA was observed to increase acid phosphatase levels at 5 mM in nasal tissue. The cytotoxicity observed with Alachlor in nasal tissue is consistent with the cell proliferation response observed in nasal tissue after administration of Alachlor, but the entity responsible for the cytotoxic response is not known with certainty.

2. Carcinogenicity Study of Alachlor in Mice:

A chronic feeding study in CD-1 mice was conducted by Bio/dynamics Inc. (June 18, 1981, Project No. 77-2064; Study No. BD-77-423).

The study design allocated groups of 50 mice per sex to dose levels of 0, 26, 78, or 260 mg/kg/day of Alachlor (Lasso technical)* for 79 weeks. The test substance used for the first 11 months of the study was stabilized with 0.5% epichlorohydrin. The test substance used for the remaining 7 months of the study was stabilized with [redacted]
[*Alachlor was supplied in two batches: one used during the first 11 months of the study was stabilized with 0.5% epichlorohydrin; and the other used during the last 7 months, was stabilized with epoxidized soybean oil.]

The statistical evaluation of mortality of the 1981 CD-1 mouse study showed no significant incremental changes with increasing doses of Alachlor in male mice (Table 13). Female mice showed a significant increasing trend in mortality with increasing doses of Alachlor (Table 14).

Table 13. Alachlor - 1981 CD-1 Mouse Study Male Mortality Rates+ and Cox or Generalized K/W Test Results

	Weeks				
Dose (mg/kg/day)	1-26	27-52	53-66	67-80[f]	Total
0	0/50	3/50	7/47	15/40	25/50(50)
26	0/49[a]	2/49	9/46[b]	21/37	32/48(67)
78	0/50	5/50	9/45	12/36	26/50(52)
260	1/48[c]	1/47	11/46	13/35	26/48(54)

+Number of animals that died during interval/Number of animals alive at the beginning of the interval. ( )Percent.
[f]Final sacrifice at week 79; [a]One accidental death at week 15, dose 26 mg/kg/day; [b]One accidental death at week 54, dose 26 mg/kg/day.
[c]Two accidental deaths, one each at weeks 8 and 18, 260 mg/kg/day.
Note: Time intervals were selected for display purposes only.
Significance of trend denoted at control.
Significance of pair-wise comparison with control denoted at dose level.
If *, then $p < 0.05$. If **, then $p < 0.01$.

Table 14. Alachlor - 1981 CD-1 Mouse Study Female Mortality Rates+ and Cox or Generalized K/W Test Results

Dose (mg/kg/day)	Weeks 1-26	27-52	53-66	67-80f	Total
0	1/47a	2/45b	4/42c	18/38	25/45(56)**
26	0/50	1/50	3/49	13/46	17/50(34)*n
78	1/49d	2/48	4/46	19/42	26/49(53)n
260	1/49e	4/48	12/44	17/32	34/49(69).

+Number of animals that died during interval/Number of animals alive at the beginning of the interval. ( )Percent.
fFinal sacrifice at week 79; nNegative change from control; aThree accidental deaths, one each at weeks 1, 20, and 26, dose 0 mg/kg/day; bOne accidental death at week 39, dose 0 mg/kg/day; cOne accidental death at week 53, dose 0 mg/kg/day; dOne accidental death at week 8, dose 78 mg/kg/day; eOne accidental death at week 10, dose 260 mg/kg/day.
Note: Time intervals were selected for display purposes only.
Significance of trend denoted at control.
Significance of pair-wise comparison with control denoted at dose level.
If *, then $p < 0.05$. If **, then $p < 0.01$.

A second carcinogenicity study in CD-1 mice was conducted by the Environmental Health Laboratory (December 8, 1994, Project Nos. MSL-13847 and EHL 91166; Study No. ML-92-001; MRID# 43507601).

The study design allocated groups of 50 mice per sex to one of four dose levels. Male mice received 0, 16.64, 65.42, or 262.40 mg/kg/day, and female mice received 0, 23.73, 90.34, or 399.22 mg/kg/day, of Alachlor for 79 weeks. An additional 10 mice per sex per dose were designated for interim sacrifice at week 53.

The statistical evaluation of mortality of the 1994 CD-1 mouse study showed no significant incremental changes with increasing doses of Alachlor in male (Table 15) or female (Table 16) mice.

Table 15. Alachlor - 1994 CD-1 Mouse Study
Male Mortality Rates[+] and Cox or Generalized K/W Test Results

Dose (mg/kg/day)	Weeks 1-26	27-52	53[i]	53-66	67-80[f]	Total
0	1/60	1/59	10/58	3/48	4/45	9/50(18)
16.64	1/60	1/59	10/58	5/48	3/43	10/50(20)
65.42	0/60	2/60	10/58	2/48	5/46	9/50(18)
262.40	0/60	1/60	10/59	3/49	5/46	9/50(18)

[+]Number of animals that died during interval/Number of animals alive at the beginning of the interval.
[i]Interim sacrifice at week 53.
[f]Final sacrifice at week 79.
( )Percent.
Note: Time intervals were selected for display purposes only.
Significance of trend denoted at control.
Significance of pair-wise comparison with control denoted at dose level.
If *, then $p < 0.05$. If **, then $p < 0.01$.

Table 16. Alachlor - 1994 CD-1 Mouse Study
Female Mortality Rates[+] and Cox or Generalized K/W Test Results

Dose (mg/kg/day)	Weeks 1-26	27-52	53[i]	53-66	67-80[f]	Total
0	0/60	4/60	10/56	3/46	3/43	10/50(20)
23.73	0/59[a]	3/59	10/56	2/46	2/44	7/49(14)[n]
90.34	0/60	0/60	10/60	5/50	9/45	14/50(28)
399.22	0/60	2/60	10/58	2/48	6/46	10/50(20)

[+]Number of animals that died during interval/Number of animals alive at the beginning of the interval.
[i]Interim sacrifice at week 53.
[f]Final sacrifice at week 79.
[n]Negative change from control.
[a]One accidental death at week 24, dose 23.73 mg/kg/day.
( )Percent.
Note: Time intervals were selected for display purposes only.
Significance of trend denoted at control.
Significance of pair-wise comparison with control denoted at dose level.
If *, then $p < 0.05$. If **, then $p < 0.01$.

A. Mouse Tumor Reevaluation

In the 1981 chronic feeding study in CD-1 mice, males had a significant increasing trend in bronchioalveolar adenomas at $p < 0.05$. There were no significant differences in the pair-wise comparisons of the dosed groups with the controls. Female mice had significant increasing trends, in addition to significant differences in the pair-wise comparisons of the 260 mg/kg/day dose group with the controls, for bronchioalveolar adenomas and adenomas and/or carcinomas combined, all at $p < 0.01$.

Table 17. Alachlor - 1981 CD-1 Mouse Study
Male Bronchioalveolar Tumor Rates[+] and Exact Trend Test and Fisher's Exact Test Results (p values)

	Dose (mg/kg/day)			
	0	26	78	260
Adenoma	6/47	1/46	4/45	10[a]/46
(%)	(13)	(2)	(9)	(22)
p =	0.013*	0.059[n]	0.398[n]	0.192
Carcinoma	3[b]/47	5/46	7/45	2/46
(%)	(6)	(11)	(16)	(4)
p =	0.210	0.345	0.141	0.510[n]
Combined	9/47	6/46	10[c]/45	12/46
(%)	(19)	(13)	(22)	(26)
p =	0.108	0.303[n]	0.457	0.291

[+]Number of tumor bearing animals/Number of animals examined, excluding those that died before week 53.
[a]First adenoma observed at week 58, dose 260 mg/kg/day.
[b]First carcinoma observed at week 75, dose 0 mg/kg/day.
[n]Negative change from control.
[c]One animal in the 78 mg/kg/day dose group had both an adenoma and a carcinoma.
Note: Significance of trend denoted at control.
Significance of pair-wise comparison with control denoted at dose level.
If *, then $p < 0.05$. If **, then $p < 0.01$.

