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

JUN 26 1987

MEMORANDUM

OFFICE OF
PESTICIDES AND TOXIC SUBSTANCES

Subject: Peer Review of Alachlor - Reconsideration of Classification

From: Judith W. Hauswirth, Ph.D. Judith W. Hauswirth
Acting Section Head, Section VI 5/18/87
Toxicology Branch/HED (TS-769C)

To: Robert Taylor
Product Manager #25
Fungicide - Herbicide Branch
Registration Division (TS-767C)

David Giamporcaro
Special Review Branch
Registration Division (TS-769C)

The Toxicology Branch Peer Review Committee met on April 15, 1987 to reconsider the classification of Alachlor as a B_2 oncogen in light of the conclusions of the Science Advisory Panel (SAP) (November 19, 1976) and the registrant's rebuttal to the Agency's Position Document 2/3.

A. Individuals in Attendance:

1. Peer Review Committee: (Signatures indicate concurrence with peer review unless otherwise stated).

Theodore M. Farber — Theodore M. Farber
Reto Engler — Reto Engler
Louis Kasza — Louis Kasza
Judith W. Hauswirth — Judith W. Hauswirth
William Marcus — William Marcus
Gary Burin — Gary Burin
Robert Beliles — Robert Beliles
Donald Barnes — Donald W. Barnes

2. Peer Review Members in Absentia: (Committee members who were not able to attend the discussion; signatures indicate concurrence with the overall conclusions of the Committee.)

3/312

John A. Quest

Esther Rinde

Anne Barton

William Burnam

Diane Beal

B. Material Reviewed:

The following material was made available to the Committee for review:

1. Toxicology Branch Peer Review Report on Alachlor (meeting of 3/25/86 and report dated 5/20/86, copy appended);

2. Report of Panel Recommendations (SAP report dated 11/25/87);

3. Partial transcript of SAP meeting (11/19/86); and

4. Comments in Reply to EPA's Federal Register Notice of October 8, 1986. The Alachlor Special Review Technical Support Document dated September 1986 (submission by the registrant).

C. Background Information:

On March 25, 1987 the Toxicology Branch Peer Review Committee met to discuss and evaluate the weight-of-the-evidence on alachlor, with particular reference to its classification by the Agency as a B_2 oncogen in the Special Review Position Document 1 (December 1984). After considering the criteria in the EPA Guidelines for classifying a carcinogen, the Committee concurred with the original classification concluding that:

> Alachlor met all but one of the criteria specified for the B-2 classification, any of which alone can be sufficient for such a classification. 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 another experiment 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 dose levels.

The SAP upheld the B_2 classification but felt that the mouse study was not positive for oncogenicity. They concluded that alachlor was a B_2 oncogen since it produced "an unusual type of neoplasm [nasal turbinate tumors] in the

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PEER REVIEW FILES

CHEMICAL NAME: Alachlor
CASWELL NO.: 011
CAS NO.: 15972-60-8
REVIEWER: Hauswirth

CURRENT AGENCY DECISION

B2; 8 x 10-2

TUMOR TYPE / SPECIES

Nasal; stomach, thyroid,
thymus; LE rats (M and/or F).
Lung; CD-1 mice (F).

REVIEWER PEER REVIEW PACKAGE	PEER REVIEW MEETING DATE	PEER REVIEW DOCUMENTS	PEER REVIEW CLASSIFICATION
5. / /	5. / /	5. / /	5.
4. / /	4. / /	4. / /	4.
3. / /	3. / /	3. / /	3.
2. 03/30/87	2. 04/15/87	2. 06/26/87	2. B2; 8 x 10-2
1. 03/17/86	1. 03/25/86	1. 06/26/86	1. B2

SAP MEETING	SAP CLASSIFICATION
2. / /	2.
1. 06/26/86	1. B2

QUALITATIVE/QUANTITATIVE RISK ASSESSMENT DOCUMENT	GENETIC TOXICITY ASSESSMENT DOCUMENT
2. / /	1. / /
1. 11/09/87	

MISCELLANEOUS:

Newspaper clippings: 03/16/89 and 11/25/87.
Stamped 2/1/90; #PR-007699; 285 p.; mha.

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Peer Review Documents
(Memo dates)

rat, coupled with the finding that two metabolites of alachlor are mutagenic." They further stated that "the data available clearly meet the criteria for a B_2 classification."

D. Reevaluation of Classification:

The Committee was asked to address the following points which summarize the registrant's conclusion that alachlor should be reclassified as category C oncogen:

1. Lack of oncogenicity in multiple species (since the mouse study was considered negative by the SAP);

2. Questionable malignant tumor response in multiple experiments (nasal turbinate tumors were mostly benign);

3. Lack of unusual degree, site, type or early onset (at doses below the MTD, there was not an unusually high incidence of nasal turbinate tumors; nasal turbinates were not routinely examined at the time of the alachlor study); and

4. Alachlor is not a genotoxic oncogen and there are species differences in its metabolism.

Point #1:

Both the SAP and the registrant felt that the mouse study was negative for oncogenicity since the incidence of lung tumors in female mice was within the historical control range for this strain of mouse as reported by Sher (Toxicology Letters 11: 103-110, 1982). The average incidence of lung tumors, as cited in this paper in CD-1 female mice is 17% with a range of 0-41%. The incidence of lung tumors at the high dose in the alachlor study was 22%. The SAP also stated that this conclusion was supported "by the lack of evidence of progression from benign to malignant tumors, and the lack of an increase in tumor multiplicity in treated mice".

The Committee disagreed with both the SAP and the registrant on this point. They felt that historical control data derived from the literature was at best tertiary information for consideration and that concurrent control data should be primarily relied upon followed by contemporaneous data from the conducting laboratory. They concluded that the mouse study was positive for oncogenicity since:

1. The incidence of lung tumors was significantly ($p < 0.05$) increased at the high dose in female mice;

2. The incidence of lung tumors in female mice that died in extremis was significantly ($p < 0.01$) induced indicating early onset; and

3. Historical control data from the performing laboratory (Bio/dynamics on studies that were conducted for at least 5-6 months longer than the alachlor study indicated that the incidence of lung tumors at the high dose (22%) was just within the historical range (0-23%). The spontaneous incidence of lung tumors is known to increase significantly with age. Therefore, it would not be

unexpected that the tumor incidence in the alachlor study would be ~~within~~ outside the historical control range of studies conducted for 18 months at Bio/dynamics.

Point #2:

The registrant claims that the nasal turbinate tumors, induced by alachlor, were mostly benign, especially at dosages which they considered to be at or below the maximum tolerated dose (42 mg/kg/day) (MTD).

The Committee agreed that at 15 and 42 mg/kg/day of alachlor, the nasal turbinate tumors were mostly benign since only two carcinomas (1 male and 1 female) were found, both at 42 mg/kg/day. However, at 126 mg/kg/day malignant nasal turbinate tumors were induced indicating that this tumor type progresses to malignancy.

Point #3:

The registrant argues that the nasal turbinate tumors were not induced to an unusual degree at dosages at or below the MTD, nor are they rare tumors especially since this tumor type was not routinely looked for at the time of the alachlor study and would not be considered uncommon today.

The Committee noted that the registrant submitted no data to support their contention that nasal turbinate tumors are no longer considered rare tumors or that they occur spontaneously in Long-Evans rats.

The Committee also reconsidered their determination of the MTD in the two alachlor studies in rats. In the high dose study (0, 14, 42 and 126 mg/kg/day), they originally concluded (see atached Peer Review Report of 5/20/86) that each of these doses exceeded the MTD. However, upon reconsideration they felt that in this study, based upon increased mortatlity, 42 mg/kg/day approximated the MTD in females and 14 mg/kg/day in males. In the low dose study (0, 0.5, 2.5 and 15 mg/kg/day), they originally concluded that the MTD was exceeded at 15 mg/kg/day. Upon reexamination of the mortality data upon which this decision was made, the Committee felt that they had erred and there was no evidence from the results of the study that 15 mg/kg/day even approached an MTD. They concluded that 42 mg/kg/day best approximated an MTD for alachlor.

Point #4:

The registrant claims that alachlor is not a genotoxic oncogen since alachlor was not mutagenic in several short-term assays. The Committee agrees that the weight-of-the-evidence indicates that alachlor, itself, is not a mutagen. However, two metabolites of alachlor, both identified in rat, were mutagenic in the Ames assay. These two metabolites are N-2-ethyl-6-(1-hydroxyethyl)-phenyl-2-(methylsulfonyl) acetamide and N-[2-ethyl-6-(1-hydroxyethyl)-pheny]-N-(methoxymethyl) acetamide.

The registrant also claims that the monkey is a better model than the rat for determining the oncgenic potential of alachlor in man. The Committee noted that the registrant has identified one of the mutagenic metabolites of alachlor in monkey urine, as well as rat, and that without any evidence on the oncogenicity of alachlor in the monkey, they must rely upon rodent data to

make a determination.

E. Conclusions on the Reevaluation:

The Committee felt upon reconsideration of the available data and review of the registrant's arguments and the SAP's decision, that alachlor should be classified as a B_2 oncogen (probable human carcinogen), corroborating their initial decision. They further felt that the conclusions reached in their initial review still stood, that is that administration of alachlor was associated with an increased incidence of benign and maligant tumors in male and female rats in multiple experiments to an unusual degree and at an unusual site (nasal turbinates) and of benign lung t rs in female CD-1 mice. These conclusion meet all three criteria of a Category B_2 classification, any one of which is sufficient for classification in this category.

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MEMORANDUM

OFFICE OF
PESTICIDES AND TOXIC SUBSTANCES

SUBJECT: Peer Review of Alachlor

FROM: Esther Rinde, Ph.D. E. Rinde 5/27/86
Scientific Mission Support Staff
Toxicology Branch/HED (TS-769c)

TO: Robert Taylor
Product Manager #25
Fungicide-Herbicide Branch
Registration Division (TS-767c)

The Toxicology Branch Peer Review Committee met on March 25, 1986 to discuss and evaluate the weight-of-the-evidence on Alachlor, with particular reference to consideration of whether there is agreement on its classification as a B-2 carcinogen.

A. Individuals in Attendance:

1. Peer Review Committee: (Signatures indicate concurrence with peer review unless otherwise stated).

Theodore M. Farber	Theodore M. Farber
Reto Engler	
Louis Kasza	
Bertram Litt	
Gary Burin	
Laurence Chitlick	
Bruce Means	
William Marcus	
Robert Belliles	
Esther Rinde	

A. Individuals in Attendance (continued)

2. Reviewers: (Non-panel members responsible for data presentation; signatures indicate technical accuracy of panel report.)

Judith Hauswirth — Judith W. Hauswirth

3. Peer review members in absentia: (Commitee members who were not able to attend the discussion; signatures indicate concurrence with the overall conclusions of the Committee.)

John A. Quest — John A. Quest

Richard Hill — [signature]

Stephen Johnson — [signature]

Anne Barton — [signature]

B. Material Reviewed:

The material available for review consisted of the following:

A. DER: A chronic Feeding study of Alachlor in Rats. Bio/Dynamics.

B. DER: A chronic Study of Alachlor Administered in feed to Long-Evans Rats. Monsanto Environmental Health Laboratory.

C. DER: A Special Chronic Feeding Study with Alachlor in Long-Evans Rats. Monsanto.

D. Tumor incidence table for the three rat studies combined; also DER on Monsanto's reevaluation of submucosal gland hyperplasia seen in study reviewed under Part 5.b. of Peer Review Memo (3/17/86).

E. DER: An 18 Month Oncogenic Study in Mice. Bio/dynamics.

F. Sher,S.P., R.D. Jensen and D.L. Bokelman. Spontaneous Tumors in Control F344 and Charles River CD Rats and Charles River CD-1 and B6C3HF1 Mice. Toxicology Letters 11: 103-110, 1982.

G. Homburger,F., A.B. Russfield, J.H. Weisburger, S. Lim, S.P. Chak and E.K. Weisburger. Aging Changes in CD-1 HaM/IC.: Mice reared Under Standard Laboratory Conditions. J. Natl. Cancer Inst. 55: 37-45, 1975.

H. Historical Control Data from Bio/dynamics on Lung Tumors and Liver Tumors in CD-1 Mice.

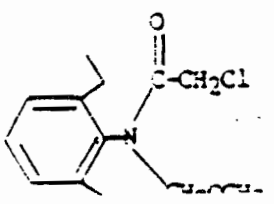
I. Table: Q_1^* Potency Estimates for Alachlor Based on Rat Tumor Data (from the PD-1).

A copy of the information reviewed is appended to this panel report.

C. Background Information:

Alachlor (2-chloro-2'6' diethyl-N-(methoxymethyl)-acetanilide) is registered for use as a selective herbicide for the control of many preemergent broadleaf weeds and grasses. In December 1984, a Special Review Position Document 1 was issued on alachlor, in which the Agency concluded Alachlor is a class B_2 oncogen based on the proposed EPA Guidelines, and that "the weight of the evidence demonstrates that alachlor is oncogenic to laboratory animals and, in the absence of data on humans, it is prudent to treat alachlor as a probable human carcinogen".

A Special Review Position Document 2,3 (PD 2,3) is now being prepared on alachlor; it was felt that it would be beneficial at this time to reevaluate alachlor through the peer review process prior to issuing the PD 2,3.



D. Evaluation of Oncogenicity Evidence for Alachlor:

1. A Chronic Feeding Study of Alachlor in Rats:

Bio/dynamics administered alachlor (Lasso Technical) in the diet to groups of 50 male and 50 female Long-Evans rats at concentrations of 0, 100, 300, or 1000 ppm (0, 14, 42 and 126 mg/kg/day, respectively) for 812 to 813 days (males) and 741 to 744 days (females). Two different lots of the technical alachlor were used during the study: Lot #XHI-167, stabilized with 0.5% epichlorohydrin* (for the first 11 months of the study) and Lot #MHK-6, [redacted] (for the remainder of the study). The following incidence of tumors were observed.

STUDY # 1

Tumor Site and Type	Sex	Dose (mg/Kg/day) 0	14	42	126
Stomach:					
leiomyosarcoma	M	0/49	0/50	0/50	1/50
	F	0/50	0/50	0/50	1/49
osteosarcoma	M	0/49	0/50	0/50	3/50
	F	0/50	0/50	0/50	4/49
gastric adenocarcinoma	M	0/49	0/50	0/50	2/50
	F	0/50	0/50	0/50	1/49
malignant mixed gastric tumor	M	0/49	0/50	0/50	11/50
	F	0/50	0/50	1/50	17/49
Thyroid:					
follicular adenoma	M	1/48	0/50	1/49	11/50
	F	0/49	0/44	2/46	2/49
follicular carcinoma	M	0/48	0/50	0/49	2/50
	F	0/49	0/44	0/46	2/49
Nasal Turbinates respiratory epithelium:					
adenomas	M	0/46	0/46	10/41	23/42
	F	0/49	0/47	4/42	10/48
carcinomas	M	0/46	0/46	1/41	0/42
	F	0/49	0/47	1/42	0/48

*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.

Nasal turbinate tumors (mainly benign) were significantly increased in both males ($p<0.001$) and females ($p<0.02$) at the mid dose level (42 mg/kg/d) and above.

Stomach malignant tumors increased significantly ($p<0.001$) in both sexes at the high dose level.

Thyroid follicular tumors (adenomas and carcinomas) were significantly increased in males at the high dose level ($p<0.001$).

The lowest dose of alachlor tested in this study probably exceeded a MTD as evidenced by high mortality, compared to controls. Increases in organ weights (liver, kidney, spleen, et al.) were also noted, as were gross findings, at all dose levels, indicative of a compound related effect.

2. A Chronic Feeding Study of Alachlor in Rats:

Monsanto administered technical alachlor (94.13%) in the diet to groups of 50 male and 50 female Long-Evans rats at concentrations of 0, 0.5, 2.5 and 15.0 mg/kg/day for 25 to 26 months. The alachlor was stabilized with [redacted] Epichlorohydrin was not used as a stabilizer. The following incidence of tumors/lesions was observed.

		STUDY #2			
		Dose (mg/kg/day)			
Tumor Type and Site	Sex	Control 0	Low 0.5	Medium 2.5	High 15.0
Thyroid					
follicular					
adenoma	M	2/49	4/50	3/49	4/49
	F	1/49	1/49	0/49	2/47
carcinoma	M	1/49	0/50	1/49	2/49
	F	3/49	1/49	1/49	1/49
Thymus					
lymphosarcoma	M	0/49	0/50	1/46	0/50
	F	0/48	1/50	2/48	3/43
Adrenal —					
pheochromocytoma					
benign	M	8/50	7/50	2/50	6/50
	F	1/49	1/50	3/50	5/49
malignant	M	2/50	2/50	0/50	2/50
	F	1/49	0/50	0/50	0/49
Nose Turbinates					
respiratory epithelium					
adenoma	M	0/45	0/48	0/45	1/45
	F	0/42	0/44	1/47	3/48
neurofibroma	M	0/45	1/48	0/43	0/45
	F	0/42	0/44	0/47	0/48
submucosal gland					
adenoma	M	0/45	0/48	1/45	0/45
	F	0/42	0/44	0/47	0/48
epith. hyperplasia/					
metaplasia	M	1/45	1/48	1/45	1/45
	F	0/42	0/44	0/47	2/48
submucosal gland					

INERT INGREDIENT INFORMATION IS NOT INCLUDED

Nasal turbinate tumors were significantly elevated ($p<0.01$) in both males and females at 15 mg/kg/day (the highest dose tested). One female rat in the mid dose group also had this tumor and one male in this group had a submucosal gland adenoma.