Table 18. Alachlor - 1981 CD-1 Mouse Study Female Bronchioalveolar Tumor Rates[+] and Peto's Prevalence Test Results (p values)

	Dose (mg/kg/day)			
	0	26	78	260
Adenoma	2/41	4/47	7/43	10[a]/37
(%)	(5)	(9)	(16)	(27)
p =	0.001**	0.340	0.052	0.003**
Carcinoma	1/23	1/35	1/28	1[b]/20
(%)	(4)	(3)	(4)	(5)
Combined	3/41	5/47	8/43	11/37
(%)	(7)	(11)	(19)	(30)
p =	0.001**	0.430	0.073	0.004**

[+]Number of tumor bearing animals/Number of animals examined, excluding those that died before observation of the first tumor.
[a]First adenoma observed at week 63, dose 260 mg/kg/day.
[b]First carcinoma observed at week 77, dose 260 mg/kg/day.
Note: Significance of trend denoted at control.
Significance of pair-wise comparison with control denoted at dose level.
If *, then $p < 0.05$. If **, then $p < 0.01$.

Monsanto submitted an addendum to this study following the original submission. This report contained an evaluation done by Bio/dynamics on the nasal turbinates of mice in the control and high dose group. Tissues from all remaining animals were examined (originally only 10 mice/sex/group had been examined). No nasal turbinate tumors were found.

The **first** meeting of the Health Effects Division Carcinogenicity Peer Review Committee concluded that a dose adequate for carcinogenicity testing "was probably reached or slightly exceeded at the high-dose in female mice, as evidenced by slight increase in mortality, 10% body weight depression, an increase in thyroid follicular atrophy and in kidney chronic interstitial fibrosis."

In the 1994 carcinogenicity study in CD-1 mice, although there were no significant increasing trends, male mice had significant differences in the pair-wise comparisons of the 16.64 and 262.40 mg/kg/day dose groups with the controls for bronchioalveolar adenomas and adenomas and/or carcinomas combined, all at $p < 0.05$ (Table 19). There were also significant differences in the pair-wise comparisons of the 65.42 mg/kg/day dose group with the controls for bronchioalveolar adenomas and adenomas and/or carcinomas combined, both at $p < 0.01$. There were no significant compound-related tumors observed in female mice (Table 20).

Table 19. Alachlor - 1994 CD-1 Mouse Study
Male Bronchioalveolar Tumor Rates[+] and Exact Trend Test and Fisher's Exact Test Results (p values)

	Dose (mg/kg/day)			
	0	16.64	65.42	262.40
Adenoma	4/48	11/49	15/50	12[a]/49
(%)	(8)	(22)	(30)	(24)
p =	0.132	0.049*	0.006**	0.029*
Carcinoma	1/48	0/49	3[b]/50	0/49
(%)	(2)	(0)	(6)	(0)
p =	0.293	0.495[n]	0.324	0.495[n]
Combined	4[c]/48	11/49	18/50	12/49
(%)	(8)	(22)	(36)	(24)
p =	0.161	0.049*	0.001**	0.029*

[+]Number of tumor bearing animals/Number of animals examined, excluding those that died before week 49; also excludes week 53 interim sacrifice animals.
[a]First adenoma observed at week 71, dose 262.40 mg/kg/day.
[b]First carcinoma observed at week 49, dose 65.42 mg/kg/day.
[n]Negative change from control.
[c]One animal in the 0 mg/kg/day dose group had both an adenoma and a carcinoma.
Note: One animal in each of the 65.42 and the 262.40 mg/kg/day dose groups of the interim sacrifice group had a bronchioalveolar adenoma. Interim sacrifice animals are not included in this analysis.
Significance of trend denoted at control.
Significance of pair-wise comparison with control denoted at dose level.
If *, then $p < 0.05$. If **, then $p < 0.01$.

**Table 20. Alachlor - 1994 CD-1 Mouse Study
Female Bronchioalveolar Tumor Rates[+] and Exact
Trend Test and Fisher's Exact Test Results (p values)**

	Dose (mg/kg/day)			
	0	23.73	90.34	399.22
Adenoma	3[a]/46	7/46	6/50	9/48
(%)	(7)	(15)	(12)	(19)
p =	0.088	0.158	0.287	0.070
Carcinoma	1/46	1/46	1[b]/50	3/48
(%)	(2)	(2)	(2)	(6)
p =	0.120	0.753	0.731	0.325
Combined	4/46	8/46	7/50	11[c]/48
(%)	(9)	(17)	(14)	(23)
p =	0.059	0.177	0.312	0.053

[+]Number of tumor bearing animals/Number of animals examined, excluding those that died before week 54; also excludes week 53 interim sacrifice animals.
[a]First adenoma observed at week 53, dose 23.73 mg/kg/day, in an interim sacrifice animal. Second adenoma observed at week 54, dose 0 mg/kg/day, in an animal that died on study.
[b]First carcinoma observed at week 60, dose 90.34 mg/kg/day.
[c]One animal in the 399.22 mg/kg/day dose group had both an adenoma and a carcinoma.
Note: One animal in each of the 23.73 and the 399.22 mg/kg/day dose groups of the interim sacrifice group had a bronchioalveolar adenoma. Interim sacrifice animals are not included in this analysis.
Significance of trend denoted at control.
Significance of pair-wise comparison with control denoted at dose level. If *, then $p < 0.05$. If **, then $p < 0.01$.

The conclusions of two expert panels with regard to the relevance of the mouse lung tumors can be summarized as follows:

Expert Panel Convened by Monsanto Company, September, 1995:

"There is extensive support for the conclusion that Alachlor is not carcinogenic in the mouse. The most significant points are as follows:

- No dose response in the second study; 16-fold increase in dose resulted in no relevant differences across treated groups.

- No evidence of treatment-related preneoplastic changes (hyperplasia or dysplasia) or other effects in the lungs of treated groups.

- No change in multiplicity of pulmonary neoplasms between treated and control groups.

- No increase in progression to malignant neoplasms in treated groups compared to control.

- Tumor incidence within historical control range.

- Lack of consistency between studies.

- Known high variability in incidence of pulmonary neoplasms in the mouse (clearly demonstrated by intergroup variability in previous mouse studies conducted by Monsanto).

- No evidence of localization in the mouse lung as determined by whole body autoradiography studies.

Deliberations of the Scientific Panel Meeting convened in Brussels June 6, 1994 to consider safety and exposure data on Monsanto's herbicide, Alachlor

The first mouse study provided a suggestion of an increase in lung tumors but this effect was judged to be unrelated to treatment. Preliminary results from the second mouse study showed an increase in lung tumors in all treatment groups with no evidence of a dose-response. The **panel** concluded that until the results of the second mouse study were subjected to peer review and considered in the light of historical data, no conclusions could be drawn from the data. It was noted that innocuous substances such as BHT induce mouse lung tumors.

Overall the panel concluded the following regarding the Alachlor mouse tumor data:

> "The increased incidences of lung tumors observed in the treated groups in the second mouse study did not demonstrate a dose-response, and require critical peer review in the light of historical data on mouse lung tumors" [it is noted that at the time of this review, the second mouse study had not been totally completed].

At the request of **Monsanto**, PATHCO Inc. convened an expert panel to review slides and data from the two 18-month feeding studies of Alachlor in mice with specific reference to the lung tumors reported in these studies. The expert panel consisted of Dr. Dawn G. Goodman who acted as Chairperson, Dr. Donald L. Dungworth, and Dr. Jerrold M. Ward.

The panel reviewed microscopic slides of lung tumors from both the Bio/dynamic and Monsanto studies. The **panel** generally agreed with the diagnoses of the study pathologists and noted that the morphology of the tumors from both studies was comparable to those seen as spontaneous age-associated tumors in untreated animals. In addition, during their slide review, the panel noted that there was no evidence of toxicity to the lung in either study.

The **panel's** conclusions regarding the lung tumors in each study and interpretation of their significance are discussed below.

A. Bio/dynamics Study

1. Male mice

The **panel** concluded that there was no evidence of a compound-related effect on the lung in male CD-1 mice in this study.

2. Female mice

In female mice there was a slight increase in the incidence of bronchioalveolar neoplasms in high dose females, primarily adenomas. There was no increase in the incidence of bronchioalveolar carcinomas or bronchioalveolar hyperplasia, nor was there any evidence of toxicity to the lung parenchyma. The morphology of the tumors in this study was comparable to the spectrum of bronchioalveolar tumors seen in controls as spontaneous age-associated lesions.

The increase in tumor incidence in females was not linearly related to the increase in dose. Although there was a ten-fold increase in dose from the low to the high dose, there was only a two-fold increase in the incidence of adenomas and no increase in the incidence of hyperplastic lesions or malignant tumors.

Bronchioalveolar neoplasms of the lung are common age-associated neoplasms in CD-1 mice with highly variable

incidences. This can be observed in the historical data presented in the study. The incidences of bronchioalveolar tumors seen in the various groups in this study are within the historical ranges reported for these tumors. The incidence observed in the controls is at the low end of the historical range while the high dose group is at the upper end, resulting in an apparent increase between the control and high dose groups which is not biologically meaningful.

The incidences of bronchioalveolar tumors in high dose females is not statistically significant with a Fishers exact test at $p < 0.01$. The panel followed the recommendation of Haseman and used a p value of $p < 0.01$ to determine statistical significance for bronchioalveolar neoplasms which are considered to be common tumors. A common tumor is defined as one with an incidence rate of 1% or more in historical controls. These authors have recommended $p < 0.01$ to indicate treatment-related differences for group-wise comparisons. At $p < 0.01$ the neoplastic response more accurately reflects an effect of the test article. Such a procedure decreases the false-positive rate to 7-8%.

The **panel's** conclusion was that there is no evidence in this study that bronchioalveolar tumors of lung in male mice are related to administration of Alachlor. The weight of evidence likewise does not indicate that bronchioalveolar tumors in female mice in this study are related to administration of Alachlor.

B. Monsanto Study

1. Male mice

In male mice, there was an increase in the incidence of bronchioalveolar tumors, primarily adenomas, in treated animals compared to controls. These increases were only significant at the mid dose level and not at the high dose. Mortality was comparable among all dose groups. Thus, the lower incidence observed in the high dose group was not related to poor survival in this group compared to other dose groups or controls. Thus, there is no evidence of a dose response. In addition, there was no increase in the incidences of bronchioalveolar hyperplasia or bronchioalveolar carcinomas, nor was there an increase in multiplicity of tumors in treated animals. There was also no evidence of toxicity to the lung parenchyma. The tumors observed in this study were typical morphologically of the spontaneous bronchioalveolar tumors commonly observed in aged CD-1 mice. Bronchioalveolar tumors in CD-1 mice have a highly variable spontaneous rate and the incidences reported in the high dose group fall within the

historical control range. Thus, the weight of the evidence does not suggest that the lung tumors in male mice are related to administration of Alachlor.

2. Female mice

In female mice, there is a slight increase in the incidences of bronchioalveolar tumors, primarily adenomas in treated animals compared to controls. These increases are not statistically significant at any dose level. There is no evidence of a dose response and the increased incidences are not supported by the presence of other effects as discussed for males.

The **panel** concluded that the weight of evidence does not indicate that bronchioalveolar tumors in male or female mice in this study are related to administration of Alachlor.

Interpretation of Results Reported in the Two Studies

In evaluating each study independently, there are slight increases in lung tumors which are not considered to be related to administration of Alachlor. Superficially, the fact that both studies showed slight increases in lung tumor incidence might suggest that there is a marginal effect which is not detected by the individual studies. The **panel** did not believe this was the case for several reasons which are discussed below.

1. Lack of reproducibility of results

There is no consistent effect between the two studies. In the Bio/dynamics study, the increase in lung tumors was in females. Males were not affected. In the Monsanto study, the increase in lung tumors was primarily in males. In males, a comparable dose (mid dose-300/400 ppm) repeated twice gave quite different results. In both males and females, an increase in high dose administered (1000 vs. 1600 ppm) did not result in a concomitant increase in tumor incidence.

2. Nonlinear dose response

Any increase in tumor incidence observed in either study in treated animals compared to controls was not proportional to the increase in dose levels.

3. Absence of lung toxicity

There was no evidence of any toxic effect on the lung, either acute or chronic, in either study.

4. Lack of progression

The tumors observed were primarily benign. There was no increase in the incidence of potential preneoplastic lesions (i.e. hyperplasia) or in the incidence of malignant tumors. In addition, there was no increase in multiplicity of tumors (i.e. multiple tumors per animal) nor was there a decrease in latency (earlier appearance of the tumors) in treated versus control animals.

5. Common tumors

The lung tumors observed in both studies were comparable morphologically to those seen as spontaneous age associated neoplasms in CD-1 mice. These tumors occur with highly variable incidences in control groups.

6. No known mechanism

There is no known mechanism to explain the patterns of increased lung tumor incidence in these studies.

a) Genotoxicity

Alachlor has been tested in numerous assays for genotoxicity. Alachlor is not genotoxic in most systems tested and is generally considered nongenotoxic.

b) Immunosuppression

There is no evidence in either chronic study that Alachlor may be immunosuppressive.

c) Lung toxicity

Alachlor is nontoxic to the lung as evidenced by the lack of tissue injury and/or cell proliferation in either study. In addition, by autoradiography, Alachlor does not appear to localize in the lung. These factors suggest that the lung is not a target tissue.

Conclusions

After considering all of the available statistical and biological information from the two studies and for the reasons enumerated above, the **panel** concluded that the weight of evidence indicates that administration of Alachlor does not cause an increase in lung tumors in mice.

E. Mutagenicity data

Studies submitted in relation to Alachlor considered by previous HED/CPRC meetings are as follows:

Alachlor was shown to be negative in an Ames *Salmonella* assay [MRID# 00109563]. Urine from Alachlor treated rats tested in an Ames *Salmonella* assay resulted in a weak mutagenic response in strain TA98 in the presence of ß-glucuronidase. A weak mutagenic response was also observed in strain TA1537 in the presence of both ß-glucuronidase and metabolic activation [MRID# 00155392]. Additional Ames *Salmonella* assays with metabolites of Alachlor showed that of five metabolites tested (t-hydroxysulfone [rat, mouse, goat, hen, rotation crops metabolite], *sec*- amide p-hydroxy methylsulfone [rat metabolite], t-sulfinyllacetic acid [corn metabolite], t-oxanilic acid [soil, water, soybean metabolite], and t-sulfonic acid [corn, soil, soybeans, water metabolite]), only the t-hydroxysulfone metabolite was observed to be mutagenic (strain TA100 at 3 and 10 mg/plate in the presence and absence of metabolic activation). Alachlor was positive in an *in vivo/in vitro* UDS assay at 1000 mg/kg, a dose approximating the LD_{50} in rats [MRID# 00014061]. Alachlor was negative in a recombinant/ conversion assay in bacteria, a DNA damage/repair assay in rat hepatocytes, an *in vitro* cytogenetics assay in rat bone marrow, and in an HGPRT gene mutation assay in CHO cells.