Thymus lymphosarcomas and adrenal pheochromocytomas were significantly increased ($p<0.05$) in the high dose females.

There was a non-significant increase in thyroid follicular cell tumors in the high dose male group.

> The highest dose tested probably exceeded a 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.)

Monsanto was requested to reevaluate the submucosal gland hyperplasia seen in both males and females. Experimental Pathology Laboratories, Inc. (EPL) performed a histological reevaluation; their report indicated that the submucosal nasal lesions (hyperplasia) were not neoplastic, however their analysis reflected a slightly higher incidence of adenomas of the nasal cavity. EPL's diagnosis is compared with that of Monsanto in the table below.

	Nasal turbinate adenomas			
	EPL's data		Monsanto's data	
Group (mg/kg/day)	Males	Females	Males	Females
0	0/44	0/42	0/45	0/42
0.5	0/47	0/42	0/48	0/44
2.5	0/44	1/47	0/45	1/47
15.0	15/45	14/48	11/45	9/48

3. A Special Chronic Feeding Study With Alachlor

In a study performed by Monsanto, alachlor was administered in the diet to Long-Evans rats at a concentration of 126 mg/kg/day. After a period of exposure (5-8 months) sufficient to induce ocular lesions (as confirmed by the consulting ophthalmologist) the treated animals were divided into 3 groups[1]. Group I animals were designated to remain on the treatment diet until the end of the two-year study period; group II animals were selected, based on the status of their ocular lesions, for interim sacrifice; and group III animals, based on predicted potential recovery from ocular lesions, were placed on untreated diets for the remainder of the study period. The control group from Study #2 discussed above under section 2 can also be considered here since the two studies were run concurrently.

[1]The grouping process was by design selective for susceptibility for ocular lesions and not a random selection, however, 99% of the females were affected with these lesions by month 13 of the study.

STUDY #3

		Control	Group I	Group III
Nasal turbinates				
respiratory epithelium adenoma	M	0/45	42/61	10/17
	F	0/42	11/25	19/46
carcinoma	M	0/45	7/61	0/17
	F	0/42	2/25	1/46
Thymus lymphosarcoma	M	0/49	1/68	1/16
	F	0/48	0/25	1/43
Adrenal pheochromocytoma benign	M	8/50	8/70	2/20
	F	1/49	0/31	2/48
malignant	M	2/50	2/70	1/20
	F	0/49	0/31	0/48
Thyroid follicular adenoma	M	2/49	8/69	1/20
	F	1/49	4/31	3/49
carcinoma	M	1/49	10/69	1/20
	F	3/49	0/31	1/49
Stomach				
mixed carcino-sarcoma	M	0/50	3/68	0/20
	F	0/50	19/31	0/49
anaplastic sarcoma	M	0/50	1/68	0/20
	F	0/50	3/31	0/49
adenocarcinoma	M	0/50	0/68	0/20
	F	0/50	10/31	0/49
leiomyosarcoma	M	0/50	0/68	0/20
	F	0/50	10/31	0/49
undiff. sarcoma	M	0/50	0/68	0/20
	F	0/50	16/31	2/49
undiff. carcinoma	M	0/50	0/68	0/20
	F	0/50	3/31	0/49
Brain neuroepithelioma	M	0/50	1/70	0/20
	F	0/50	1/31	1/49

(continued)

STUDY #3 (continued)

		Control	Group I	Group III
Liver				
hepatoma	M	1/50	3/70	0/20
	F	0/50	1/31	0/49
neoplastic nodule	M	0/50	0/70	0/70
	F	0/50	1/31	1/49
hepatocellular carcinoma	M	2/50	2/70	0/20
	F	0/50	2/31	1/49

Note that nasal turbinate adenomas developed in rats exposed to alachlor for only 5-6 months at the beginning of the study (Group III).

The MTD was exceeded in female rats, as evidenced by a statistically significant increase in mortality; (In males, this single dose tested probably approached MTD.)

Monsanto submitted a reevaluation of the neuroepitheliomas seen in this study; electron microscopy of such a tumor from one of the animals showed "intermediate fiber typical of keratin", from which Monsanto concluded that the tumor was epithelial, not neural. C.I.I.T. also reevaluated all three brain tumors and concluded that they were extensions of nasal adenocarcinomas and not brain tumors. However, a discrepancy in animal numbers and diagnoses remains to be resolved before either Monsanto's or C.I.I.T.'s conclusions can be accepted (J. Hauswirth Memo). Monsanto has been informed of this discrepancy.

4. An 18 Month Oncogenic Study in Mice

In a study performed by Bio/dynamics, alachlor (Lasso technical)* was administered in the diet to groups of fifty male and fifty female CD-1 mice at dosages corresponding to the following levels: 0, 26, 78 and 260 mg/kg/day. The incidence of pertinent non-neoplastic and neoplastic changes are tabulated below.

STUDY #4

		Dose (mg/kg/day)			
		Control 0	Low 26	Mid 78	High 260
Lung bronchiolar-alveolar	M	6/50	1/50	4/50	10/50
adenoma	F	2/50	4/50	7/50	10/50
carcinoma	M	3/50	5/50	7/50	2/50
	F	1/50	1/50	1/50	1/50
fibrosarcoma	M	0/50	0/50	0/50	0/50
	F	0/50	0/50	0/50	1/50
congestion	M	1/50	13/50	13/50	12/50
	F	5/50	5/50	12/50	16/50
Liver					
adenoma	M	5/50	1/50	4/50	7/50
	F	0/50	0/50	0/50	1/50
carcinoma	M	0/50	3/50	1/50	4/50
	F	0/50	0/50	1/50	0/50
Uterus					
leiomyoma	F	0/50	2/50	0/50	0/50
leiomyosarcoma	F	1/50	0/50	2/50	3/50
endometrial carcinoma	F	0/50	1/50	0/50	0/50
endometrial polyp	F	1/50	3/50	0/50	3/50
granular cell myoblastoma	F	0/50	0/50	0/50	1/50

*Alachlor was supplied in two batches:
Lot XHI-167 used during the first 11 months of the study was stabilized with 0.5% epichlorohydrin; Lot MHK-6, used during the last 7 months, was stabilized with [redacted]

The major target organ for oncogenicity was the lung. The incidence of lung bronchioalveolar tumors was significantly increased in the high dose females ($p<0.05$) and was also significant ($p<0.01$) for the high dose females which died in extremis during the study. The incidence of lung tumors in females which died during the study was:

Control	0/30
Low	1/17
Mid	3/27
High	7/35

The MTD 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.

Monsanto submitted an addendum to this study on 2/25/85. The report contains 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.

5. Historical Control Information

Historical control data on lung tumors in CD-1 mice could be found in the open literature:

I MSD Study: Sher et al. Toxicology Letters 11:103-110, 1982.

N - animals:	M	1232	N - groups:	M	24	Age:	81-105 weeks
	F	1240		F	24		
adenoma	M	0-38%					
	F	0-41%					
adenocarcinoma	M	0-16%					
	F	0-12%					

II Homburger Data: Homburger et al. J. Natl. Cancer Inst. 35:37-43, 1975.

N - animals	M	99	
	F	102	
		18 months	
	M	2	
adenoma	F	4	
adenocarcinoma	M	-	
	F	1	

The MSD study duration was too long, so that comparisons based on these controls could not be made, however the study length from which the Homburger Data was derived, was appropriate. These latter control values were exceeded in the treated animals of study #4; furthermore, the Homburger data appear to indicate that the response seen in concurrent male controls was high, which could be masking the true response in the treated males.

Additional historical control data obtained from Bio-dynamics on the incidence of lung and liver tumors in CD-1 mice for concurrently run studies were discussed but were also found to be inappropriate because the length of the studies was 23-25 months, exceeding the 18 months of the Alachlor study.

Historical control data for the rats was requested from Monsanto, but has not been made available at this time.

E. Additional Toxicology Data on Alachlor:

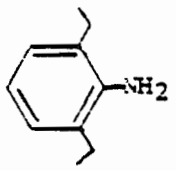
1. Metabolism:

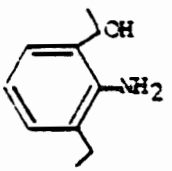
Fourteen metabolites of alachlor have been found in the urine and 13 in feces of Sprague-Dawley rats fed alachlor. Only three of these were found in both urine and feces (Figure 1 & 2). Approximately 89% of the radioactivity is eliminated in urine and feces (1:1) within four days; the rate of elimination is biphasic. Mercapturic acid, glucuronic acid, sulfate conjugation and side chain hydroxylation are important metabolic pathways in the rat. One metabolite found in rat urine, N-[2-ethyl-6-(1-hydroxyethyl)-phenyl]-N-(methoxymethyl)-2(methylsulfonyl)acetamide (metabolite XII), was mutagenic in the Ames salmonella assay, both with and without metabolic activation.

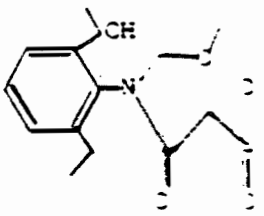
In Rhesus monkeys, 5 conjugates were identified in urine only (Figure 3) when alachlor was given intravenously: 92-94% of the total radioactivity was excreted in the urine during the first 24 hours and 91-94% in the feces during the first 48 hours (9-10:1). Studies via 2 other routes (intramuscular and topical) were considered unacceptable.

In human biomonitoring studies, metabolites which contained diethyl aniline (DEA) and hydroxy-ethyl, ethyl aniline (HEEA) moieties of alachlor were identified in urine.

Note that metabolites with both the HEEA and DEA moieties were found in both humans and rats (metabolite XII also contains the HEEA moiety); and while Monsanto claims that the monkey is a "better model for man in the case of alachlor"* in monkeys, only metabolites with the DEA moiety were found.

DEA: 

HEEA: 

Metabolite XII: 

2. Non-Oncogenic Toxicological Effects

The acute oral LD_{50}'s in the rat of alachlor (90%) and technical alachlor are 2.3 g/kg and 0.93-1.2 g/kg, respectively. In mice the acute oral LD_{50} of technical alachlor is 2.1 g/kg.

In a 3-generation reproduction study in Charles River Sprague-Dawley CD rats, the NOEL was 10 mg/kg based on kidney effects (chronic nephritis, hydronephrosis) seen in F_2 adult males and F_{3b} male pups.

In a one year subchronic beagle dog study the NOEL was 1 mg/kg/day based on hemosiderosis seen in liver, kidney and spleen of dogs in the 3 and 10 mg/kg/day groups.

Alachlor was not teratogenic to rats at 400 mg/kg/day (HDT).

A NOEL for non-neoplastic toxicity was established for alachlor in a 2-year chronic feeding/oncogenicity study in Long-Evans rats. The NOEL was 2.5 mg/kg/day based upon molting of retina pigmentation and increased mortality rate in the females and abnormal disseminated foci in male liver.

*[Monsanto's Rebuttal to Alachlor PD-1]

3. Mutagenicity:

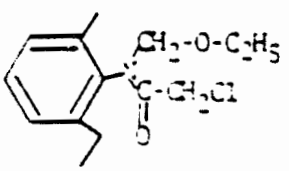
The results of mutagenicity testing conducted on alachlor are summarized in the following table.

Test	Core Classification	Result	Comments
Ames Assay	acceptable	negative	a positive response was seen at 5000 ug/plate in TA 1535 but the response was not repeated for consecutive doses.
Gene mutation in CHO cells HGPRT locus	acceptable	negative	
In-vivo bone marrow chromosome aberration assay	acceptable	negative	no structureal or numerical chromosomal aberrations
In-vivo - in vitro hepatocyte DNA repair assay	acceptable	positive	positive at highest dose tested (2.0g/kg/day) - "weakly genotoxic"
DNA damage in B. subtilis M45 and H17	acceptable	negative	did not cause DNA damage. (20-20,00 ug/plate)

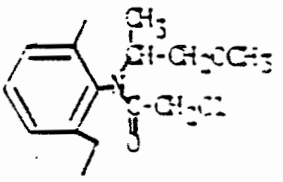
As noted in the metabolism section of this report one metabolite of alachlor tested positive in the Ames assay (TA 100 - both with and without metabolic activation over six test doses).

4. Structure-Activity Correlations:

Alachlor is structurally related to metolachlor and acetochlor, structures of which are shown below.



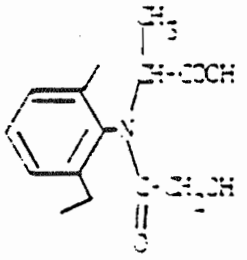
ACETOCHLOR



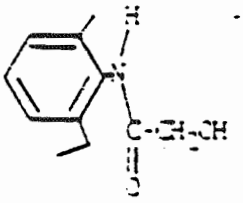
METOLACHLOR

PENDING REGISTRATION INFORMATION IS NOT INCLUDED

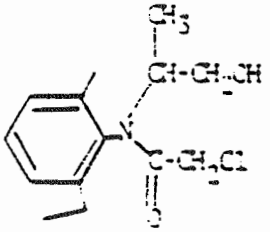
Limited mutagenicity data are available on metolachlor. It has been reported to be negative in the Ames salmonella assay and did not have any effects on fertility, zygote or embryo survival in the in vivo developing sperm mouse assay. Metolachlor, when fed to CD rats at levels of 30, 300 and 3000 ppm caused an increase in proliferative liver lesions (neoplastic nodules) in the high dose female rats. In this study nasal turbinate tumors were seen in two high dose males and one high dose female. Metolachlor was negative for oncogenicity in the mouse. Metolachlor has been evaluated in Peer Review as a class C carcinogen. Identified metabolites of metolachlor are shown below:



Urine & Feces



Urine only



Feces only

F. Weight of Evidence Considerations:

The committee considered the following facts regarding toxicology data on alachlor to be of importance in a weight of evidence determination of oncogenic potential.

1. Administration of alachlor in the diet to Long-Evans rats is associated with statistically significant increases in incidence over the control in the following tumors:

   Nasal turbinate tumors (mostly benign) at mid and high doses, in both sexes.

   Thyroid follicular tumors in male rats.

   Malignant stomach tumors in male and female rats.

2. Administration of alachlor in the diet to female CD-1 mice is associated with a statistically significant increase in lung tumors (bronchiolar-alveolar adenomas and carcinomas) in female mice.

3. Alachlor was tested in several in vitro and in vivo assays for mutagenicity and/or DNA damage. Of these only the in vivo - in vitro hepatocyte DNA repair assay was positive - and only at the HDT. It was judged, therefore, to be "weakly genotoxic", however a metabolite of alachlor was found to be positive in the Ames Test (Strain TA 100), both with and without metabolic activation over 6 test doses.

4. The metabolite referred to above is a moiety common to metabolites found in both humans and rats (but not in monkeys). This data is significant in so much as Monsanto maintains that the monkey is a "better model for man in the case of alachlor" [Monsanto's Rebuttal to Alachlor PD-1].

5. [redacted]

6. Metolachlor, another structurally related herbicide, when fed to CD rats, caused an increase in liver neoplastic nodules in the high dose females. In this same study, nasal turbinate tumors were seen in 2 high dose males and 1 high dose female, however metolachlor was negative for oncogenicity in the mouse.

G. Classification of Oncogenic Potential:

Criteria contained in the final draft of the proposed EPA Guidelines (12/1/85) for classifying a carcinogen were considered. These Guidelines state that "Sufficient evidence of carcinogenicity indicates that there is an increased incidence of malignant tumors or combined malignant and benign tumors: a) in multiple species [MET] or strains; b) in multiple experiments [MET] (e.g., with different routes of administration or using different dose levels; or c) to an unusual degree in a single experiment with regard to high incidence [MET], unusual site or type of tumor [MET], or early age of onset [MET]. Additional evidence may be provided by data on dose-response effects [MET], as well as information from short-term tests [partially MET] or on chemical structure [MET]".

Alachlor met all but one of the criteria specified for the B-2 classification, any of which alone can be sufficient for such a classification. 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 another experiment with Long-Evans rats, nasal turbinate tumors occured 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. Additionaly, a metabolite of alachlor was mutagenic in the Ames Test at 6 dose levels.

[redacted] Metolachlor when fed to CD rats, caused an increased incidence of neoplastic nodules in females at the high dose; metolachlor was negative for oncogenicity in the mouse.

The committee concluded that the data available for alachlor (from animal studies) is sufficient for its classification as a B-2 "Probable Human Carcinogen".

H. Major Rebuttals by Monsanto

The committee also addressed the following major points:

1. It is contended that the rat is not the appropriate model for assessing potential effects on humans; rather the monkey is more appropriate.

The committee disagrees since for this chemical it appears that the rat produces metabolites similar to those observed in man. Moreover, these very metabolites belong to the class of alachlor metabolites which seem to have mutagenic activity (refer to sections on Metabolism and Mutagenicity).