The following Alachlor mutagenicity studies were submitted subsequent to previous CPRC meetings:

Alachlor itself was negative in an Ames *Salmonella typhimurium* mammalian microsome plate incorporation assay [MRID# 42651301], conducted in the absence of S9 and with S9 prepared from uninduced rat, mouse, or monkey nasal turbinates, at concentrations ranging from 50 to 5000 µg/plate. Tester strains TA98, TA100, TA1535, and TA1537 were used.

Included with the above study [MRID# 42651301] were mutagenicity data on 2',6'-Diethyl-2-methylthioacetanilide (DMTA), a proposed metabolic intermediate of Alachlor during metabolism to the s-methylsulfoxide, and 2,6-diethylaniline (DEA), a proposed toxic metabolite of Alachlor. These data showed the following:

- DMTA was positive in strain TA1535 in three independent *Salmonella typhimurium* mammalian microsome plate incorporation assays, conducted with S9 prepared from mouse nasal turbinates. In addition, there was a tendency for increased numbers of revertants of TA1535 to occur following exposure to higher dose levels (1500 and/or 5000 µg/plate) of

DMTA, and this was also observed in one of two assays conducted with rat nasal turbinate S9. Although only marginal increases were observed, they were reproducible and statistically significant. There was no response in tester strains TA98, TA100 or TA1537 with the nonactivated test material or in the presence of S9 prepared from mouse, rat or monkey nasal turbinates. These data are not considered relevant since DMTA is not a stable product of Alachlor metabolism.

•DEA was positive in strains TA1535 and TA100 in at least two independent *Salmonella typhimurium*/mammalian microsome plate incorporation assays, conducted with S9 prepared from mouse nasal turbinates. The nonactivated test material and the test material activated with rat nasal turbinate S9 were also positive in strain TA100. Although only marginal increases were observed, they were reproducible and statistically significant. There was no consistent response in tester strains TA98 and TA1537.

Alachlor was positive for inducing unscheduled DNA synthesis (UDS) in hepatocytes [MRID# 42651302] recovered from male Fischer-344 rats at 12 hours after oral gavage administration of 1000 mg/kg. The average number of net nuclear grains increased by >5 compared with the controls, and >10% of the cells were in repair. Similarly, a comparison of the individual data from treated animals and the vehicle control group showed that hepatocytes recovered from 3 of 5 animals were positive for UDS, cells from one animal showed a borderline positive response, and liver cells from the remaining animal was negative. Based on Mirsalis et al. (1982) and the reviewer's experience, these data are suggestive of a genotoxic response, but it is noted that the dose at which a positive response was observed approximates the LD_{50} of Alachlor in rats. There was no indication of UDS activity at 12 hours after oral gavage administration of lower doses (50, 200, or 500 mg/kg) or at 2 hours following gavage with 1000 mg/kg.

Alachlor was negative in a micronucleus assay in Long-Evans rats [MRID# 42651303] conducted with a single intraperitoneal injection of 150, 300, or 600 mg/kg and 24-, 48-, and 72-hour sacrifice times. Two males and one female receiving the high dose died, and clinical signs of toxicity were observed in males at all doses and in mid- and high-dose females. A separate experiment in the same study with radiolabeled Alachlor provided evidence that the test material reached the target organ when administered intraperitoneally.

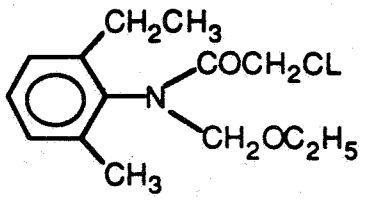
Alachlor was fed to rats and mice in the diet for up to 60 days and cell proliferation was assessed by 3H-thymidine labeling *in vivo* [MRID# 42852102]. Dose levels ranged from 0.5 to 260 mg/kg/day. Cell proliferation was seen at doses of 126 and 260 mg/kg/day in the olfactory epithelium of the nasal turbinates of rats but not mice. The response in rats was reversible. The NOEL for cell proliferation in the olfactory epithelium of the nasal turbinates in rats was 46 mg/kg/day and the LOEL was 154 mg/kg/day.

Related to these studies is a study entitled *Study of the Effects of Alachlor on Cellular Stress Response Genes in Rat Nasal Turbinate Tissue* (Monsanto Company, The Agricultural Group, Environmental Health Laboratory for Monsanto Company, Monsanto Study# ML-94-160, Monsanto EHL Study# EHL94081, February 28, 1995, EPA MRID# 43590002) where two stress response genes were examined, the heat shock protein 70 (hsp70) and the NAD(P)H: quinone oxidoreductase also known as NAD(P)H: menadione oxidoreductase1 (nmo). Long Evans rats were treated with Alachlor in the diet at 1600 to 2450 ppm (equivalent to 226 mg/kg/day) for 30 or 60 days. The respiratory and olfactory epithelia from the nasal turbinates were harvested for the examinations. The investigators found that hsp70 and nmo are expressed constitutively in nasal turbinate tissue of the Long Evans rats. Treatment of these rats with 126 mg Alachlor/kg body weight/day for 60 days caused a statistically significant induction of nmo in both respiratory and olfactory epithelia and a statistically significant induction of hsp70 in the olfactory epithelia. They state that the stress gene induction observed in Alachlor treated nasal turbinates indicates that the nasal turbinate cell are under physiological stress. Cell damage resulting from such stress may lead to increased cell turnover in these tissues, which may in turn lead to promotion of nasal tumors.

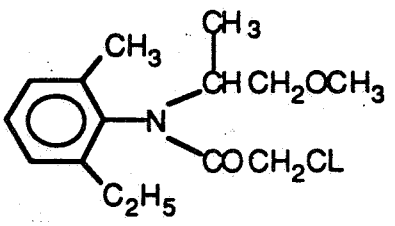
Overall, the **registrant** suggests that the submitted mutagenicity data provides evidence that Alachlor itself is not genotoxic, and that of all the Alachlor metabolites tested, few metabolites, including diethylaniline and t-hydroxysulfone demonstrate mutagenic activity. The mutagenic activity demonstrated by these metabolites occurs only at higher concentrations of test material and the data indicate only weakly positive effects in the *Salmonella* assays. The UDS activity observed occurs only at a dose approximating the LD_{50} in rats. These data are supportive of a non-genotoxic mechanism of action in induction of tumors of the nasal epithelium, gastric mucosa, and thyroid from administration of Alachlor.

F. Structure Activity Data Related to Alachlor

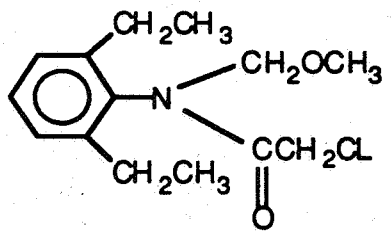
Alachlor is structurally related to Acetochlor (from two manufacturers; separate products although the same chemical structurally), Allidochlor, Butachlor, Metolachlor, Propachlor, and SAN 582H, as illustrated on the next page:



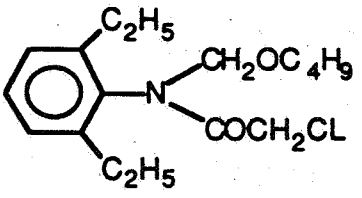
Acetochlor



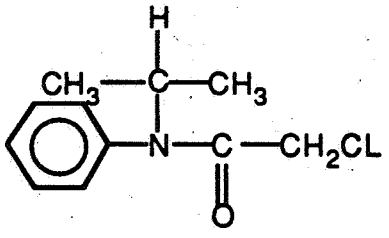
Metolachlor



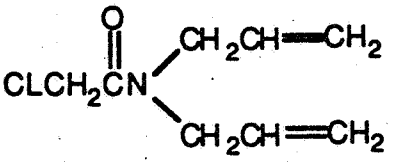
Alachlor



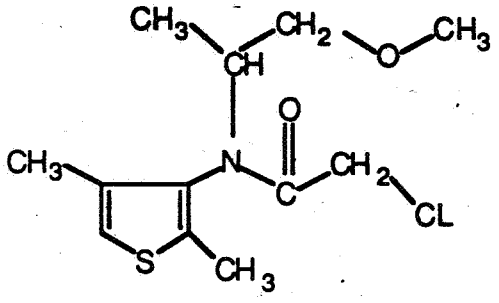
Butachlor



Propachlor

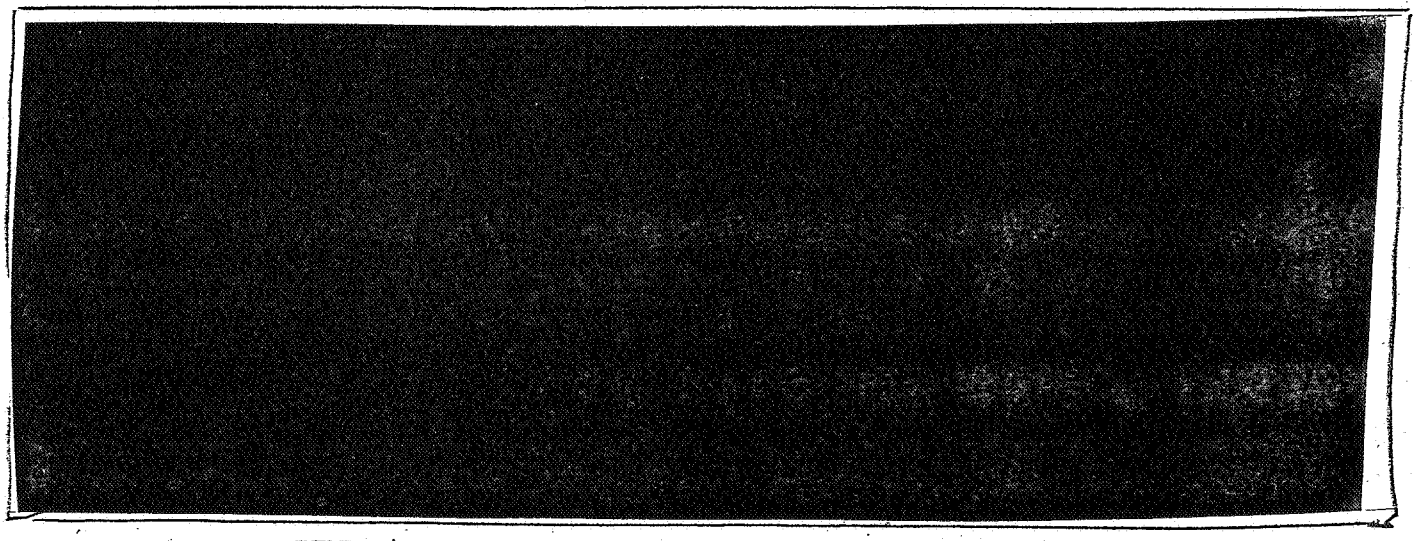


Allidochlor



SAN 582H

Acetochlor has been classified as a Group B2 Carcinogen (Probable Human Carcinogen) by the HED Carcinogenicity Peer Review Committee (CPRC), CRAVE and the Science Advisory Panel (SAP). This is based on the evidence that administration of acetochlor causes increased incidence of benign and malignant tumors at multiple sites in Sprague-Dawley rats (papillary adenomas of the nose/turbinates in both sexes; hepatocellular carcinomas in both sexes and thyroid follicular cell adenomas in males at excessively toxic dose levels). Also, increased incidence of benign and malignant tumors at multiple sites in Swiss albino CD-1 mice (hepatocellular carcinoma in both sexes; lung carcinomas in females; uterine histiocytic sarcoma and benign ovarian tumors in females; kidney adenomas in females). Acetochlor was positive for mutations in Chinese hamster ovary cells (with and without metabolic activation) and in mouse lymphoma cells (with metabolic activation). In addition, positive results were found for aberrations in cultured human lymphocytes and a weak positive response in an *in vivo/in vitro* UDS assay. Suggestive results were found in an Ames *Salmonella* assay and negative results in an *in vitro* UDS assay, an *in vivo* cytogenetics test in rat bone marrow, and a mouse micronucleus test.



PENDING REGISTRATION INFORMATION IS NOT INCLUDED

Propachlor in a two year chronic/carcinogenicity study in rats showed evidence of an increase incidence of thyroid and ovarian neoplasia; however, the study did not test at high enough levels to adequately assess the carcinogenic potential of Propachlor. Also, a carcinogenicity study in mice did not test at high enough levels to adequately assess the carcinogenicity potential. Propachlor was positive in an aberrations test in CHO cells, suggestive in a CHO/hgprt gene mutation assay, and inconclusive in an *in vivo* cytogenetics test. It was negative in a UDS assay.

Metolachlor administration in the diet to Charles River rats resulted in statistically significant increases in liver adenomas and combined liver adenomas/carcinomas in female rats. The same liver neoplasia in female rats was also observed in a separate repeat study. In male rats there was a statistically significant trend, but not pair-wise significance, for liver tumors. There was no apparent increase in tumors when Metolachlor was administered in the diet to CD-1 mice in two separate studies. Metolachlor was negative in the *Salmonella* assay, mouse lymphoma gene mutation assay, and the mouse micronucleus assay. It induced cell proliferation in hepatocytes. Based on these and other submitted data, the CPRC concluded that the classification of Metolachlor is a Group C - possible human carcinogen and that a Margin of Exposure methodology be used for estimation of human risk.

SAN 582H, in a chronic toxicity/carcinogenicity study in rats, was found to cause increased incidence of benign tumors of the liver in male rats at 700 and 1500 ppm. In female rats, benign tubular adenomas of the ovary were observed in increased incidence at 1500 ppm. In a 94 week dietary administration study in mice, no increase in the incidence of treated mice with benign or malignant tumors was observed. SAN 582H was not mutagenic in the Ames Salmonella assay, but caused positive UDS activity at dose levels well below the cytotoxic level in one study. A second UDS study showed that SAN 582H did not induce any significant increase in net nuclear grain counts. Other studies on the mutagenicity of SAN 582H (a third UDS assay, *in vitro* transformation of BALB/3T3 cells with S9 activation, and *in vitro* micronucleus test in mouse bone marrow) did not show any mutagenic effects, but were all classified as unacceptable by the Agency.