H. Major Rebuttals (continued)

2. It is contended that nasal turbinate tumors are strain specific (Long-Evans Rat).

The committee found no evidence that this is anything other than conjecture - ~~no other rat strain has been tested~~ IBT has tested 2 strains although not concurrently. Furthermore, nasal turbinate tumors were not the only response in Long-Evans rats.

3. It is contended that the "effects" are not seen in monkey and dog.

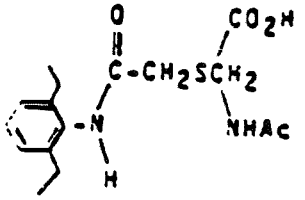
The committee concluded that data for subchronic (less than lifetime) exposure of other species can not refute oncogenic effect in a lifetime study.

4. It is contended that the mouse study did not show any oncogenic effect for alachlor.

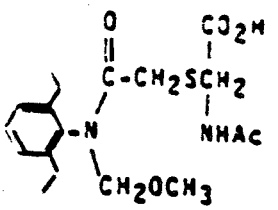
The committee disagrees with this conclusion (see review of mouse study, section D.4)

EPA's detailed response to Monsanto's Rebuttal is appended to this panel report.

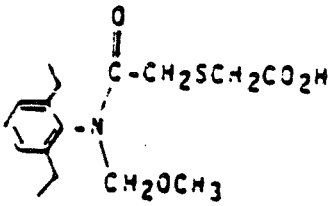
FIGURE 3 METABOLITES OF ALACHLOR



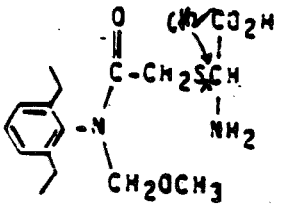
Secondary Mercapturate (4)



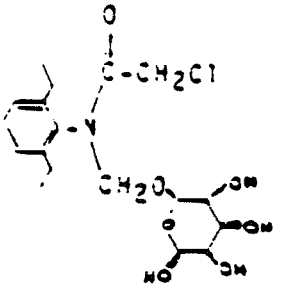
Tertiary Mercapturate (5)



Thioacetic Acid Conjugate (6)



Cysteine Conjugate (7)



Glucuronide Conjugate (8)

Page_____ is not included in this copy.

Pages 25 through 26 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.

_____ Description of quality control procedures.

_____ Identity of the source of product ingredients.

_____ Sales or other commercial/financial information.

_____ A draft product label.

_____ The product confidential statement of formula.

_____ Information about a pending registration action.

✓ FIFRA registration data.

_____ The document is a duplicate of page(s) _____.

_____ The document is not responsive to the request.

The information not included is generally considered confidential by product registrants. If you have any questions, please contact the individual who prepared the response to your request.

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

MAY 19 1987

OFFICE OF
PESTICIDES AND TOXIC SUBSTANCES

MEMORANDUM

SUBJECT: Toxicology Branch Peer Review Committee Final Document on Alachlor

FROM: Reto Engler, Chief
Mission Support Staff
Toxicology Branch/HED (TS-769C)

TO: Addressees

Attached for your review and Final Signature is the Peer Review Document on Alachlor. This document was not sent out for Draft Signature since it is a reconsideration of the original Peer Review decision made on this pesticide. No new data was considered by the Committee. The original Peer Review Document is also attached.

Attachment

ADDRESSEES:

Farber
Kasza
Marcus
Burin
Beliles
Barnes
Quest
Rinde
Barton
Burnam
Beal

6/26/86

007699

FILE COPY

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

MEMORANDUM

OFFICE OF
PESTICIDES AND TOXIC SUBSTANCES

SUBJECT: Peer Review of Alachlor

FROM: Esther Rinde, Ph.D. E. Rinde 5/27/86
Scientific Mission Support Staff
Toxicology Branch/HED (TS-769c)

TO: Robert Taylor
Product Manager #25
Fungicide-Herbicide Branch
Registration Division (TS-767c)

The Toxicology Branch Peer Review Committee met on March 25, 1986 to discuss and evaluate the weight-of-the-evidence on Alachlor, with particular reference to consideration of whether there is agreement on its classification as a B-2 carcinogen.

A. Individuals in Attendance:

1. Peer Review Committee: (Signatures indicate concurrence with peer review unless otherwise stated).

Theodore M. Farber	Theodore M. Farber
Reto Engler	
Louis Kasza	Louis Kasza
Bertram Litt	
Gary Burin	
Laurence Chitlick	W. Tuttle for L. Chitlick
Bruce Means	Bruce K. Means
William Marcus	
Robert Beliles	Robert P Beliles
Esther Rinde	E. Rinde

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A. Individuals in Attendance (continued)

2. Reviewers: (Non-panel members responsible for data presentation; signatures indicate technical accuracy of panel report.)

Judith Hauswirth — Judith W. Hauswirth

3. Peer review members in absentia: (Commitee members who were not able to attend the discussion· signatures indicate concurrence with the overall conclusions of the Committee.)

John A. Quest — John A. Quest

Richard Hill — Richard Hill

Stephen Johnson — Stephen Johnson

Anne Barton — Anne Barton

B. Material Reviewed:

The material available for review consisted of the following:

A. DER: A chronic Feeding study of Alachlor in Rats. Bio/Dynamics.

B. DER: A chronic Study of Alachlor Administered in feed to Long-Evans Rats. Monsanto Environmental Health Laboratory.

C. DER: A Special Chronic Feeding Study with Alachlor in Long-Evans Rats. Monsanto.

D. Tumor incidence table for the three rat studies combined; also DER on Monsanto's reevaluation of submucosal gland hyperplasia seen in study reviewed under Part 5.b. of Peer Review Memo (3/17/86).

E. DER: An 18 Month Oncogenic Study in Mice. Bio/dynamics.

F. Sher,S.P., R.D. Jensen and D.L. Bokelman. Spontaneous Tumors in Control F344 and Charles River CD Rats and Charles River CD-1 and B6C3HF1 Mice. Toxicology Letters 11: 103-110, 1982.

G. Homburger,F., A.B. Russfield, J.H. Weisburger, S. Lim, S.P. Chak and E.K. Weisburger. Aging Changes in CD-1 HaM/ICR Mice reared Under Standard Laboratory Conditions. J. Natl. Cancer Inst. 55: 37-45, 1975.

H. Historical Control Data from Bio/dynamics on Lung Tumors and Liver Tumors in CD-1 Mice.

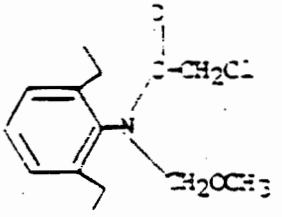
I. Table: Q_1^* Potency Estimates for Alachlor Based on Rat Tumor Data (from the PD-1).

A copy of the information reviewed is appended to this panel report.

C. Background Information:

Alachlor (2-chloro-2'6' diethyl-N-(methoxymethyl)-acetanilide) is registered for use as a selective herbicide for the control of many preemergent broadleaf weeds and grasses. In December 1984, a Special Review Position Document 1 was issued on alachlor, in which the Agency concluded Alachlor is a class B_2 oncogen based on the proposed EPA Guidelines, and that "the weight of the evidence demonstrates that alachlor is oncogenic to laboratory animals and, in the absence of data on humans, it is prudent to treat alachlor as a probable human carcinogen".

A Special Review Position Document 2,3 (PD 2,3) is now being prepared on alachlor; it was felt that it would be beneficial at this time to reevaluate alachlor through the peer review process prior to issuing the PD 2,3.



ALACHLOR

3.)

D. Evaluation of Oncogenicity Evidence for Alachlor:

1. A Chronic Feeding Study of Alachlor in Rats:

Bio/dynamics administered alachlor (Lasso Technical) in the diet to groups of 50 male and 50 female Long-Evans rats at concentrations of 0, 100, 300, or 1000 ppm (0, 14, 42 and 126 mg/kg/day, respectively) for 812 to 813 days (males) and 741 to 744 days (females). Two different lots of the technical alachlor were used during the study: Lot #XHI-167, stabilized with 0.5% epichlorohydrin* (for the first 11 months of the study) and Lot #MHK-6, [redacted] (for the remainder of the study). The following incidence of tumors were observed.

STUDY # 1

Tumor Site and Type	Sex	Dose (mg/Kg/day) 0	14	42	126
Stomach:					
leiomyosarcoma	M	0/49	0/50	0/50	1/50
	F	0/50	0/50	0/50	1/49
osteosarcoma	M	0/49	0/50	0/50	3/50
	F	0/50	0/50	0/50	4/49
gastric adenocarcinoma	M	0/49	0/50	0/50	2/50
	F	0/50	0/50	0/50	1/49
malignant mixed gastric tumor	M	0/49	0/50	0/50	11/50
	F	0/50	0/50	1/50	17/49
Thyroid:					
follicular adenoma	M	1/48	0/50	1/49	11/50
	F	0/49	0/44	2/46	2/49
follicular carcinoma	M	0/48	0/50	0/49	2/50
	F	0/49	0/44	0/46	2/49
Nasal Turbinates respiratory epithelium:					
adenomas	M	0/46	0/46	10/41	23/42
	F	0/49	0/47	4/42	10/48
carcinomas	M	0/46	0/46	1/41	0/42
	F	0/49	0/47	1/42	0/48

*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.

Nasal turbinate tumors (mainly benign) were significantly increased in both males ($p<0.001$) and females ($p<0.02$) at the mid dose level (42 mg/kg/d) and above.

Stomach malignant tumors increased significantly ($p<0.001$) in both sexes at the high dose level.

Thyroid follicular tumors (adenomas and carcinomas) were significantly increased in males at the high dose level ($p<0.001$).

The lowest dose of alachlor tested in this study probably exceeded a MTD as evidenced by high mortality, compared to controls. Increases in organ weights (liver, kidney, spleen, et al.) were also noted, as were gross findings, at all dose levels, indicative of a compound related effect.

2. A Chronic Feeding Study of Alachlor in Rats:

Monsanto administered technical alachlor (94.13%) in the diet to groups of 50 male and 50 female Long-Evans rats at concentrations of 0, 0.5, 2.5 and 15.0 mg/kg/day for 25 to 26 months. The alachlor was stabilized with [redacted] Epichlorohydrin was not used as a stabilizer. The following incidence of tumors/lesions was observed.

		STUDY #2			
		Dose (mg/kg/day)			
Tumor Type and Site	Sex	Control 0	Low 0.5	Medium 2.5	High 15.0
Thyroid follicular					
adenoma	M	2/49	4/50	3/49	4/49
	F	1/49	1/49	0/49	2/47
carcinoma	M	1/49	0/50	1/49	2/49
	F	3/49	1/49	1/49	1/49
Thymus lymphosarcoma	M	0/49	0/50	1/46	0/50
	F	0/48	1/50	2/48	3/43
Adrenal pheochromocytoma					
benign	M	8/50	7/50	2/50	6/50
	F	1/49	1/50	3/50	5/49
malignant	M	2/50	2/50	0/50	2/50
	F	1/49	0/50	0/50	0/49
Nose/Turbinates respiratory epithelium					
adenoma	M	0/45	0/48	0/45	11/45
	F	0/42	0/44	1/47	9/43
neurofibroma	M	0/45	1/48	0/45	0/45
	F	0/42	0/44	0/47	0/43
submucosal gland adenoma	M	0/45	0/48	1/45	0/45
	F	0/42	0/44	0/47	0/43
epith. hyperplasia/ metaplasia	M	1/45	1/48	1/45	1/45
	F	0/42	0/44	0/47	2/43
submucosal gland hyperplasia	M	2/45	1/48	3/45	21/45
	F	2/47	5/44	5/47	11/43

Nasal turbinate tumors were significantly elevated ($p<0.01$) in both males and females at 15 mg/kg/day (the highest dose tested). One female rat in the mid dose group also had this tumor and one male in this group had a submucosal gland adenoma.

Thymus lymphosarcomas and adrenal pheochromocytomas were significantly increased ($p<0.05$) in the high dose females.

There was a non-significant increase in thyroid follicular cell tumors in the high dose male group.

> The highest dose tested probably exceeded a 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.)

Monsanto was requested to reevaluate the submucosal gland hyperplasia seen in both males and females. Experimental Pathology Laboratories, Inc. (EPL) performed a histological reevaluation; their report indicated that the submucosal nasal lesions (hyperplasia) were not neoplastic, however their analysis reflected a slightly higher incidence of adenomas of the nasal cavity. EPL's diagnosis is compared with that of Monsanto in the table below.

	Nasal turbinate adenomas			
	EPL's data		Monsanto's data	
Group (mg/kg/day)	Males	Females	Males	Females
0	0/44	0/42	0/45	0/42
0.5	0/47	0/42	0/48	0/44
2.5	0/44	1/47	0/45	1/47
15.0	15/45	14/48	11/45	9/48

3. A Special Chronic Feeding Study With Alachlor

In a study performed by Monsanto, alachlor was administered in the diet to Long-Evans rats at a concentration of 126 mg/kg/day. After a period of exposure (5-8 months) sufficient to induce ocular lesions (as confirmed by the consulting ophthalmologist) the treated animals were divided into 3 groups[1]. Group I animals were designated to remain on the treatment diet until the end of the two-year study period; group II animals were selected, based on the status of their ocular lesions, for interim sacrifice; and group III animals, based on predicted potential recovery from ocular lesions, were placed on untreated diets for the remainder of the study period. The control group from Study #2 discussed above under section 2 can also be considered here since the two studies were run concurrently.

[1]The grouping process was by design selective for susceptibility for ocular lesions and not a random selection, however, 99% of the females were affected

STUDY #3

		Control	Group I	Group III
Nasal turbinates respiratory epithelium adenoma	M	0/45	42/61	10/17
	F	0/42	11/25	19/46
carcinoma	M	0/45	7/61	0/17
	F	0/42	2/25	1/46
Thymus lymphosarcoma	M	0/49	1/68	1/16
	F	0/48	0/25	1/43
Adrenal pheochromocytoma benign	M	8/50	8/70	2/20
	F	1/49	0/31	2/48
malignant	M	2/50	2/70	1/20
	F	0/49	0/31	0/48
Thyroid follicular adenoma	M	2/49	8/69	1/20
	F	1/49	4/31	3/49
carcinoma	M	1/49	10/69	1/20
	F	3/49	0/31	1/49
Stomach mixed carcino-sarcoma	M	0/50	3/68	0/20
	F	0/50	19/31	0/49
anaplastic sarcoma	M	0/50	1/68	0/20
	F	0/50	3/31	0/49
adenocarcinoma	M	0/50	0/68	0/20
	F	0/50	10/31	0/49
leiomyosarcoma	M	0/50	0/68	0/20
	F	0/50	10/31	0/49
undiff. sarcoma	M	0/50	0/68	0/20
	F	0/50	16/31	2/49
undiff. carcinoma	M	0/50	0/68	0/20
	F	0/50	3/31	0/49
Brain neuroepithelioma	M	0/50	1/70	0/20
	F	0/50	1/31	1/49

(continued)

STUDY #3 (continued)

		Control	Group I	Group III
Liver				
hepatoma	M	1/50	3/70	0/20
	F	0/50	1/31	0/49
neoplastic nodule	M	0/50	0/70	0/70
	F	0/50	1/31	1/49
hepatocellular carcinoma	M	2/50	2/70	0/20
	F	0/50	2/31	1/49

Note that nasal turbinate adenomas developed in rats exposed to alachlor for only 5-6 months at the beginning of the study (Group III).

The MTD was exceeded in female rats, as evidenced by a statistically significant increase in mortality; (In males, this single dose tested probably approached MTD.)

Monsanto submitted a reevaluation of the neuroepitheliomas seen in this study; electron microscopy of such a tumor from one of the animals showed "intermediate fiber typical of keratin", from which Monsanto concluded that the tumor was epithelial, not neural. C.I.I.T. also reevaluated all three brain tumors and concluded that they were extensions of nasal adenocarcinomas and not brain tumors. However, a discrepancy in animal numbers and diagnoses remains to be resolved before either Monsanto's or C.I.I.T.'s conclusions can be accepted (J. Hauswirth Memo). Monsanto has been informed of this discrepancy.

4. An 18 Month Oncogenic Study in Mice

In a study performed by Bio/dynamics, alachlor (Lasso technical)* was administered in the diet to groups of fifty male and fifty female CD-1 mice at dosages corresponding to the following levels: 0, 26, 78 and 260 mg/kg/day. The incidence of pertinent non-neoplastic and neoplastic changes are tabulated below.

		STUDY #4 Dose (mg/kg/day)			
		Control 0	Low 26	Mid 78	High 260
Lung bronchiolar-alveolar adenoma	M	6/50	1/50	4/50	10/50
	F	2/50	4/50	7/50	10/50
carcinoma	M	3/50	5/50	7/50	2/50
	F	1/50	1/50	1/50	1/50
fibrosarcoma	M	0/50	0/50	0/50	0/50
	F	0/50	0/50	0/50	1/50
congestion	M	1/50	13/50	13/50	12/50
	F	5/50	5/50	12/50	16/50
Liver adenoma	M	5/50	1/50	4/50	7/50
	F	0/50	0/50	0/50	1/50
carcinoma	M	0/50	3/50	1/50	4/50
	F	0/50	0/50	1/50	0/50
Uterus leiomyoma	F	0/50	2/50	0/50	0/50
leiomyosarcoma	F	1/50	0/50	2/50	3/50
endometrial carcinoma	F	0/50	1/50	0/50	0/50
endometrial polyp	F	1/50	3/50	0/50	3/50
granular cell myoblastoma	F	0/50	0/50	0/50	1/50

*Alachlor was supplied in two batches:
Lot XHI-167 used during the first 11 months of the study was stabilized with 0.5% epichlorohydrin; Lot MHK-6, used during the last 7 months, was stabilized [redacted]

The major target organ for oncogenicity was the lung. The incidence of lung bronchioalveolar tumors was significantly increased in the high dose females ($p<0.05$) and was also significant ($p<0.01$) for the high dose females which died in extremis during the study. The incidence of lung tumors in females which died during the study was:

Control	0/30
Low	1/17
Mid	3/27
High	7/35

The MTD 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.

Monsanto submitted an addendum to this study on 2/25/85. The report contains 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.

5. Historical Control Information

Historical control data on lung tumors in CD-1 mice could be found in the open literature:

I MSD Study: Sher et al. Toxicology Letters 11:103-110, 1982.

N - animals:	M	1232	N - groups:	M	24	Age: 81-105 weeks
	F	1240		F	24	
adenoma	M	0-38%				
	F	0-41%				
adenocarcinoma	M	0-16%				
	F	0-12%				

II Homburger Data: Homburger et al. J. Natl. Cancer Inst. 35:37-43, 1975.

N - animals	M	99	
	F	102	
			18 months
adenoma	M		2
	F		4
adenocarcinoma	M		-
	F		1

The MSD study duration was too long, so that comparisons based on these controls could not be made, however the study length from which the Homburger Data was derived, was appropriate. These latter control values were exceeded in the treated animals of study #4; furthermore, the Homburger data appear to indicate that the response seen in concurrent male controls was high, which could be masking the true response in the treated males.

Additional historical control data obtained from Bio-dynamics on the incidence of lung and liver tumors in CD-1 mice for concurrently run studies were discussed but were also found to be inappropriate because the length of the studies was 23-25 months, exceeding the 18 months of the Alachlor study.

Historical control data for the rats was requested from Monsanto, but has not been made available at this time.

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E. Additional Toxicology Data on Alachlor:

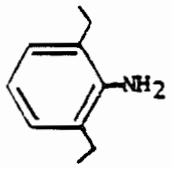
1. Metabolism:

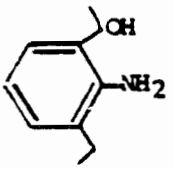
Fourteen metabolites of alachlor have been found in the urine and 13 in feces of Sprague-Dawley rats fed alachlor. Only three of these were found in both urine and feces (Figure 1 & 2). Approximately 89% of the radioactivity is eliminated in urine and feces (1:1) within four days; the rate of elimination is biphasic. Mercapturic acid, glucuronic acid, sulfate conjugation and side chain hydroxylation are important metabolic pathways in the rat. One metabolite found in rat urine, N-[2-ethyl-6-(1-hydroxyethyl)-phenyl]-N-(methoxymethyl)-2(methylsulfonyl)acetamide (metabolite XII), was mutagenic in the Ames salmonella assay, both with and without metabolic activation.

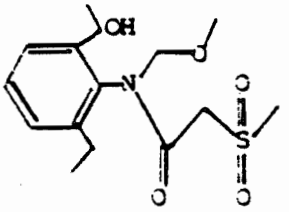
In Rhesus monkeys, 5 conjugates were identified in urine only (Figure 3) when alachlor was given intravenously: 92-94% of the total radioactivity was excreted in the urine during the first 24 hours and 91-94% in the feces during the first 48 hours (9-10:1). Studies via 2 other routes (intramuscular and topical) were considered unacceptable.

In human biomonitoring studies, metabolites which contained diethyl aniline (DEA) and hydroxy-ethyl, ethyl aniline (HEEA) moieties of alachlor were identified in urine.

Note that metabolites with both the HEEA and DEA moieties were found in both humans and rats (metabolite XII also contains the HEEA moiety); and while Monsanto claims that the monkey is a "better model for man in the case of alachlor"* in monkeys, only metabolites with the DEA moiety were found.

DEA: 

HEEA: 

Metabolite XII: 

2. Non-Oncogenic Toxicological Effects

The acute oral LD_{50}'s in the rat of alachlor (90%) and technical alachlor are 2.3 g/kg and 0.93-1.2 g/kg, respectively. In mice the acute oral LD_{50} of technical alachlor is 2.1 g/kg.

In a 3-generation reproduction study in Charles River Sprague-Dawley CD rats, the NOEL was 10 mg/kg based on kidney effects (chronic nephritis, hydronephrosis) seen in F_2 adult males and F_{3b} male pups.

In a one year subchronic beagle dog study the NOEL was 1 mg/kg/day based on hemosiderosis seen in liver, kidney and spleen of dogs in the 3 and 10 mg/kg/day groups.

Alachlor was not teratogenic to rats at 400 mg/kg/day (HDT).

A NOEL for non-neoplastic toxicity was established for alachlor in a 2-year chronic feeding/oncogenicity study in Long-Evans rats. The NOEL was 1.5 mg/kg/day based upon molting of retina pigmentation and increased mortality rate in the females and abnormal disseminated foci in male liver.

3. Mutagenicity:

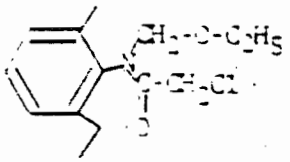
The results of mutagenicity testing conducted on alachlor are summarized in the following table.

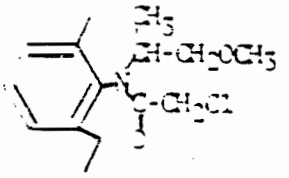
Test	Core Classification	Result	Comments
Ames Assay	acceptable	negative	a positive response was seen at 5000 ug/plate in TA 1535 but the response was not repeated for consecutive doses.
Gene mutation in CHO cells HGPRT locus	acceptable	negative	
In-vivo bone marrow chromosome aberration assay	acceptable	negative	no structureal or numerical chromosomal aberrations
In-vivo - in vitro hepatocyte DNA repair assay	acceptable	positive	positive at highest dose tested (1.0g/kg/day) - "weakly genotoxic"
DNA damage in B. subtilis M45 and H17	acceptable	negative	did not cause DNA damage. (20-20,00 ug/plate)

As noted in the metabolism section of this report one metabolite of alachlor tested positive in the Ames assay (TA 100 - both with and without metabolic activation over six test doses).

4. Structure-Activity Correlations:

Alachlor is structurally related to metolachlor and acetochlor, structures of which are shown below.

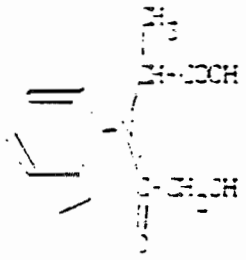




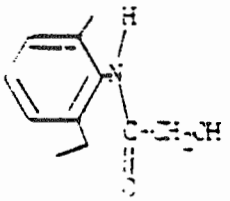
PENDING REGISTRATION INFORMATION IS NOT INCLUDED

[redacted]

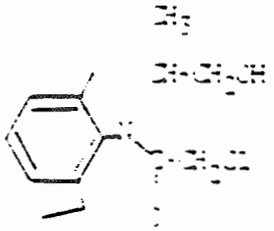
Limited mutagenicity data are available on metolachlor. It has been reported to be negative in the Ames salmonella assay and did not have any effects on fertility, zygote or embryo survival in the in vivo developing sperm mouse assay Metolachlor, when fed to CD rats at levels of 30, 300 and 3000 ppm caused an increase in proliferative liver lesions (neoplastic nodules) in the high dose female rats. In this study nasal turbinate tumors were seen in two high dose males and one high dose female. Metolachlor was negative for oncogenicity in the mouse. Metolachlor has been evaluated in Peer Review as a class C carcinogen. Identified metabolites of metolachlor are shown below:



Urine & Feces



Urine only



Feces only

F. Weight of Evidence Considerations:

The committee considered the following facts regarding toxicology data on alachlor to be of importance in a weight of evidence determination of oncogenic potential.

1. Administration of alachlor in the diet to Long-Evans rats is associated with statistically significant increases in incidence over the control in the following tumors:

   Nasal turbinate tumors (mostly benign) at mid and high doses, in both sexes.

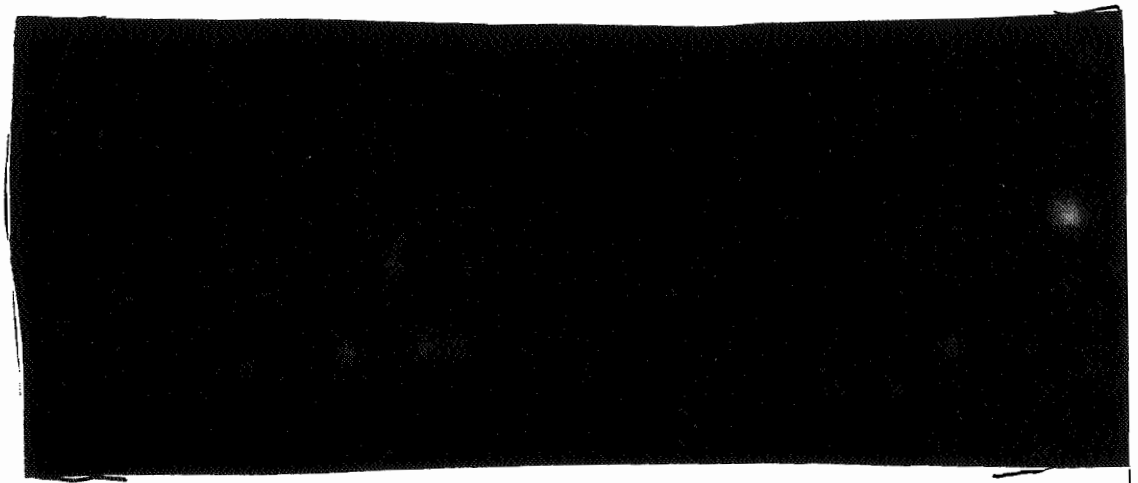
   Thyroid follicular tumors in male rats.

   Malignant stomach tumors in male and female rats.

2. Administration of alachlor in the diet to female CD-1 mice is associated with a statistically significant increase in lung tumors (bronchiolar-alveolar adenomas and carcinomas) in female mice.

3. Alachlor was tested in several in vitro and in vivo assays for mutagenicity and/or DNA damage. Of these only the in vivo - in vitro hepatocyte DNA repair assay was positive - and only at the HDT. It was judged, therefore, to be "weakly genotoxic", however a metabolite of alachlor was found to be positive in the Ames Test (Strain TA 100), both with and without metabolic activation over 6 test doses.

4. The metabolite referred to above is a moiety common to metabolites found in both humans and rats (but not in monkeys). This data is significant in-so-much as Monsanto maintains that the monkey is a "better model for man in the case of alachlor" [Monsanto's Rebuttal to Alachlor PD-1].

5. 

6. Metolachlor, another structurally related herbicide, when fed to CD rats, caused an increase in liver neoplastic nodules in the high dose females. In this same study, nasal turbinate tumors were seen in 2 high dose males and 1 high dose female, however metolachlor was negative for oncogenicity in the mouse.

G. Classification of Oncogenic Potential:

Criteria contained in the final draft of the proposed EPA Guidelines (12/1/85) for classifying a carcinogen were considered. These Guidelines state that "Sufficient evidence of carcinogenicity indicates that there is an increased incidence of malignant tumors or combined malignant and benign tumors: a) in multiple species [MET] or strains; b) in multiple experiments [MET] (e.g., with different routes of administration or using different dose levels; or c) to an unusual degree in a single experiment with regard to high incidence [MET], unusual site or type of tumor [MET], or early age of onset [MET]. Additional evidence may be provided by data on dose-response effects [MET], as well as information from short-term tests [partially MET] or on chemical structure [MET]".

Alachlor met all but one of the criteria specified for the B-2 classification, any of which alone can be sufficient for such a classification. 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 another experiment with Long-Evans rats, nasal turbinate tumors occured 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. Additionaly, a metabolite of alachlor was mutagenic in the Ames Test at 6 dose levels.

[redacted] Metolachlor when fed to CD rats, caused an increased incidence of neoplastic nodules in females at the high dose; metolachlor was negative for oncogenicity in the mouse.

The committee concluded that the data available for alachlor (from animal studies) is sufficient for its classification as a B-2 "Probable Human Carcinogen".

H. Major Rebuttals by Monsanto

The committee also addressed the following major points:

1. It is contended that the rat is not the appropriate model for assessing potential effects on humans; rather the monkey is more appropriate.

   The committee disagrees since for this chemical it appears that the rat produces metabolites similar to those observed in man. Moreover, these very metabolites belong to the class of alachlor metabolites which seem to have mutagenic activity (refer to sections on Metabolism and Mutagenicity).

H. Major Rebuttals (continued)

2. It is contended that nasal turbinate tumors are strain specific (Long-Evans Rat).

The committee found no evidence that this is anything other than conjecture - ~~no other rat strain has been tested.~~ IBT has tested 2 strains although not Wistar. Furthermore, nasal turbinate tumors were not the only response in Long-Evans rats.

3. It is contended that the "effects" are not seen in monkey and dog.

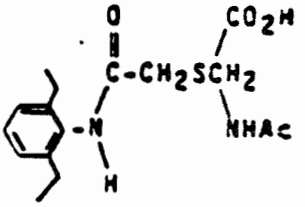
The committee concluded that data for subchronic (less than lifetime) exposure of other species can not refute oncogenic effect in a lifetime study.

4. It is contended that the mouse study did not show any oncogenic effect for alachlor.

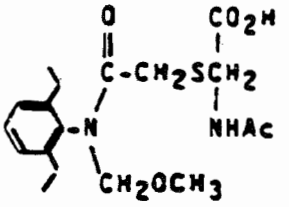
The committee disagrees with this conclusion (see review of mouse study, section D.4)

EPA's detailed response to Monsanto's Rebuttal is appended to this panel report.

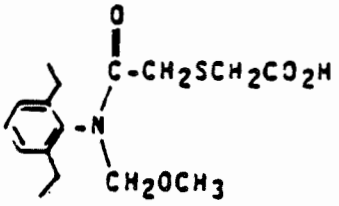
FIGURE 3 METABOLITES OF ALACHLOR



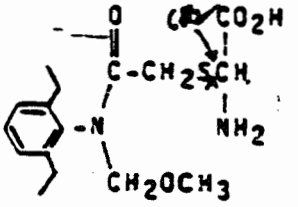
Secondary Mercapturate (4)



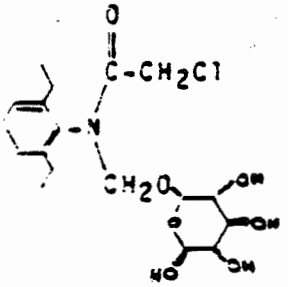
Tertiary Mercapturate (5)



Thioacetic Acid Conjugate (6)



Cysteine Conjugate (7)



Glucuronide Conjugate (8)

Page_____ is not included in this copy.

Pages 45 through 46 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.

____ Description of quality control procedures.

____ Identity of the source of product ingredients.

____ Sales or other commercial/financial information.

____ A draft product label.

____ The product confidential statement of formula.

____ Information about a pending registration action.

__✓__ FIFRA registration data.

____ The document is a duplicate of page(s) _____.

____ The document is not responsive to the request.

The information not included is generally considered confidential by product registrants. If you have any questions, please contact the individual who prepared the response to your request.

SAP Executive Summary

6/26/86

007699

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

JUL 7 1986

OFFICE OF
PESTICIDES AND TOXIC SUBSTANCES

MEMORANDUM

SUBJECT: Transmittal of the Final FIFRA Scientific Advisory Panel Reports on the June 25-26, 1986, Meeting

TO: Douglas D. Campt, Director
Office of Pesticide Programs (TS-766)

The above mentioned meeting of the FIFRA Scientific Advisory Panel (SAP) was an open meeting held in Arlington, Virginia, to review the Office of Drinking Water's Health Advisory documents for the following pesticides:

(1) Alachlor
(2) Aldicarb
(3) Carbofuran
(4) Chlordane
(5) 2,4-D
(6) Dibromochloropropane (DBCP)
(7) 1,2-Dichloropropane
(8) 2,4,5-TP
(9) Endrin
(10) Ethylene Dibromide (EDB)
(11) Heptachlor/Heptachlor Epoxide
(12) Lindane
(13) Methoxychlor
(14) Oxamyl
(15) Toxaphene

Please find attached the SAP's final reports on the draft Health Advisories discussed at the meeting.