G. Weight-of-the-Evidence Considerations

1. The 1981 rat study:

The following observations were noted in male rats in this study:

i) **Significant pair-wise increase** in nasal respiratory epithelial adenomas and adenomas and/or adenocarcinomas combined observed at 42 and 126 mg/kg/day Alachlor vs control ($p < 0.01$) as well as **significant trends** for these tumor types.

ii) **Significant pair-wise increase** in malignant mixed gastric tumors and gastric adenocarcinomas and/or malignant mixed gastric tumors combined at 126 mg/kg Alachlor vs control ($p < 0.01$) as well as **significant trends** for these tumor types.

iii) **Significant pair wise increase** in thyroid follicular cell adenomas and adenomas and/or carcinomas combined at 126 mg/kg Alachlor ($p < 0.01$) as well as **significant trends** for these tumor types.

iv) **Significant trends** were observed for brain oligodendrogliomas of the hypothalamus, stomach osteosarcomas, and thyroid follicular cell carcinomas, all at $p < 0.01$. There were also **significant increasing trends** in brain ependymomas and ependymomas and/or malignant ependymomas combined, and stomach gastric adenocarcinomas, all at $p < 0.05$.

v) There were **significant differences in the pair-wise comparisons** of the 126 mg/kg/day dose group with the controls for stomach osteosarcomas, and thyroid follicular cell carcinomas, both at $p < 0.05$.

For female rats in this study, the following tumor data were reported:

i) **Significant pair-wise differences** in the incidence of nasal turbinate adenomas and adenomas and/or adenocarcinomas combined at 42 and 126 mg/kg/day Alachlor ($p < 0.01$ at 126 mg/kg/day; $p < 0.05$ at 42 mg/kg/day) as well as **significant trends** for these tumor types.

ii) **Significant pair-wise differences** in the incidence of stomach malignant mixed gastric tumors and gastric adenocarcinomas and/or malignant mixed gastric tumors combined ($p < 0.01$) at 126 mg/kg/day Alachlor, as well as **significant trends** for these tumor types. At 42 mg/kg/day, one malignant mixed gastric tumor (a rare tumor) was observed in female rats.

iii) **Significant increasing trends** in liver adenomas, stomach osteosarcomas, and thyroid follicular cell adenomas and/or adenocarcinomas combined, all at $p < 0.01$.

iv) **Significant pair-wise differences** vs control at 126 mg/kg/day Alachlor in the incidence of mammary gland adenofibromas, adenofibromas and/or fibroadenomas combined, and adenofibromas, fibroadenomas, and papillary adenocarcinomas combined ($p < 0.05$).

v) **Significant pair-wise differences** at 14 mg/kg/day in the incidence of mammary gland adenofibromas and/or fibroadenomas combined ($p < 0.05$).

Of all the tumors listed above, the **increasing trend** observed in brain oligodendrogliomas of the hypothalamus, and the **significant trend** in brain ependymomas and ependymomas and/or malignant ependymomas combined in **male** rats and the **significant pair-wise comparisons** for mammary gland adenofibromas, adenofibromas and/or fibroadenomas combined, and adenofibromas, fibroadenomas, and papillary adenocarcinomas combined and liver adenomas in **female** rats **were considered to have occurred at excessively toxic doses** and were not discussed further.

2. The 1983 rat study:

In this study, male rats were observed with the following tumor types:

i) **Significant pair-wise differences** in the incidence of nasal respiratory epithelium adenomas at 15 mg/kg/day Alachlor vs control ($p < 0.01$) as well as a significant trend for this tumor type.

Female rats in this study were observed with the following tumor types:

i) **Significant pair-wise differences** in the incidence of adrenal benign pheochromocytomas and nasal respiratory epithelium adenomas at the 15 mg/kg/day dose level ($p < 0.05$ and $p < 0.01$, respectively) as well as **significant trends** for these tumor types.

ii) **Significant pair-wise differences** in the incidence of thymus malignant lymphosarcomas at the 15 mg/kg/day dose level vs control ($p < 0.05$).

The Carcinogenicity Peer Review Committee considered the nasal respiratory adenomas in the weight-of-the-evidence determination for alachlor.

3. The 1981 mouse study:

a. Male mice had a **significant increasing trends** in bronchioalveolar adenomas at $p < 0.05$. There were **no** significant differences in the pair-wise comparisons of the dosed groups with the controls.

b. Female mice had **significant increasing trends**, in addition to **significant differences in the pair-wise comparisons** of the 260 mg/kg/day dose group with the controls, for bronchioalveolar adenomas and adenomas and/or carcinomas combined, all at $p < 0.01$.

4. The 1994 mouse study:

a. Although there were **no** significant increasing trends, male mice had **significant differences in the pair-wise comparisons** of the 16.64 and 262.40 mg/kg/day dose groups with the controls for bronchioalveolar adenomas and adenomas and/or carcinomas combined, all at $p < 0.05$.

b. There were also **significant differences in the pair-wise comparisons** of the 65.42 mg/kg/day dose group with the controls for bronchioalveolar adenomas and adenomas and/or carcinomas combined, both at $p < 0.01$.

c. There were **no** significant compound-related tumors observed in female mice.

5. Mutagenicity

a. Alachlor was classified as weakly genotoxic by the CPRC based on previously submitted mutagenicity studies.

b. Recently submitted studies showed weak positive responses of two Alachlor metabolites in the Ames salmonella assay, and a weak positive response of Alachlor in a UDS assay in Fischer-344 rat hepatocytes at 1000 mg/kg, a dose approximating the LD_{50} in rats. It is noted that the UDS activity observed occurred at a dose in which significant hepatotoxicity was also observed.

c. Low level binding to rat nasal DNA was observed after oral administration of 123 mg/kg, but the results of this study were inconclusive.

6. Cell Proliferation/Protein Binding

a. In a study measuring cell proliferation in nasal mucosa, liver, thyroid, and glandular stomach in Long-Evans rats, Alachlor produced a dose-related increase in cell proliferation in nasal mucosa, but cell proliferation in other tissues was observed only at the highest dose levels tested. The effect was reversible with cessation of treatment. No cell proliferation was observed in nasal mucosa of CD-1 mice.

b. Tissue glutathione levels were monitored in the liver, brain, blood, eyes, thyroid, nasal mucosa, and stomach following oral, and intraperitoneal doses of Alachlor (0-700 mg/kg) to rats. Depletion of hepatic and stomach glutathione was observed at 350 mg/kg i.p. followed by recovery in the liver, while stomach glutathione was decreased in a time-related fashion. Oral administration of Alachlor resulted in liver and stomach glutathione depletion at 2 hours post-dose at 126 and 350 mg/kg. Dietary administration of Alachlor up to 126 mg/kg had no detrimental effect on tissue glutathione levels.

c. Female Long-Evans rats fed Alachlor in the diet at 126 mg/kg/day and sacrificed on days 1, 3, 7, and 13 showed Alachlor binding to nasal tissue proteins. The level of binding correlated with the length of treatment. The adduct was identified as the 3, 5-diethylbenzoquinone-4-imine cysteine adduct, consistent with the role of 2,6-diethyl-aniline in the formation of nasal tumors.

d. Acid phosphatase was used as a marker for cytotoxicity to nasal tissue following in vitro administration of Alachlor, diethylaniline, secondary sulfide, and secondary amide to rat nasal turbinate preparations. Alachlor and diethylaniline were observed to increase acid phosphatase levels in rat nasal tissue preparations. This observation is consistent with the cell proliferation response observed in nasal tissue after Alachlor administration.

The data on mutagenicity and cell proliferation as a whole suggest that while genotoxic species of Alachlor may be produced, such genotoxic activity is observed at doses of Alachlor in which GSH depletion and/or saturation of protein binding has occurred. This conclusion is supported from the submitted data in which GSH and protein reaction with Alachlor and/or Alachlor metabolites is observed at lower concentrations than the genotoxic activity.

7. Structure-Activity Relationships

Alachlor is structurally related to Acetochlor (Group B2), Butachlor (not classified), Metolachlor (Group C), and SAN 582H (Group C).