Stephen L. Johnson, Executive Secretary
FIFRA Scientific Advisory Panel (TS-769)

Attachments

cc: Panel Members
John A. Moore
James Lamb
Al Heier
Susan H. Sherman
John W. Melone
James Akerman
Joseph Cotruvo
Arnold Kuzmack
EPA Participants

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FEDERAL INSECTICIDE, FUNGICIDE, AND RODENTICIDE ACT

SCIENTIFIC ADVISORY PANEL

Consideration of Health Advisory on Alachlor

The Federal Insecticide, Fungicide, and Rodenticide Act (FIFRA) Scientific Advisory Panel (SAP) has completed review of the Office of Drinking Water's (ODW) Health Advisory for Alachlor. The review was conducted in an open meeting held in Arlington, Virginia on June 26, 1986. All Panel members were present for the review, except Dr. Rosmarie von Rumker and Dr. Thomas W. Clarkson.

Public notice of the meeting was published in the Federal Register on Wednesday, June 4, 1986.

Oral statements were received from staff of the Environmental Protection Agency and from Mr. Chuck Pace, representing the National Audubon Society.

In consideration of all matters brought out during the meeting and careful review of all documents presented by the Agency, the Panel unanimously submits the following report.

REPORT OF SAP RECOMMENDATIONS

General Comments

The Panel agrees, in concept, with the EPA/ODW program of preparing health advisories on selected pesticides and other chemicals that have been or could be found in drinking water supplies. These health advisories could be useful to regional, state and local officials in assessing the severity of incidents involving drinking water contamination. However, the Panel also agrees with several comments offered by the public that these documents, though prepared by EPA only as "advisories," will be adopted as real or de facto laws and regulations by other agencies. Consequently, it is extremely important that these health advisories present the most scientifically defensible positions possible. Further, these documents should be reviewed and updated on a regular basis.

The Panel believes that health advisory documents should present multiple calculations of health advisories to show the extent of agreement between different studies and endpoints. These should include the use of human data, the most appropriate NOAEL, and the

most appropriate LOAEL for toxic effects in multiple species including man. The health advisories should then conclude which of the above is most appropriate and present the range upon which the conclusion is based. One approach that should be considered strongly is the presentation of the above data in a tabular form similar to the National Academy of Sciences drinking water document, 1977 Drinking Water and Health, Volume I, National Academy Press, Washington, D.C. Furthermore, specific criteria should be established for how and when each uncertainty factor, i.e., 5, 10, 100, 1000, or 10000, is to be used. Presently, the entire process appears to be proceeding in a rather whimsical manner. For instance in one example, even though several carcinogenicity studies on a chemical had been completed and deemed adequate, the lifetime health advisory was calculated using subchronic data and then divided by an additional factor of 10 for uncertainty. This resulted in a 100- to 200-fold lower health advisory than would have been calculated from the available chronic data. Since the health advisories are likely to become real or de facto regulations, much greater attention must be given to the scientific validity of these documents.

In some of the health advisories it was stated that "the chemical" may be classified as a Group B, C, etc., carcinogen. Will some of these compounds be classified by the Agency as of the effective dates of the health advisories? If so, this information should be included in the health advisories. In addition, when carcinogenic endpoints have been demonstrated for a chemical, upper and lower confidence limits and maximum likely estimates (MLE) should be included.

Although the Panel agrees with the potential utility of the 1-day health advisories, their reliability may be greatly affected by the types of data used to calculate them. Particularly, one must be certain that the endpoint, where an effect level is interpreted, is a toxic effect resulting from the chemical in question. It must be realized that the 1-day health advisories are subject to error if the effect endpoints reflect merely a physiological variation.

Lastly, the Panel recommends that the word "protective" be removed from the definition of the DWEL and substituted with a "no-effect-level."

Alachlor

The Agency requested the Panel to focus its attention upon a set of issues relating to the Health Advisory for Alachlor and provide any comments on the scientific and technical merit of the document, focusing principally upon those sections of the document devoted to risk assessment, both qualitative and quantitative.

Panel Response:

Included in the June 12, 1986, transmittal of materials for the June 25-26, 1986, FIFRA Scientific Advisory Panel (SAP) meeting were the following chemical specific issues for alachlor:

1. In considering its carcinogenic potential, the Agency has placed alachlor into Group B2: Probable Human Carcinogen, according to EPA's proposed scheme. Is this the appropriate categorization?

2. Are the tumors noted both during and at the termination of the mouse oncogenicity study supportive of a conclusion that alachlor is carcinogenic in this species?

Since alachlor is in Special Review and the Office of Pesticide Programs (OPP) is planning to issue a PD 2/3 the summer of 1986, the Office of Drinking Water (ODW) and the OPP have requested that the Panel's review and comments on the above mentioned chemical specific issues be deferred until after the issuance of the PD 2/3. The Panel concurs with this request.

The Panel has no specific comments to make in addition to the general comments presented above.

FOR THE CHAIRMAN

Certified as an accurate report of Findings:

Stephen L. Johnson
Executive Secretary
FIFRA Scientific Advisory Panel

Date: 7/7/86

Qualitative/Quantitative Risk Assessment

Nov. 9, 1987

007699

Memo

RE: Alachlor Q^*

Engler, Lewy & Hamsworth have selected a single estimate of Q^*, 8×10^{-2} based on the 2nd rat study using nasal turbinate tumors. This decision was made because the current policy is that only one value for Q^* should be selected, based on the qualitative aspects of the data. In the case of Alachlor it was deemed inappropriate to combine tumors or tumor incidence across sex.

The second study was considered more appropriate for risk assessment since:

1) the 1st study exceeded the MTD @ high-dose.

2) the 2nd study used the current ~~mixture~~ technical mixture

3) the 2nd study was designed and conducted with knowledge that nasal tumors were likely.

Reviewer's Package for Second Meeting

007699

UNITED STATES ENVIRONMENTAL PROTECTION AGENCY
WASHINGTON, D C 20460

MAR 30 1987

FILE COPY

MEMORANDUM

SUBJECT: Alachlor - Reevaluation by Peer-Review Group
OFFICE OF PESTICIDES AND TOXIC SUBSTANCES

FROM: Reto Engler, Chief
Scientific Mission Support Staff
Toxicology Brancn/HED (TS-769)

TO: Addressees

Attached for your review is a package prepared by Dr. Hauswirth concerning issues on Alachlor raised by both the SAP and Monsanto. - Please evaluate our previous Peer-Review and the questions raised in Dr. Hauswirtn's memo.

A meeting to discuss these issues is scheduled for Wednesday, April 15, 1987, at 1:30 PM in Dr. Farber's office.

Attachment

ADDRESSEES:

T. Farber
W. Burnam
L. Kasza
R. Levy
J. Quest
E. Kinde
J. Hauswirtn
A. Barton
W. Markus
G. Burin
D. Beal
R. Deliles
D. Barnes

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

MEMORANDUM

OFFICE OF
PESTICIDES AND TOXIC SUBSTANCES

Subject: Alachlor - Classification as a B_2 Oncogen

From: Judith W. Hauswirth, Ph.D. Judith W. Hauswirth
Acting Head, Section VI 3/12/87
Toxicology Branch/HFD

To: Peer Review Committee
Toxicology Branch/HFD

Background:

On March 25, 1986, the Toxicology Branch Peer Review Committee met to evaluate the weight-of-the-evidence on Alachlor and to consider the classification of Alachlor as a B_2 oncogen in the Position Document - 1 on Alachlor. The Committee agreed that Alachlor was a B_2 oncogen. Their basis for this classification is summarized below (taken from Peer Review Report on Alachlor, Appendix I):

> Alachlor met all but one of the criteria specified for the B_2 classification, any of which alone can be sufficient for such a classification. 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 two dose levels. In another experiment 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 dose levels.

On November 19, 1986 this classification was the subject of a Science Advisory Panel (SAP) meeting. The SAP agreed that Alachlor was a B_2 oncogen as defined by the EPA Cancer Guidelines but questioned whether it was a probable human carcinogen (see Appendix II, Report of Panel Recommendations). However, they did not feel that Alachlor elicited a positive oncogenic response in the mouse (see pertinent parts of transcript from the meeting, Appendix III and Appendix II), since the incidence of lung tumors in the female high dose group was within the historical control rate for this strain of mouse according to Sher et al. (Toxicology Letters 11:103-110, 1982). This data is summarized on page 10 of the Toxicology Peer Review Report on Alachlor (Appendix I).

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For Committee Consideration:

As a result of the SAP decision and the issuance of the Position Document-2,3 (Federal Register Notice of October 8, 1986 The Alachlor Special Review Technical Support Document Dated September, 1986), the registrant has asked for reconsideration of the oncogenicity classification of Alachlor from a B_2 to a category C. Their justification can be found in Appendix IV. Briefly it is as follows:

- o Lack of oncogenicity in multiple species (since mouse study was considered negative by the SAP)
- o Questionable malignant tumor response in multiple experiments (nasal turbinate tumors were mostly benign)
- o Lack of unusual degree, site, type or early onset (at doses below the MTD there was not an unusually high inicidence of nasal turbinate tumors, nasal turbinates were not routinely examined at the time of the alachlor study)
- o Alachlor is not a genotoxic oncogen and there are species differences in its metabolism.

The Committee is asked to consider the registrant's arguments for a C classification of the oncogenic potential of Alachlor and determine whether their arguments are sufficient to change the categorization from B_2 to C.

Appendix I

FILE COPY

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

MEMORANDUM

OFFICE OF PESTICIDES AND TOXIC SUBSTANCES

SUBJECT: Peer Review of Alachlor

FROM: Esther Rinde, Ph.D. E. Rinde 5/22/86
Scientific Mission Support Staff
Toxicology Branch/HED (TS-769c)

TO: Robert Taylor
Product Manager #25
Fungicide-Herbicide Branch
Registration Division (TS-767c)

The Toxicology Branch Peer Review Committee met on March 25, 1986 to discuss and evaluate the weight-of-the-evidence on Alachlor, with particular reference to consideration of whether there is agreement on its classification as a B-2 carcinogen.

A. Individuals in Attendance:

1. Peer Review Committee: (Signatures indicate concurrence with peer review unless otherwise stated).

Theodore M. Farber	Theodore M. Farber
Reto Engler	
Louis Kasza	
Bertram Litt	
Gary Burin	
Laurence Chitlick	
Bruce Mears	
William Marcus	
Robert Belles	
Esther Rinde	

6)

A. Individuals in Attendance (continued)

2. Reviewers: (Non-panel members responsible for data presentation; signatures indicate technical accuracy of panel report.)

Judith Hauswirth — Judith W. Hauswirth

3. Peer review members in absentia: (Commitee members who were not able to attend the discussion; signatures indicate concurrence with the overall conclusions of the Committee.)

John A. Quest — John A. Quest

Richard Hill — Richard Hill

Stephen Johnson — Stephen Johnson

Anne Barton — Anne Barton

B. Material Reviewed:

The material available for review consisted of the following:

A. DER: A chronic Feeding study of Alachlor in Rats. Bio/Dynamics.

B. DER: A chronic Study of Alachlor Administered in feed to Long-Evans Rats. Monsanto Environmental Health Laboratory.

C. DER: A Special Chronic Feeding Study with Alachlor in Long-Evans Rats. Monsanto.

D. Tumor incidence table for the three rat studies combined; also DER on Monsanto's reevaluation of submucosal gland hyperplasia seen in study reviewed under Part 5.b. of Peer Review Memo (3/17/86).

E. DER: An 18 Month Oncogenic Study in Mice. Bio/dynamics.

F. Sher,S.P., R.D. Jensen and D.L. Bokelman. Spontaneous Tumors in Control F344 and Charles River CD Rats and Charles River CD-1 and B6C3HF1 Mice. Toxicology Letters 11: 103-110, 1982.

G. Homburger,F., A.B. Russfield, J.H. Weisburger, S. Lim, S.P. Chak and E.K. Weisburger. Aging Changes in CD-1 HaM/ICR Mice reared Under Standard Laboratory Conditions. J. Natl. Cancer Inst. 55: 37-45, 1975.

H. Historical Control Data from Bio/dynamics on Lung Tumors and Liver Tumors in CD-1 Mice.

I. Table: Q_1^* Potency Estimates for Alachlor Based on Rat Tumor Data from the PD-1).

A copy of the information reviewed is appended to this panel report.

C. Background Information:

Alachlor (2-chloro-2'6' diethyl-N-(methoxymethyl)-acetanilide) is registered for use as a selective herbicide for the control of many preemergent broadleaf weeds and grasses. In December 1984, a Special Review Position Document 1 was issued on alachlor, in which the Agency concluded Alachlor is a class B_2 oncogen based on the proposed EPA Guidelines, and that "the weight of the evidence demonstrates that alachlor is oncogenic to laboratory animals and, in the absence of data on humans, it is prudent to treat alachlor as a probable human carcinogen".

A Special Review Position Document 2,3 (PD 2,3) is now being prepared on alachlor; it was felt that it would be beneficial at this time to reevaluate alachlor through the peer review process prior to issuing the PD 2,3.

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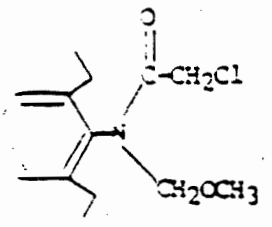

D. Evaluation of Oncogenicity Evidence for Alachlor:

1. A Chronic Feeding Study of Alachlor in Rats:

Bio/dynamics administered alachlor (Lasso Technical) in the diet to groups of 50 male and 50 female Long-Evans rats at concentrations of 0, 100, 300, or 1000 ppm (0, 14, 42 and 126 mg/kg/day, respectively) for 812 to 813 days (males) and 741 to 744 days (females). Two different lots of the technical alachlor were used during the study: Lot #XHI-167, stabilized with 0.5% epichlorohydrin* (for the first 11 months of the study) and Lot #MHK-6, [redacted] (for the remainder of the study). The following incidence of tumors were observed.

STUDY # 1

Tumor Site and Type	Sex	Dose (mg/Kg/day) 0	14	42	126
Stomach:					
leiomyosarcoma	M	0/49	0/50	0/50	1/50
	F	0/50	0/50	0/50	1/49
osteosarcoma	M	0/49	0/50	0/50	3/50
	F	0/50	0/50	0/50	4/49
gastric adenocarcinoma	M	0/49	0/50	0/50	2/50
	F	0/50	0/50	0/50	1/49
malignant mixed gastric tumor	M	0/49	0/50	0/50	11/50
	F	0/50	0/50	1/50	17/49
Thyroid:					
follicular adenoma	M	1/48	0/50	1/49	11/50
	F	0/49	0/44	2/46	2/49
follicular carcinoma	M	0/48	0/50	0/49	2/50
	F	0/49	0/44	0/46	2/49
Nasal Turbinates respiratory epithelium:					
adenomas	M	0/46	0/46	10/41	23/42
	F	0/49	0/47	4/42	10/48
carcinomas	M	0/46	0/46	1/41	0/42
	F	0/49	0/47	1/42	0/48

*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.

Nasal turbinate tumors (mainly benign) were significantly increased in both males ($p<0.001$) and females ($p<0.02$) at the mid dose level (42 mg/kg/d) and above.

Stomach malignant tumors increased significantly ($p<0.001$) in both sexes at the high dose level.

Thyroid follicular tumors (adenomas and carcinomas) were significantly increased in males at the high dose level ($p<0.001$).

The lowest dose of alachlor tested in this study probably exceeded a MTD as evidenced by high mortality, compared to controls. Increases in organ weights (liver, kidney, spleen, et al.) were also noted, as were gross findings, at all dose levels, indicative of a compound related effect.

2. A Chronic Feeding Study of Alachlor in Rats:

Monsanto administered technical alachlor (94.13%) in the diet to groups of 50 male and 50 female Long-Evans rats at concentrations of 0, 0.5, 2.5 and 15.0 mg/kg/day for 25 to 26 months. The alachlor was stabilized with [redacted] Epichlorohydrin was not used as a stabilizer. The following incidence of tumors/lesions was observed.

		STUDY #2 Dose (mg/kg/day)			
Tumor Type and Site	Sex	Control 0	Low 0.5	Medium 2.5	High 15.0
Thyroid					
follicular					
adenoma	M	2/49	4/50	3/49	4/49
	F	1/49	1/49	0/49	2/47
carcinoma	M	1/49	3/50	1/49	2/49
	F	3/49	1/49	1/49	1/49
Thymus					
lymphosarcoma	M	0/49	3/50	1/46	0/50
	F	0/48	1/50	2/48	3/43
Adrenal					
pheochromocytoma					
benign	M	8/50	7/50	2/50	6/50
	F	1/49	1/50	3/50	5/49
malignant	M	2/50	2/50	0/50	2/50
	F	1/49	3/50	0/50	0/49
Nose/Turbinates					
respiratory epithelium					
adenoma	M	0/45	1/48	0/45	11/45
	F	0/42	0/44	1/47	9/48
neurofibroma	M	0/45	1/48	0/45	0/45
	F	0/42	0/44	0/47	0/48
submucosal gland					
adenoma	M	0/45	1/48	1/45	0/45
	F	0/42	0/44	0/47	3/48
epith. hyperplasia/					
metaplasia	M	1/45	1/48	1/45	1/45
	F	0/42	0/44	0/47	1/48
submucosal gland					
hyperplasia	M	1/45	1/48	3/45	11/45

Nasal turbinate tumors were significantly elevated ($p<0.01$) in both males and females at 15 mg/kg/day (the highest dose tested). One female rat in the mid dose group also had this tumor and one male in this group had a submucosal gland adenoma.