8. Conclusions of the CPRC regarding the proposed mechanism of nasal, gastric, and thyroid tumor formation from Alachlor:

The Health Effects Division Carcinogenicity Peer Review Committee has reviewed the data contained within the Peer Review Document with respect to the proposed mechanism(s) for induction of nasal, gastric, and thyroid tumors as presented. As previously noted, nasal tumors are believed to be the result of formation of the benzoquinone imine metabolite of Alachlor, binding of the imine to cellular protein, and subsequent cell damage with compensatory increase in cell turnover and eventual production of benign tumors. The Long-Evans rat has been demonstrated to be highly sensitive to production of nasal tumors, based on the ability to form this metabolite of Alachlor and localization of this metabolite to the nasal tissue. The CPRC concluded that the data in support of the mechanism for the nasal turbinates is indicative of a rat specific response. Although the rat and human were recognized to possess the same enzyme(s) involved in production of the putative toxic species from Alachlor, it was also recognized that the activity of these enzymes was substantially greater in the rat compared to the human. Thus, the

model of rat nasal tumorigenesis may not be relevant for human cancer assessment.

Thyroid tumors have been proposed to be the result of induction of hepatic glucuronyl transferase with subsequent decrease in circulating T3 and T4, a subsequent increase in TSH, and eventual hyperplastic response of the thyroid. The mechanistic data and tumor type meets the criteria established by the Agency and the use of the MOE approach for human cancer assessment is consistent with Agency policy.

Stomach tumors from Alachlor administration are believed to result from mucosal toxicity leading to atrophy of the mucosa and parietal cells resulting in hypochlorhydria, increased stomach pH, and compensatory increase in gastrin levels. Gastrin is known to exert a trophic effect on fundic mucosal cells. Under these conditions, a proliferative response is initiated, resulting in tumor formation. The CPRC stated that this is a direct contact effect, non-genotoxic mechanism which parallels human pathological conditions (chronic atrophic gastritis and Zollinger-Ellison syndrome; see Appendix). These tumors result from an indirect response to change in pH (decrease). At doses below which gastric pathology is observed, one would not expect a tumorigenic response. The use of the MOE approach for human cancer assessment is consistent with Agency policy.

9. Margin of Exposure Values:

A no-observed-effect-level [NOEL] of 14 mg/kg/day, and a lowest observed effect level [LOEL] of 42 mg/kg/day were selected on the basis of increased incidence of malignant mixed gastric tumors (a rare tumor). These doses are to be used for the M.O.E. calculations. The above doses were selected based on the relevance of the gastric tumors to humans and in the absence of information on pre-neoplastic gastric lesions (mucosal atrophy was noted in the mechanistic study). For comparison purposes, the Reference Dose (RfD) for Alachlor is 0.01 mg/kg/day usihg the 1 year feeding study in dogs with a NOEL of 1 mg/kg/day and a LOEL of 3 mg/kg/day based on increased incidence of hemosiderosis and hemolytic anemia with an uncertainty factor of 100.

The following are the endpoints, NOEL's and LOEL's in mg/kg/day used in selecting a value to be used in the calculations of **MOE's** for evaluating Alachlor:

STUDY	ENDPOINT	NOEL(mg/kg/day)	LOEL(mg/kg/day)
Neoplastic endpoints			
2 year rat(1981)	nasal respiratory epithelium adenomas & adenomas &/or carcinomas	14	42
	malignant mixed gastric tumors	14	42
	thyroid follicular cell adenoma & adenomas &/or carcinomas	Males=42	Males=126
2 year rat(1983)	nasal respiratory epithelium adenomas	2.5	15
79 week mouse(1981)	bronchioalveolar adenomas & adenomas &/or carcinomas	Females=78	Females=260
79 week mouse(1994)	bronchioalveolar adenomas & adenomas &/or carcinomas		Males=<16.64 (no trend)
Nonneoplastic endpoints			
2 year rat(1981)	mortality	42	126
2 year rat(1981)	decreased body weight gain	Males=14 Females=42	Males=42 Females=126
2 year rat(1981)	decreased water consumption	Females=42	Females=126
2 year rat(1981)	uveal degeneration syndrome	Males=14	Males=42
2 year rat(1981)	cataracts	42	126
2 year rat(1981)	retinal degeneration	42	126
2 year rat(1981)	iris atrophy	42	126
2 year rat(1981)	increased liver weights	42	126
2 year rat(1981)	increased spleen weights	42	126
2 year rat(1981)	mediastinal & mesenteric lymph nodes-plasma cell hyperplasia	Females=42	Females=126
2 year rat(1981)	thyroid-follicular atrophy	Males=14 Females=42	Males=42 Females=126
2 year rat(1981)	periportal hepatocyte hypertrophy	14	42
2 year rat(1981)	centrolobular hepatocyte necrosis	42	126
2 year rat(1983)	submucosal gland hyperplasia (nasal)	2.5	15.0

STUDY	ENDPOINT	NOEL(mg/kg/day)	LOEL(mg/kg/day)
Nonneoplastic endpoints			
2 year rat(1983)	inflammation of nasal passages	2.5	15.0
2 year rat(1983)	retinal pigment degeneration	2.5	15.0
2 year rat(1983)	mortality	Females=2.5	Females=15.0
2 year rat(1983)	abnormal disseminated liver foci	Males=2.5	Males=15.0
2 year rat(1983)	myocardial necrosis/scar	Females=2.5	Females=15.0
2 year rat(1983)	kidney-mononuclear infiltration	Females=2.5	Females=15.0
2 year rat(1983)	kidney-tubular sclerosis	Females=2.5	Females=15.0
2 year rat(1983)	adrenal cortical telangiectasis	Females=2.5	Females=15.0
18 month mouse(1981)	mortality	Females=78	Females=260
18 month mouse(1981)	decreased body weight gain	Females=78	Females=260
18 month mouse(1981)	increased liver weights	78	260
18 month mouse(1981)	increased kidney weights	Males=78	Males=260
18 month mouse(1981)	kidney-chronic interstitial fibrosis	78	260
18 month mouse(1994)	decreased body weight gain	Males=65.42 Females=90.34	Males=262.40 Females=399.22
18 month mouse(1994)	decreased food efficiency	Females=90.34	Females=399.22
18 month mouse(1994)	mass/nodule of the liver	Males=16.64 Females=90.34	Males=65.42 Females=399.22
18 month mouse(1994)	mass/nodule of the lung		Males=<16.64
18 month mouse(1994)	increased liver weights	Males=65.42	Males=262.40
18 month mouse(1994)	increased kidney weights		Males=<16.64
18 month mouse(1994)	kidney hyperplasia (tubular epithelium) & nephritis	Males=65.42	Males=262.40
18 month mouse(1994)	hepatocellular hypertrophy (centrilobular)	Males=16.64	Males=65.42
18 month mouse(1994)	fibrous osteodystrophy of the sternum	Females=90.34	Females=399.22

STUDY	ENDPOINT	NOEL(mg/kg/day)	LOEL(mg/kg/day)
Nonneoplastic	**endpoints**		
1 year dog	hemosiderosis & hemolytic anemia	1	3
Reproductive-rat	parental & pup systemic toxicity-increased kidney weights, discoloration	10	30
Developmental-rat	increased mortality, increased post-implantation loss, reduced # live fetuses, decreased fetal body weights	150	400
Develop-rabbit	decreased maternal body weight gain	100	150