Thymus lymphosarcomas and adrenal pheochromocytomas were significantly increased ($p<0.05$) in the high dose females.

There was a non-significant increase in thyroid follicular cell tumors in the high dose male group.

> The highest dose tested probably exceeded a 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.)

Monsanto was requested to reevaluate the submucosal gland hyperplasia seen in both males and females. Experimental Pathology Laboratories, Inc. (EPL) performed a histological reevaluation; their report indicated that the submucosal nasal lesions (hyperplasia) were not neoplastic, however their analysis reflected a slightly higher incidence of adenomas of the nasal cavity. EPL's diagnosis is compared with that of Monsanto in the table below.

	Nasal turbinate adenomas			
	EPL's data		Monsanto's data	
Group (mg/kg/day)	Males	Females	Males	Females
0	0/44	0/42	0/45	0/42
1.5	0/47	0/42	0/48	0/44
3.5	0/44	1/47	0/45	1/47
15.0	15/45	14/48	11/45	9/48

3. A Special Chronic Feeding Study With Alachlor

In a study performed by Monsanto, alachlor was administered in the diet to Long-Evans rats at a concentration of 126 mg/kg/day. After a period of exposure (5-8 months) sufficient to induce ocular lesions (as confirmed by the consulting ophthalmologist) the treated animals were divided into 3 groups*. Group I animals were designated to remain on the treatment diet until the end of the two-year study period; group II animals were selected, based on the status of their ocular lesions, for interim sacrifice; and group III animals, based on predicted potential recovery from ocular lesions, were placed on untreated diets for the remainder of the study period. The control group from Study #2 discussed above under section 2 can also be considered here since the two studies were run concurrently.

*The grouping process was by design selective for susceptibility for ocular lesions and not a random selection, however, 39% of the females were affected

STUDY #3

		Control	Group I	Group III
Nasal turbinates				
respiratory epithelium adenoma	M	0/45	42/61	10/17
	F	0/42	11/25	19/46
carcinoma	M	0/45	7/61	0/17
	F	0/42	2/25	1/46
Thymus lymphosarcoma	M	0/49	1/68	1/16
	F	0/48	0/25	1/43
Adrenal pheochromocytoma				
benign	M	8/50	8/70	2/20
	F	1/49	0/31	2/48
malignant	M	2/50	2/70	1/20
	F	0/49	0/31	0/48
Thyroid follicular				
adenoma	M	2/49	8/69	1/20
	F	1/49	4/31	3/49
carcinoma	M	1/49	10/69	1/20
	F	3/49	0/31	1/49
Stomach				
mixed carcino-sarcoma	M	0/50	3/68	0/20
	F	0/50	19/31	0/49
anaplastic sarcoma	M	0/50	1/68	0/20
	F	0/50	3/31	0/49
adenocarcinoma	M	0/50	0/68	0/20
	F	0/50	10/31	0/49
leiomyosarcoma	M	0/50	0/68	0/20
	F	0/50	10/31	0/49
undiff. sarcoma	M	0/50	0/68	0/20
	F	0/50	16/31	2/49
undiff. carcinoma	M	0/50	0/68	0/20
	F	0/50	3/31	0/49
Brain				
neuroepithelioma	M	0/50	1/70	0/20
	F	0/50	1/31	1/49

(continued)

STUDY #3 (continued)

		Control	Group I	Group III
Liver				
hepatoma	M	1/50	3/70	0/20
	F	0/50	1/31	0/49
neoplastic nodule	M	0/50	0/70	0/70
	F	0/50	1/31	1/49
hepatocellular carcinoma	M	2/50	2/70	0/20
	F	0/50	2/31	1/49

Note that nasal turbinate adenomas developed in rats exposed to alachlor for only 5-6 months at the beginning of the study (Group III).

The MTD was exceeded in female rats, as evidenced by a statistically significant increase in mortality; (In males, this single dose tested probably approached MTD.)

Monsanto submitted a reevaluation of the neuroepitheliomas seen in this study; electron microscopy of such a tumor from one of the animals showed "intermediate fiber typical of keratin", from which Monsanto concluded that the tumor was epithelial, not neural. C.I.I.T. also reevaluated all three brain tumors and concluded that they were extensions of nasal adenocarcinomas and not brain tumors. However, a discrepancy in animal numbers and diagnoses remains to be resolved before either Monsanto's or C.I.I.T.'s conclusions can be accepted (J. Hauswirth Memo). Monsanto has been informed of this discrepancy.

4. An 18 Month Oncogenic Study in Mice

In a study performed by Bio/dynamics, alachlor (Lasso technical)* was administered in the diet to groups of fifty male and fifty female CD-1 mice at dosages corresponding to the following levels: 0, 26, 78 and 260 mg/kg/day. The incidence of pertinent non-neoplastic and neoplastic changes are tabulated below.

		STUDY #4 Dose (mg/kg/day)			
		Control 0	Low 26	Mid 78	High 260
Lung bronchiolar-alveolar	M	6/50	1/50	4/50	10/50
adenoma	F	2/50	4/50	7/50	10/50
carcinoma	M	3/50	5/50	7/50	2/50
	F	1/50	1/50	1/50	1/50
fibrosarcoma	M	0/50	0/50	0/50	0/50
	F	0/50	0/50	0/50	1/50
congestion	M	1/50	13/50	13/50	12/50
	F	5/50	5/50	12/50	16/50
Liver					
adenoma	M	5/50	1/50	4/50	7/50
	F	0/50	0/50	0/50	1/50
carcinoma	M	0/50	3/50	1/50	4/50
	F	0/50	0/50	1/50	0/50
Uterus					
leiomyoma	F	0/50	2/50	0/50	0/50
leiomyosarcoma	F	1/50	0/50	2/50	3/50
endometrial carcinoma	F	0/50	1/50	0/50	0/50
endometrial polyp	F	1/50	3/50	0/50	3/50
granular cell myoblastoma	F	0/50	0/50	0/50	1/50

*Alachlor was supplied in two batches: Lot MHI-167 used during the first 11 months of the study was stabilized with 0.5% epichlorohydrin; Lot MHK-6, used during the last 7 months, was stabilized with [redacted]

The major target organ for oncogenicity was the lung. The incidence of lung bronchioalveolar tumors was significantly increased in the high dose females ($p<0.05$) and was also significant ($p<0.01$) if the high dose females which died in extremis during the study. The incidence of lung tumors in females which died during the study was:

Control	0/30
Low	1/37
Mid	0/27
High	7/35

The MTD 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.

Monsanto submitted an addendum to this study on 2/25/85. The report contains 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.

5. Historical Control Information

Historical control data on lung tumors in CD-1 mice could be found in the open literature:

I MSD Study: Sher et al. Toxicology Letters 11:103-110, 1982.

N - animals:	M	1232	N - groups:	M	24	Age: 81-105 weeks
	F	1240		F	24	
adenoma	M	0-38%				
	F	0-41%				
adenocarcinoma	M	0-16%				
	F	0-12%				

II Homburger Data: Homburger et al. J. Natl. Cancer Inst. 55:37-43, 1975.

N - animals	M	99
	F	102

		18 months
adenoma	M	2
	F	4
adenocarcinoma	M	-
	F	1

The MSD study duration was too long, so that comparisons based on these controls could not be made, however the study length from which the Homburger Data was derived, was appropriate. These latter control values were exceeded in the treated animals of study #4; furthermore, the Homburger data appear to indicate that the response seen in concurrent male controls was high, which could be masking the true response in the treated males.

Additional historical control data obtained from Bio-dynamics on the incidence of lung and liver tumors in CD-1 mice for concurrently run studies were discussed but were also found to be inappropriate because the length of the studies was 23-25 months, exceeding the 18 months of the Alachlor study.

Historical control data for the rats was requested from Monsanto, but has not been made available at this time.

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E. Additional Toxicology Data on Alachlor:

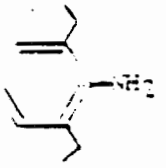
1. Metabolism:

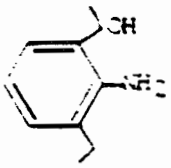
Fourteen metabolites of alachlor have been found in the urine and 13 in feces of Sprague-Dawley rats fed alachlor. Only three of these were found in both urine and feces (Figure 1 & 2). Approximately 89% of the radioactivity is eliminated in urine and feces (1:1) within four days; the rate of elimination is biphasic. Mercapturic acid, glucuronic acid, sulfate conjugation and side chain hydroxylation are important metabolic pathways in the rat. One metabolite found in rat urine, N-[2-ethyl-6-(1-hydroxyethyl)-phenyl]-N-(methoxymethyl)-2(methylsulfonyl)acetamide (metabolite XII), was mutagenic in the Ames salmonella assay, both with and without metabolic activation.

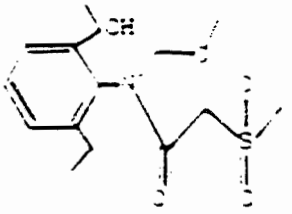
In Rhesus monkeys, 5 conjugates were identified in urine only (Figure 3) when alachlor was given intravenously: 92-94% of the total radioactivity was excreted in the urine during the first 24 hours and 91-94% in the feces during the first 48 hours (9-10:1). Studies via 2 other routes (intramuscular and topical) were considered unacceptable.

In human biomonitoring studies, metabolites which contained diethyl aniline (DEA) and hydroxy-ethyl, ethyl aniline (HEEA) moieties of alachlor were identified in urine.

Note that metabolites with both the HEEA and DEA moieties were found in both humans and rats (metabolite XII also contains the HEEA moiety); and while Monsanto claims that the monkey is a "better model for man in the case of alachlor"* in monkeys, only metabolites with the DEA moiety were found.

DEA: 

HEEA: 

Metabolite XII: 

2. Non-Oncogenic Toxicological Effects

The acute oral LD_{50}'s in the rat of alachlor (90%) and technical alachlor are 1.8 g/kg and 0.93-1.2 g/kg, respectively. In mice the acute oral LD_{50} of technical alachlor is 2.1 g/kg.

In a 3-generation reproduction study in Charles River Sprague-Dawley CD rats, the NOEL was 10 mg/kg based on kidney effects (chronic nephritis, hydronephrosis) seen in F_2 adult males and F_{3b} male pups.

In a one year subchronic beagle dog study the NOEL was 1 mg/kg/day based on hemosiderosis seen in liver, kidney and spleen of dogs in the 3 and 10 mg/kg/day groups.

Alachlor was not teratogenic to rats at 400 mg/kg/day (HDT).

A NOEL for non-neoplastic toxicity was established for alachlor in a 2-year chronic feeding/oncogenicity study in Long-Evans rats. The NOEL was 1.5 mg/kg/day based upon molting of retina pigmentation and increased mortality rate in the females and abnormal disseminated foci in male liver.

*[Monsanto's Rebuttal to Alachlor PD-1]

3. Mutagenicity:

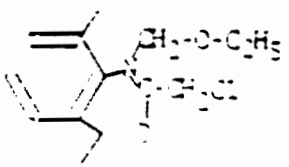
The results of mutagenicity testing conducted on alachlor are summarized in the following table.

Test	Core Classification	Result	Comments
Ames Assay	acceptable	negative	a positive response was seen at 5000 ug/plate in TA 1535 but the response was not repeated for consecutive doses.
Gene mutation in CHO cells HGPRT locus	acceptable	negative	
In-vivo bone marrow chromosome aberration assay	acceptable	negative	no structureal or numerical chromosomal aberrations
In-vivo - in vitro hepatocyte DNA repair assay	acceptable	positive	positive at highest dose tested (1.0g/kg/day) - "weakly genotoxic"
DNA damage in B. subtilis M45 and H17	acceptable	negative	did not cause DNA damage. (20-20,00 ug/plate)

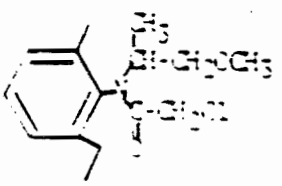
As noted in the metabolism section of this report one metabolite of alachlor tested positive in the Ames assay (TA 100 - both with and without metabolic activation over six test doses).

4. Structure-Activity Correlations:

Alachlor is structurally related to metolachlor and acetochlor, structures of which are shown below.



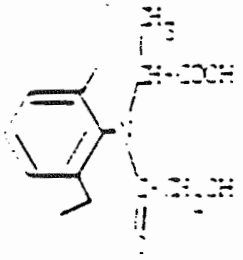
ACETOCHLOR



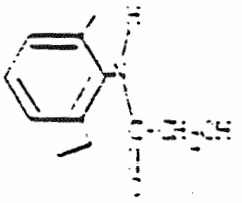
METOLACHLOR

[redacted]

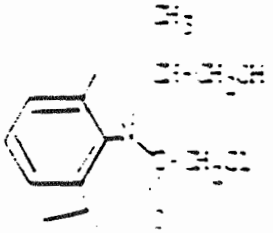
Limited mutagenicity data are available on metolachlor. It has been reported to be negative in the Ames salmonella assay and did not have any effects on fertility, zygote or embryo survival in the in vivo developing sperm mouse assay. Metolachlor, when fed to CD rats at levels of 30, 300 and 3000 ppm caused an increase in proliferative liver lesions (neoplastic nodules) in the high dose female rats. In this study nasal turbinate tumors were seen in two high dose males and one high dose female. Metolachlor was negative for oncogenicity in the mouse. Metolachlor has been evaluated in Peer Review as a class C carcinogen. Identified metabolites of metolachlor are shown below:



Urine & Feces



Urine only



Feces only

F. Weight of Evidence Considerations:

The committee considered the following facts regarding toxicology data on alachlor to be of importance in a weight of evidence determination of oncogenic potential.

1. Administration of alachlor in the diet to Long-Evans rats is associated with statistically significant increases in incidence over the control in the following tumors:

   Nasal turbinate tumors (mostly benign) at mid and high doses, in both sexes.

   Thyroid follicular tumors in male rats.

   Malignant stomach tumors in male and female rats.

2. Administration of alachlor in the diet to female CD-1 mice is associated with a statistically significant increase in lung tumors (bronchiolar-alveolar adenomas and carcinomas) in female mice.

3. Alachlor was tested in several in vitro and in vivo assays for mutagenicity and/or DNA damage. Of these only the in vivo - in vitro hepatocyte DNA repair assay was positive - and only at the HDT. It was judged, therefore, to be "weakly genotoxic", however a metabolite of alachlor was found to be positive in the Ames Test (Strain TA 100), both with and without metabolic activation over 6 test doses.

4. The metabolite referred to above is a moiety common to metabolites found in both humans and rats (but not in monkeys). This data is significant in-so-much as Monsanto maintains that the monkey is a "better model for man in the case of alachlor" [Monsanto's Rebuttal to Alachlor PD-1].

5. [redacted]

6. Metolachlor, another structurally related herbicide, when fed to CD rats, caused an increase in liver neoplastic nodules in the high dose females. In this same study, nasal turbinate tumors were seen in 2 high dose males and 1 high dose female, however metolachlor was negative for oncogenicity in the mouse.

PENDING REGISTRATION INFORMATION IS NOT INCLUDED

G. Classification of Oncogenic Potential:

Criteria contained in the final draft of the proposed EPA Guidelines (12/1/85) for classifying a carcinogen were considered. These Guidelines state that "Sufficient evidence of carcinogenicity indicates that there is an increased incidence of malignant tumors or combined malignant and benign tumors: a) in multiple species [MET] or strains; b) in multiple experiments [MET] (e.g., with different routes of administration or using different dose levels; or c) to an unusual degree in a single experiment with regard to high incidence [MET], unusual site or type of tumor [MET], or early age of onset [MET]. Additional evidence may be provided by data on dose-response effects [MET], as well as information from short-term tests [partially MET] or on chemical structure [MET]".

Alachlor met all but one of the criteria specified for the B-2 classification, any of which alone can be sufficient for such a classification. 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 another experiment with Long-Evans rats, nasal turbinate tumors occured 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. Additionaly, a metabolite of alachlor was mutagenic in the Ames Test at 6 dose levels.

[redacted] Metolachlor when fed to CD rats, caused an increased incidence of neoplastic nodules in females at the high dose; metolachlor was negative for oncogenicity in the mouse.

The committee concluded that the data available for alachlor (from animal studies) is sufficient for its classification as a B-2 "Probable Human Carcinogen".

H. Major Rebuttals by Monsanto

The committee also addressed the following major points:

.. It is contended that the rat is not the appropriate model for assessing potential effects on humans; rather the monkey is more appropriate.

The committee disagrees since for this chemical it appears that the rat produces metabolites similar to those observed in man. Moreover, these very metabolites belong to the class of alachlor metabolites which seem to have mutagenic activity (refer to sections on Metabolism and Mutagenicity).

PENDING REGISTRATION INFORMATION IS NOT INCLUDED

H. Major Rebuttals (continued)

2. It is contended that nasal turbinate tumors are strain specific (Long-Evans Rat).

The committee found no evidence that this is anything other than conjecture - ~~no other rat strain has been tested~~ IBT has applied to SIRB although not yet. Furthermore, nasal turbinate tumors were not the only response in Long-Evans rats.

3. It is contended that the "effects" are not seen in monkey and dog.

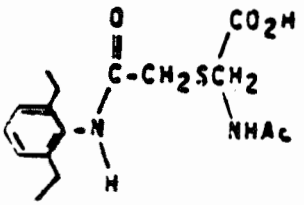
The committee concluded that data for subchronic (less than lifetime) exposure of other species can not refute oncogenic effect in a lifetime study.

4. It is contended that the mouse study did not show any oncogenic effect for alachlor.

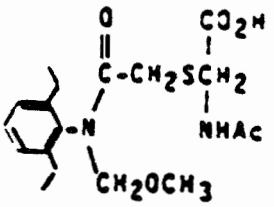
The committee disagrees with this conclusion (see review of mouse study, section D.4)

EPA's detailed response to Monsanto's Rebuttal is appended to this panel report.

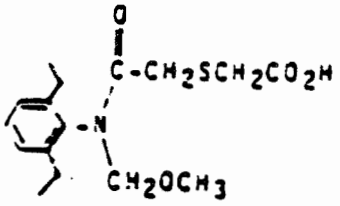
FIGURE 3 METABOLITES OF ALACHLOR



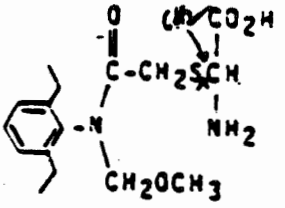
Secondary Mercapturate (4)



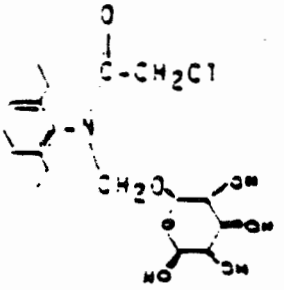
Tertiary Mercapturate (5)



Thioacetic Acid Conjugate (6)



Cysteine Conjugate (7)



Glucuronide Conjugate (8)

Page_____ is not included in this copy.

Pages 77 through 78 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.

____ Description of quality control procedures.

____ Identity of the source of product ingredients.

____ Sales or other commercial/financial information.

____ A draft product label.

____ The product confidential statement of formula.

____ Information about a pending registration action.

✓ FIFRA registration data.

____ The document is a duplicate of page(s) _____.

____ The document is not responsive to the request.

The information not included is generally considered confidential by product registrants. If you have any questions, please contact the individual who prepared the response to your request.

Appendix II

007699

FEDERAL INSECTICIDE, FUNGICIDE, AND RODENTICIDE ACT

SCIENTIFIC ADVISORY PANEL

A Set of Scientific Issues Being Considered by the Agency in Connection with the Special Review of Alachlor

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The Federal Insecticide, Fungicide, and Rodenticide Act (FIFRA) Scientific Advisory Panel (SAP) has completed review of the data base supporting the Environmental Protection Agency's (EPA) preliminary decision to cancel the registrations of all pesticide products containing the active ingredient Alachlor unless certain modifications to the terms and conditions of registrations are made by the registrants. The review was conducted in an open meeting held in Arlington, Virginia, on November 19, 1986. All Panel members except Dr. Harold L. Bergman, Dr. John J. Lech, and Dr. Thomas W. Clarkson were present for the review. Although Dr. Lech was not present at the meeting, he provided his comments via the telephone to the Chairman of the Scientific Advisory Panel and agreed with the Panel's recommendations.

Public notice of the meeting was published in the Federal Register on Friday, October 24, 1986.

Oral statements were received from staff of the Environmental Protection Agency and from Mr. Robert Harness, Monsanto Company.

In consideration of all matters brought out during the meeting and careful review of all documents presented by the Agency, the Panel unanimously submits the following report.

REPORT OF PANEL RECOMMENDATIONS

Alachlor

The Agency requested the Panel to focus its attention upon a set of scientific issues relating to the Special Review of Alachlor. There follows a list of the issues and the Panel's response to each issue:

. . Toxicological Issues

The Agency has classified Alachlor as a category B_2 probable human carcinogen.

1. Part of the basis for this classification is a statistically significant increase in lung bronchioalveolar tumors at the highest dose tested in female mice.

Panel Response:

The Panel does not agree with the Agency's interpretation of the female mouse lung tumor data. The technical support document incorrectly suggests that the [1] Sher paper's data are from mice 81 to 105 weeks of age. In reality, the paper clearly states that "Almost all studies were of 81 weeks duration in mice ...". The Panel recognizes that lung tumor incidence in mice represents one of the more variable endpoints in carcinogenesis bioassays. The control incidence for adenomas/carcinomas in the Monsanto study was 6%, whereas the average incidence in 1240 control mice was 17% (range 0-41%). Thus, the finding of a lung tumor incidence of 22% in the high dose group was considered by the Panel to be within the limits of normal variation. Additional support for this interpretation was provided by the lack of evidence of progression from benign to malignant tumors, and the lack of an increase in tumor multiplicity in treated mice.

2. The rat is an appropriate model for predicting human risk for alachlor.

Panel Response:

The Panel agrees that more data are available on metabolism of alachlor and on the carcinogenic potential of this chemical in rats than in other species. The Panel concurs that alachlor is carcinogenic for the rat and that it produces nasal adenomas and adenocarcinomas, an unusual type of neoplasm that has a low spontaneous incidence. However, whether the rat (or any other nonhuman species) is an appropriate model for predicting human risk for alachlor is not presently an answerable question. The Panel believes that the monkey may be a better metabolic surrogate for man than is the rat; unfortunately, data are not available on the tumorigenicity of alachlor in the monkey, so the circle of evidence for carcinogenesis risk evaluation in this species cannot be closed. Thus, the only positive evidence on which to evaluate human risk of carcinogenicity is the rat data. (The Panel does not believe that the mouse data show that

[1] Sher, S.P., et al. (1982) Spontaneous Tumors in Control F344 and Charles River-CD Rats and Charles River CD-1 and B6C3HF1 Mice. Toxicology Letters 11:103-110

alachlor is carcinogenic in this species.) The Panel suggests, however, that metabolic data from the monkey may be used to scale the interpretation of risk from the rat data.

The Panel believes that alachlor should be classified as a B_2 carcinogen based on the production of an unusual type of neoplasm in the rat, coupled with the finding that two metabolites of alachlor are mutagenic. While the Panel is not comfortable with the implied conclusion of the EPA Guideline that this classification means that alachlor is a probable human carcinogen, the data available clearly meet the criteria for B_2 classification.

B. Exposure Issues

The Agency estimated applicator dosage by pooling Monsanto patch data and surrogate patch data from published exposure studies to calculate a range of exposure, and then applied this range to the biomonitoring dosage.

Panel Response:

It was encouraging to see the use of a limited amount of biomonitoring data, both by the Agency and by the Monsanto Company. Even though the data are limited in scope, it can serve to corroborate the exposure assessment generated by the Agency. We compliment the Agency on expediting the incorporation of the 1985 Monsanto biomonitoring study in its exposure and risk assessment evaluation.

FOR THE CHAIRMAN

Certified as an accurate report of Findings:

Stephen L. Johnson,
Executive Secretary
FIFRA Scientific Advisory Panel

Date: 4/25/86

Appendix III

Now, if you use time to tumor, you have another factor that you are throwing in, and it does not necessarily make the risk go down by using maximum dose now, because the timing is different. In other words, the timing comes in so early, so much earlier, that the monotonicity is restored.

DR. SWENBERG: Is that Monsanto's position also, that the 126 exceeded the MTD?

MR. HARNESS: Yes, it is.

DR. SWENBERG: And what about 42; is that accepted by everyone as meeting an MTD?

DR. HAUSWIRTH: We feel as if it probably slightly exceeded an MTD; 14 and 15 milligrams probably approached the MTD.

DR. SWENBERG: Does Monsanto agree with that?

MR. HARNESS: This is Thomas Furman. Dr. Furman is in our Toxicology group.

DR. FURMAN: Yes, we do agree that 126 exceeded the MTD. I would say that 42 is probably about the MTD according to my definitions. But there are a lot of definitions or interpretations of MTD. But if I were repeat... this study solely for purposes of doing that, I would probab...

run it with about the 40 milligram per kilogram level as an MTD dosage.

DR. SWENBERG: Okay. Well, with that in mind, I am looking on Table 4 of the support document, and we only end up with one tumor type that is statistically significant, and that is the adenoma, the nasal turbinate; is that correct?

DR. HAUSWIRTH: At 14 or 15 milligrams per kilogram.

DR. SWENBERG: And at 42.

DR. HAUSWIRTH: And at 42. That's correct.

DR. SWENBERG: And so, if we disagree with the mouse data, and this is what we end up with, this doesn't end up as a B2 carcinogen, I don't believe.

DR. HAUSWIRTH: If you look at the cancer guideline of the Agency.

DR. SWENBERG: Right.

DR. HAUSWIRTH: You are saying get rid of the mouse data. If you disallow that, if you disallow the tumors that were seen at 126 milligrams per kg --

DR. SWENBERG: That's correct.

DR. HAUSWIRTH: Can I get out an overhead of

the guidelines -- or is it in your package.

DR. SWENBERG: We've got the guidelines here.

DR. HAUSWIRTH: Okay. It will just take me a minut to find my copy.

DR. SWENBERG: "A malignant tumor response in a single, well-conducted experiment that does not meet conditions for sufficient evidence. A tumor response of marginal statistical significance" -- "benign but not malignant tumors" -- "and an agent that shows no response in short-term tests" -- well, that doesn't quite fit.

DR. HAUSWIRTH: Criteria A would drop out, with what you've just said. Criteria B and multiple experiments, that would hold. We would have two experiments showing a positive result.

Under Criteria C, to an unusual degree -- I'd have to look at the data -- this is not a commonly-occurrin tumor, so it would be considered to be to an unusual degree It is also not commonly-occurring, so it would be considere to be an unusual site or type of tumor. We also, not at this dosage but at the higher dosage, have evidence of ear onset..

So we do meet Criteria B and C of the guideline

Appendix IV

COMMENTS IN REPLY TO EPA'S FEDERAL REGISTER NOTICE OF OCTOBER 8, 1986 THE ALACHLOR SPECIAL REVIEW TECHNICAL SUPPORT DOCUMENT DATED SEPTEMBER, 1986

Monsanto wishes to acknowledge that the September, 1986 technical support document (TSD) for alachlor products, recognized the significant benefits derived from the use of these products, and concluded that exposures and theoretical risks, were generally lower than previously stated in the January, 1985 PD-1 Document.

In responding to the alachlor TSD, Monsanto believes there are two major issues that warrant reconsideration by the Agency: I. The proposed restricted use classification and, II. The closed system requirement for 300 or more acres. We believe that information not previously considered by the Agency, and the conclusions reached by the EPA Scientific Advisory Panel (SAP) provide a sound basis for the Agency to modify their proposed regulatory position on these two points. Additionally, line by line comments to the TSD are provided.

I PROPOSED RESTRICTED USE CLASSIFICATION

Monsanto disagrees with the Agency proposal to restrict the use of alachlor to certified applicators or persons under their direct supervision. There are two important considerations that bear on this point: A) the categorization of alachlor in the current EPA classification system for carcinogenicity, and B) the assessment of the most up-to-date applicator exposure data.

A. Categorization of Overall Weight of Evidence for Human Carcinogenicity.

In the TSD the Agency has proposed a 'B2, Probable Human Carcinogen' categorization for alachlor. However, as

emphasized by the (SAP) in its recent review, the appropriate categorization of alachlor in the EPA classification scheme is not entirely clear. Categorization is a subjective process that is very much dependent upon interpretation of the weight of the evidence data combined with a certain perspective on the various category requirements. In contrast to the Agency's position, Monsanto contends that an equally plausible view of the alachlor data base can lead to the conclusion that the criteria for a B2 classification are not met. Monsanto submits that the following analysis is plausible and leads to the conclusion that alachlor is best categorized as 'C, Possible Human Carcinogen'. This must be considered in the Agency's final determination of proposed product restrictions.

The "Guidelines for Carcinogen Risk Assessment" (FR Vol. 51, No. 185, September 24, 1986) propose in "Table 1. Illustrative Categorization of Evidence Based on Animal and Human Data", that the following criteria would result in a 'B2, Probable Human Carcinogen' categorization:

a. there is "inadequate evidence" or "no data" from epidemiologic studies.

b. the weight of evidence of carcinogenicity based on animal studies is "sufficient".

These guidelines state that "sufficient" evidence of carcinogenicity in animal studies is attained when an agent causes an increased incidence of malignant tumors or combined malignant and benign tumors:

i. in multiple species or strains; or

ii. in multiple experiments (e.g. with different routes of administration or using different dose levels); or

iii. to an unusual degree in a single experiment with regard to high incidence, unusual site or tumor type, or early age at onset.

The following criteria apply for a 'C, Possible Human Carcinogen' categorization:

c. there is "inadequate evidence" or "no data" from epidemiologic studies

d. the weight of evidence of carcinogenicity based on animal studies is "limited".

Furthermore, the Agency has stated that the category assignments presented in these guidelines (i.e. Table 1. IV.C) "... are for illustrative purposes. There may be nuances in the classification of both animal and human data indicating that different categorizations than those given in the table should be assigned. Furthermore, these assignments are tentative and may be modified by ancillary evidence. In this regard all relevant information should be evaluated to determine if the designation of the overall weight of evidence needs to be modified. Relevant factors to be included along with the tumor data from human and animal studies include structure-activity relationships, short-term test findings, results of appropriate physiological, biochemical, and toxicological observations, and comparative metabolism and pharmacokinetic studies. The nature of these findings may cause an adjustment of the overall categorization of the weight of evidence."

In view of these considerations, it is Monsanto's opinion that alachlor does not definitively meet the criteria for "sufficient" evidence of oncogenicity based on animal studies, and thus does not meet the requirements for a B2 classification. There is also a lack of general genotoxic potential for alachlor in mammals and there are significant species differences in its metabolism and elimination. In consideration of all data available for alachlor (i.e. 'overall weight of evidence'), there is only limited evidence of oncogenicity.

1. Lack of oncogenicity in multiple species:

Monsanto submits that alachlor does not increase the incidence of malignant and/or benign neoplasms in multiple species or strains. In PD-1 and the TSD the Agency stated that alachlor was oncogenic in both rats and mice. The Agency concluded that a statistically significant increase in the incidence of lung bronchio-alveolar tumors observed in the high dose level female mice was attributable to alachlor administration.

Monsanto, SAP, and expert consultants disagree with the Agency's conclusion regarding the mouse study and maintain that the observed lung tumors are spontaneous in origin and that alachlor is not oncogenic in the mouse. It is important that interpretation of the mouse data not be influenced by the rat data since the number of species affected is important in a weight of evidence evaluation. The results of experiments conducted in each species must be evaluated independently. This view was supported by the SAP.

Monsanto has included a detailed assessment of the mouse study in Appendix A and requests the EPA to carefully review this information. The cumulative evidence contained

in this appendix supports the conclusion that the statistically significant increase in lung adenomas in female mice is unrelated to administration of alachlor and can be summarized as follows:

- these tumors are very common in this strain of mouse;
- the incidence in the control female mice was unusually low when compared to both concurrent control male mice and historical control values;
- there was no increased incidence of the lung tumors in male mice
- the change in incidence pattern with increasing age that is known to occur was atypical in the case of the control females;
- there was no increased multiplicity of tumors;
- there was no increase in hyperplasia or progression from benign to malignant lung tumors.

The SAP in reaching the conclusion that alachlor is not oncogenic in the mouse cited the following:

- The panel concurred with with Monsanto regarding the appropriateness of the historical control data provided by Sher, et al. Although the data cited in the Sher paper are noted to be from mice, 81 to 105 weeks of age, the article clearly stated that "almost all studies were of 81 weeks duration in the mice".

- Lung tumor incidence in mice is one of the more variable end-points in carcinogenesis bioassays. The control incidence for adenomas/adenocarcinomas in the Monsanto study was 6%, whereas the average incidence in 1240 control mice was

17% (range 0 to 41%). Thus the finding of a lung tumor incidence of 22% at a high dose group in the Monsanto study was within the limits of normal variation.

- Additional support was provided by the lack of evidence of progression from benign to malignant tumors, having no effect on survival due to lung tumors, and the lack of an increase in tumor multiplicity in treated mice.

During the SAP review, Dr. Robert A. Squire, DVM, PhD, of Johns Hopkins stated that in his opinion the tumors observed were not related to treatment. Also important to his determination was the singular nature of the observed lesions.

Therefore, it is Monsanto's belief that the Agency must conclude that alachlor is not oncogenic in the mouse and, does not meet the multiple species or strain criteria for "sufficient" evidence.

2. Questionable Malignant Tumor Response in Multiple Experiments:

It is Monsanto's position that malignant tumors, or combined malignant and benign tumors, have not been observed in multiple bioassays conducted with levels of alachlor at or below the maximum tolerated dose (MTD). During the course of the alachlor SAP hearing, both the Agency and Monsanto were questioned about the MTD in the alachlor chronic rat studies. Both the Agency and Monsanto agreed that the dose level of 126 mg/kg/day was unquestionably beyond the MTD. The Agency further stated that the dose level of 42 mg/kg/day also exceeded the MTD.