H. Proposed Reclassification of the Carcinogenic Potential of Alachlor:

Upon consideration of all of the submitted data regarding the carcinogenicity potential of Alachlor and in consideration of the full weight-of-the-evidence, the Health Effects Division Carcinogenicity Peer Review Committee could not reach a consensus as to the classification of Alachlor as a carcinogen. Therefore the CPRC recommended to defer the classification of Alachlor as a carcinogen and reconsider the classification in the near future using the new Cancer Assessment Guidelines when such guidelines are in effect. In addition, the CPRC recommended not to utilize the linear low dose approach, but to utilize the Margin of Exposure (MOE) methodology for the estimation of human risk. The CPRC concluded that the data in support of the mechanism for the nasal turbinates is indicative of a rat specific response. Although the rat and human were recognized to possess the same enzyme(s) involved in production of the putative toxic species from Alachlor, it was also recognized that the activity of these enzymes was substantially greater in the rat compared to the human. Thus, the model of rat nasal tumorigenesis may not be relevant for human cancer assessment. Thyroid tumors have been proposed to be the result of induction of hepatic glucuronyl transferase with subsequent decrease in circulating T3 and T4, a subsequent increase in TSH, and eventual hyperplastic response of the thyroid. The mechanistic data for thyroid tumor formation meet the criteria established by the Agency and the use of the MOE approach for human cancer assessment is consistent with Agency policy. The CPRC stated that the stomach tumor formation is a direct contact effect, non-genotoxic mechanism which parallels human pathological conditions. These tumors result from an indirect response to change in pH. The use of the MOE approach for human cancer assessment is consistent with Agency policy.

APPENDIX 1:

IMPLICATIONS OF GASTRIC TUMORS IN RODENTS TO HUMANS

An important question to be answered regarding the finding of gastric tumors in Alachlor dosed rodents is whether the animal tumor model is qualitatively referable to humans. If it is, one needs to evaluate the potential mode of action and evaluate potential risks. If the animal model is not relevant to humans, then effects would be deemed specific to animals. Certain observations aid in this determination.

Alachlor and its structural analogue Butachlor have both been reported to produce a number of different tumors of the glandular stomach in rats. However, in-depth analysis on Butachlor indicates that most if not all of the tumors appear to be poorly differentiated carcinoids of the fundus, neoplasms of enterochromaffin-like (ECL) cells that normally produce histamine (Hard et al., 1995). In rats, these carcinoid tumors appear to be the result of effects on the gastric mucosa with loss of hydrogen ion-producing parietal cells (Thake et al., 1995). This leads to a significant elevation of gastric pH, which results in stimulation and proliferation of gastrin secreting (G) cells in the pylorus (antrum). Increases in circulating gastrin ensue which stimulates ECL cells to proliferate and produce histamine. Normally, the histamine would stimulate parietal cells to produce more acid and reestablish gastric pH balance. However, under chronic Alachlor stimulation, cellular proliferation of ECL cells predominates with the ultimate formation of carcinoid tumors.

Humans have anatomical structures and cell types as do rodents for the production and regulation of gastric acidity (Wolfe & Soll, 1988). Aberrations in gastric functioning are often associated with peptic ulcer disease. Treatment includes the use of agents that reduce acidity, including hydrogen ion pump inhibitors (e.g., omeprazole) and H2-histamine antagonists (e.g., Ranitidine) (Feldman & Burton, 1990; Soll, 1990). These compounds plus some other agents, like phenoxyisobutyrate hypolipidemics [antihyperlipidemics](e.g., Clofibrate) have produced carcinoid tumors in rodents (Havu et al., 1990; Ekman et al., 1985; Poynter & Selway, 1991), seemingly associated with a block in gastric acid secretion and stimulation of G cells and then ECL cells to produce the tumors. Widespread clinical experience, albeit still somewhat limited, has not demonstrated a linkage between the use of the drugs and the development of carcinoids or an increase in precursor lesions of those tumors (Bardram et al., 1986; Helander, 1986; La Vecchia et al., 1990; Sölvell, 1990).

There are, however, two diseases in humans where increases in carcinoids have been found. In persons with chronic atrophic gastritis, there is a significant loss of parietal cells and chief cells from the body of the stomach with loss of their attended secretions (i.e., acid, pepsinogen). Some of these patients also have pernicious anemia, with the absence of intrinsic factor which normally aids in the absorption of vitamin B12 from the gut, and megaloblastic anemia. Achlorhydria develops, and the normal acid-secreting response to food is blunted. Intestinal metaplasia, a premalignant lesion, forms with replacement of normal gastric mucosa with an epithelium like that of the intestine (goblet cells). Associated with the elevated gastric pH, G cells commonly proliferate to form clusters of cells (micronodules), not only in the fundus where they are normally distributed, but also in the body of the stomach (Rubin, 1969; Creutzfeldt et al., 1971). Rarely, G cell proliferation seems to progress to gastrinomas (Russo et al., 1980). In addition, a proportion of chronic gastritis patients go on to develop stomach cancer: carcinoids associated with hypergastrinemia and adenocarcinomas, probably developing from areas of intestinal metaplasia (Carney et al., 1983; Borch et al., 1985; Sjöblom et al., 1988).

Patients with the Zollinger-Ellison syndrome have a tumor of gastrin secreting cells (gastrinoma), often in the pancreas, but also at other sites. These people have hypergastrinemia and severe and unrelenting peptic ulcers. The ulcers are due to the over stimulation of ECL cells to release histamine, with the subsequent stimulation of parietal cells to hypersecrete hydrochloric acid (Wolfe & Jensen, 1987). Patients regularly develop hyperplasia of ECL cells, sometimes of a pronounced degree, and rare cases of gastric carcinoid have also been reported (Bardram et al., 1986).

These experiences with chronic atrophic gastritis and Zollinger-Ellison syndrome point out that humans can develop gastric carcinoid tumors associated with hypergastrinemia. Only the chronic atrophic gastritis patients also manifest elevations in gastric pH, as do the rats treated with Alachlor/Butachlor and the peptic ulcer medications. It seems that humans, like rodents, can respond to reduced acid secretion with proliferation and hyperfunction of both G cells and ECL cells and with ECL hyperplasia sometimes resulting in carcinoid formation. It is interesting that chronic atrophic gastritis patients are also noted to develop adenocarcinomas of the stomach. A few adenocarcinomas were originally reported in the Alachlor and Butachlor chronic rat studies but, as stated previously, probably these fundic tumors are actually carcinoids. In addition, there

is no report of an increase in intestinal metaplasia in the rodent studies as there is in the gastritis patients (G. Hard, personal communication, November 22, 1995).

Some have questioned whether data on carcinoid formation in rodents from pharmaceuticals used for peptic ulcer therapy are referable to humans. These agents are designed to raise the pH of the stomach so that pepsinogen is not activated. It is stated that the relationship between pH elevation, G cell stimulation, hypergastrinemia and ECL cell hyperplasia and neoplasia (carcinoid) is incomplete and may be potentially misleading, as other hypotheses are tenable for tumor induction (Wormsley, 1988). For instance, gastric pH above 3 is associated with the presence of bacteria in the stomach and intestine that would not normally be there (Drasar et al., 1969; Cook, 1985). Some bacteria can reduce nitrates and nitrites to form nitrosamines which may induce gastric cancers. MNNG is one such compound which is carcinogenic in the rodent stomach, although it has produced adenocarcinomas of the body of the stomach, not fundic carcinoids. Then again, various chemicals such as proton pump inhibitors may have direct stimulatory effects on ECL cells or may have mutagenic activity that may aid in the carcinogenic process (Penston & Wormsley, 1987; Wormsley, 1988). Regardless of how the investigations on the anti-ulcer drugs unfold, there is still indication that humans have many of the same gastric responses as do rodents, and two human diseases with hypergastrinemia (with or without increases in gastric pH), are associated with carcinoid of the stomach. Thus, it seems that one cannot reject the animal model in the case of Alachlor: the carcinoids of the stomach in rodents may portend some carcinogenic hazard potential for humans.