Evaluation of tumor response patterns at dosage levels that do not exceed the MTD results in the finding of statistically significant increases in combined malignant and benign tumors on only one study. The incidence of nasal adenomas/adenocarcinomas in males and females administered 42 mg/kg/day on the first alachlor chronic rat study (BD-77-421) and nasal adenomas in males and females administered 15 mg/kg/day on the second alachlor chronic rat study (ML-80-186) were increased when compared to control incidences. No benign or malignant tumors considered to be treatment related were observed at the dosage level of 14 mg/kg/day on the first alachlor chronic rat study and no malignant tumors considered to be treatment related were observed at any of the dosage levels (i.e. 0.5, 2.5 and 15 mg/kg/day) on the second chronic rat study. Malignant tumors were observed on only one study; a response which does not meet the criteria for 'malignant or combined malignant and benign tumors' in multiple experiments required for "sufficient" evidence.

If the dosage level of 42 mg/kg/day is also considered to have exceeded the MTD, as stated by the Agency, then the only tumor response that displayed statistical significance is an increase in benign nasal adenomas in male and female rats on one study (ML-80-186). This response does not meet the multiple experiment criteria for "sufficient" evidence. Further, an increase in only benign tumors is considered an indication of "limited" evidence.

Monsanto recognizes that there are many questions and uncertainties related to the interpretation of oncogenicity data obtained at exposure levels exceeding the MTD. During the SAP hearing on oxadiazon the panel stated that "...data from an additional mouse study and a rat study were considered compromised due to dosing regimes that exceeded the Maximum Tolerated Dose (MTD)". In the recently issued

"Guidelines for Carcinogen Risk Assessment" and in its "Standard Evaluation Procedure" for rodent oncogenicity studies the Agency has stated that: "Positive studies at levels above the MTD should be carefully reviewed to ensure that the responses are not due to factors which do not operate at exposure levels below the MTD." This question is presently not answerable for alachlor.

These unanswered questions raise doubt about the appropriateness of using data obtained at dosage levels exceeding the MTD for purposes of categorizing the human oncogenicity potential of alachlor. Monsanto's position is that the "multiple experiment" criteria for "sufficient" evidence have not been definitively met for alachlor.

3. Lack of Unusual Degree, Site, Type or Early Onset:

It is Monsanto's opinion that alachlor has not induced an unusually high incidence, unusual site or unusual type of tumor in rats when administered dose levels which did not exceed the MTD. In addition, there is no evidence that alachlor administration results in the appearance of tumors at an early age.

In order for a given tumor type to be considered of an unusual nature or to occur at an unusually high incidence it is necessary to have a suitable data base for comparison. Without routine examination of a particular site/tissue on chronic bioassays there cannot be an adequate data base for assessing baseline incidences or the degree to which a tumor is unusual. It is Monsanto's belief that prior to the conduct and submission of the alachlor chronic rat study, the nasal turbinates of rats were not routinely or thoroughly examined microscopically. With an inacceptable historical database for nasal turbinates to compare with alachlor nasal tumor incidences,

any relative assessment of nasal tumor incidences is inappropriate. In review of the more recent literature, it is apparent that numerous compounds induce nasal turbinate tumors which suggests that this tumor type is not unusual. Furthermore, one would have to conclude from the categorization of metolachlor (which also causes nasal turbinate tumors in rats) as a class C oncogen, that the Agency doesn't consider nasal tumors to be unusual. Otherwise, metolachlor would have presumably met the criteria for "sufficient" evidence of oncogenicity to warrant a B2 categorization.

Recognizing that a greater number and variety of tumors were observed in the chronic rat studies at dosage levels of alachlor exceeding the MTD, one is left with the uncertainties of interpreting responses observed under these conditions. Monsanto's position is that the 'unusual degree of the response' criteria for "sufficient" evidence has not been definitively met.

4. Conclusion Related to Categorization for Oncogenic Potential:

Monsanto contends that alachlor does not definitively meet any of the criteria for "sufficient" evidence of carcinogenicity based on animal studies and thus can not be classified B2. Alachlor is not oncogenic in multiple species. Alachlor does not produce malignant tumors in multiple experiments at dose levels that do not exceed the MTD. Alachlor does not induce unusual tumors or numbers of tumors at dose levels that do not exceed the MTD. Only on consideration of responses observed at levels exceeding the MTD are some of these criteria met. Because of the uncertainties involved in attempting to interpret responses observed under these conditions, it is Monsanto's opinion that the criteria for "sufficient" evidence are not definitively met.

When other relevant factors are included in consideration of the alachlor categorization, the B2 classification appears even less appropriate. Results of genotoxicity testing from well validated assay systems with relevance to mammals present a consistent pattern of negative results which indicate that alachlor does not have a general genotoxic potential for mammals. Further, additional work completed by Monsanto does not support the conclusion that a genotoxic mode of action is associated with oncogenic effects observed in the rat. The demonstrated lack of clear genotoxic potential for alachlor in mammals provides evidence to support the conclusion that, despite evidence for oncogenic activity in the rat, alachlor is not a probable human carcinogen.

While not conclusive, the numerous metabolism and pharmacokinetic studies that have been conducted demonstrate a pattern of species differences in the metabolism and elimination of alachlor. This observation should be used to further scale interpretation, of the alachlor data as it applies to human risk as recommended by the SAP.

In summary, this determination of "limited" evidence for carcinogenicity in animal studies coupled with the absence of any positive human data suggest that a 'C, Possible Human Carcinogen' classification is the more appropriate if either of the two classifications are correct. This is consistent with the Agency's action on another registered product which is also a member of this chemical class.

Furthermore, it is wrong to perpetuate the label "probable human carcinogen" as an outcome of a rather mechanical fit into what was intended to be a flexible classification scheme and in the face of substantial information to the contrary. If alachlor is in a gray area between Class B2 and C as the SAP seemed to indicate in their dialogue

during the review, but must be forced fit into one class or the other, Monsanto requests that it be classed as a 'C, Possible Human Carcinogen'. If the Agency ultimately concludes that alachlor remain a B2, Monsanto requests the term "possible human carcinogen" be used as the category label for alachlor, based on the weight of all the evidence.

Alternatively, the Agency could elect to leave alachlor unclassified until a revision of the classification guidelines is undertaken as recommended by the SAP on February 11, 1986.

B. APPLICATOR EXPOSURE

Further evidence supporting Monsanto's position that alachlor should not be classified as Restricted Use lies in the expected extremely low levels of applicator exposure. To arrive at their estimates of exposure, the Agency has pooled surrogate patch data and Monsanto patch data and has also factored in biomonitoring data provided by Monsanto. The Agency states in the alachlor TSD that biomonitoring data, if supported by adequate and appropriate metabolism studies, generally provide a better measure of actual dosage received in the body than patch data. The Agency concludes from the bio-monitoring and pooled patch data that a range of applicator exposure correctly describes real world conditions. The Agency assumes this range to be two orders of magnitude. This range accommodates exposures from closed cab to open cab tractor and good agronomic practices to less careful work habits. Monsanto agrees that the variability in applicator exposure can span two orders of magnitude. However, the Agency in the TSD uses the value 0.0066 ug/kg bw per lb active ingredient as their

7/20/87

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CASWELL FILE

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

006013

JUL 20 1987

OFFICE OF
PESTICIDES AND TOXIC SUBSTANCES

MEMORANDUM

SUBJECT: **Alachlor** Metabolism Study in Monkeys submitted by Monsanto Company November 10, 1986
Accession Number 400009-01

Tox. Chem. No.: 11

FROM: Judith W. Hauswirth, Ph.D
Section Head, Section VI
Toxicology Branch/HED (TS-769C)

Frank W. Hauswirth 7/20/87

TO: David Giamporcaro, PM #79
Special Review Branch
Registration Division (TS-767C)

THRU: Theodore M. Farber, Ph.D., Chief
Toxicology Branch/HED (TS-769C)

Action Requested: Review Study entitled "The Metabolism of Alachlor in Rhesus Monkeys Part II Identification, Characterization and Quantification of Alachlor and It's Metabolites after Intravenous Administration to Monkeys."

Background: A preliminary report of this study was submitted to the agency and reviewed in a Toxicology Branch memorandum signed 2/14/86 (Appendix I). The conclusions based upon this review were as follows:

1. Five metabolites of alachlor were identified in the urine of monkeys injected intravenously with 0.7 or 7.0 mg/kg alachlor. They consisted of a secondary and tertiary mercapturate conjugate and a cysteine, thioacetic acid and glucuronide conjugate of alachlor. The different doses of alachlor administered appeared to quantitatively but not qualitatively alter the metabolic profile.

2. The following metabolic differences between the rat and monkey can be noted:

   - number of identifiable urinary metabolites; five in the monkey and 14 in the rat
   - the ratio of urinary to fecal radioactivity recovered: rat 1:1 and monkey 9-10:1
   - only two urinary metabolites were common to both the rat and monkey, namely the secondary and tertiary mercapturic acid conjugates
   - sulfate conjugation and side chain hydroxylation metabolites were found in the urine of the rat but not the monkey.

3. The results obtained in this study and in previously conducted intramuscular and topical metabolism studies do not support Monsanto's contention that the metabolites of alachlor "formed do not change as a function of the route of administration". This contention is not supported

   - because the metabolites of alachlor as reported for the intramuscular and topical studies appear to differ quantitatively as well qualitatively (number of metabolites found) and
   - because the limited nature of the intramuscular and topical studies in the monkey make it difficult to extrapolate the results obtained in those studies to the intravenous metabolism study.

4. The results of this study do not support Monsanto's argument that the monkey is a better model than the rat for assessing the effects of alachlor in man. No side chain hydroxylated metabolites of alachlor were identified in the urine of monkeys administered alachlor by the intravenous, intramuscular or topical routes, however, these metabolites have been identified in the urine of rats given alachlor orally and in man administered alachlor topically.

   Only that portion of this study not previously reviewed will be discussed in this memorandum.

Conclusions:

1. The only identified fecal metobolite in the monkey was a cysteine conjugate which was also identified in urine.

2. Neutral metabolites found in urine comprise approximately 5% of the administered dose.

3. A mutagenic metabolite of alachlor was identified in the urine of the monkey namely N-[2-ethyl-6-(1-hydroxyethyl)-phenyl]-2-(methylsulfonyl)-acetamide. This metabolite was also reported to be found in rat and mouse urine, is mutagenic in the Ames Salmonella assay (Strain TA 100) and is a HEEA metabolite not previously identified in the monkey.

Core Grade: Acceptable

8/6/87

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

COMPLETED

OFFICE OF WATER

MEMORANDUM

SUBJECT: Monsanto's Comments on Alachlor Health Advisory

FROM: Amal Mahfouz, Ph.D., Senior Toxicologist
Health Effects Branch, CSD/ODW (WH-550D)

TO: Reto Engler, Ph.D., Chief
Mission Support Staff
Toxicologist Branch, Hazard Evaluation Division
Office of Pesticide Programs (TS-7698C)

THRU: Edward V. Ohanian, Ph.D., Chief
Health Effects Branch, CSD/ODW (WH-550D)

The Office of Drinking Water (ODW) received on July 16, 1987, the attached comments on the Alachlor draft Health Advisory. Your comments on Monsanto's submission are needed by August 7, 1987.

The final draft of the Alachlor HA (copy attached) has been just edited as the previous draft was sent to Monsanto for comments. Therefore, some of Monsanto's comments relative to errors in calculations are irrelevant at this time. However, the issues related to Monsanto's use of the 95% lower confidence bound on risk in the oncogenicity discussion section, as well as the discussion of the rabbit teratology studies, the developmental studies, and the monkey studies need to be addressed. Please contact me if you need any further information.

Attachments

Reto
Your comments/help will be very valuable

Ed Ohanian

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007699 Engler

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

MEMORANDUM

OFFICE OF
PESTICIDES AND TOXIC SUBSTANCES

SUBJECT: Monsanto Comments on Alachlor Health Advisory

FROM: for Reto Engler, Chief E. Reinde 8/6/87
Scientific Mission Support Staff
Toxicology Branch/HED (TS-769)

TO: Edward V. Ohanian, Chief
Health Effects Branch, CSD
Office of Drinking Water (WH-550)

The following comments are in response to your July 23, 1987 request:

I. With respect to the risk calculations:

1. Monsanto's comments on page 16 regarding the 10-day Health Advisory

a) Monsanto used a NOAEL = 60 mg/kg/day and an uncertainty factor of 100.

b) ODW page 9 of Health Advisory uses a LOAEL of 10 mg/kg/day and a safety factor of 1000.

2. Monsanto's estimated risk of 6×10^{-4} on page 18 is correct but.

a) The Health Advisory estimate on page 10 has a relative source contribution (RSC) that must account for the difference.

Any questions on the above should be directed to Mr. Richard Levy (557-3715) or Mr. C. J. Nelson, of my staff.

II. With respect to toxicology issues: the following comments were prepared by Dr. Judith Hauswirth:

Page 5 of Monsanto's comments: p. 4 Metabolism
Monsanto is correct that monkey urine contains an HEEA metabolite.

Page 6 of Monsanto's comments: p. 4 Metabolism
The monkey is similar to man in ratio of HEEA:DEA metabolites (except for one individual with a ratio of 2:1). Man may be defective in mercapturic acid formation - an active pathway in monkey, indicating a difference between the two.

Page 7 of Monsanto's comments: Artificial insemination is appropriate method for dosing in rabbit teratology studies.

The statement by ODW on total implantations was misquoted from the OPP review.

"There was an increased incidence of the following" anomalies.....

A LOEL and NOEL was not determined by OPP for the rabbit teratology study. The first and second rabbit teratology studies should not be compared since different vehicles were used in each study. Monsanto should be made aware that statistical significance is not necessary in calling an increase in an anomaly compound related.

Page 8 of Monsanto's comments: Carcinogenicity Point (4)
Monsanto is correct. Also see OPP memo dated June 4, 1987 p. 4 which is attached.

Page 9-12 of Monsanto's comments: Amal reviewed the rat teratology study. Again Monsanto should be made aware that statistical significance is not always necessary in calling an increase or decrease in a particular parameter in a teratology study or any study compound related.

Page 12 of Monsanto's comments: OPP stands on their conclusion that the mouse study on diacnior was positive based upon statistically significant increase in lung tumors at the high dose in female mice. There was also evidence of decreased latency in this study for this tumor type.

OPP has accepted Monsanto's argument that the brain tumors originally diagnosed in the first rat study were in actuallity extensions of nasal turbinate tumors. This was determined by reevaluation by different pathologists.

Page 13 of Monsanto's comments: OPP didn't have any argument with Monsanto's presentation on the dose-resporse relationship of the nasal turbinate tumors from the two chronic rat studies. See our comments in attached memo p. 4.

Attachment

cc: Amal Mahfouz - WH 550
Judith Hauswirth - TS-769
Richard Levy - TS-769
C.J. Nelson - TS-769

RDI: 6/87 sp

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SECTION HEAD

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

JUN 4 1987

MEMORANDUM

OFFICE OF PESTICIDES AND TOXIC SUBSTANCES

Subject: Toxicology Branch Comments on Various Responses to the Agency's Federal Register Notice Dated October 8, 1986 and the Technical Support Document on Alachlor

To: Amy S. Rispin, Ph.D., Chief
Science Integration Staff
Hazard Evaluation Division (TS-769C)

David Giamporcaro
Special Review Branch
Registration Division (TS-767C)

From: Judith W. Hauswirth, Ph.D. Judith W. Hauswirth 6/3/87
Section Head, Section VI
Toxicology Branch/HED (TS-769C)

Thru: Theodore M. Farber, Ph.D., Chief
Toxicology Branch/HED (TS-769C) 6/4/87

A response to the Agency's Federal Register Notice dated October 8, 1986 and their Technical Support Document on Alachlor was received from each of the following: Monsanto Company; the National Audubon Society; Natural Resources Defense Council; and the National Network to Prevent Birth Defects. Responses pertinent to the Agency's position on the toxicological issues regarding Alachlor will be dealt with individually by commentor in this memorandum.

Monsanto Company

Monsanto's comments on the mouse oncogenicity study on alachlor, the genotoxicity of alachlor, the MTD as related to nasal turbinate tumor incidence in Long-Evans rats, species differences in the metabolism of alachlor and the EPA classification of alachlor as a B_2 oncogen were considered by the Toxicology Branch Peer Review Committee on 4/15/87 and are addressed in the Peer Review Document resulting from this meeting which is attached.

1. Line by Line Comments in Response to the Alachlor Technical Support Document.

Page I-1, ¶ 4: Results of a recently submitted monkey metabolism study (MSL 5727) indicate that the monkey does metabolize alachlor to at least one HEEA metabolite. Two of these have been found to be mutagenic in the Ames Salmonella assay. The metabolite tentatively identified in monkey urine was also identified in rat and mouse and is one of the two mutagenic metabolites.

In addition, in their Health Assessment prepared for Canada, the NRDC cites a reference which indicates that humans may be defective in mercapturic acid formation, a pathway which is active in the monkey, leading to the formation

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a preponderance of mercapturic acid and thiol metabolites (See for example, Tox. Br. Comments on Monsanto's rebuttal to the Agency's PD 1 on alachlor, dated April 3, 1986).

The fact remains that the oncogenicity studies were carried out in rodents and this data along with metabolism and mutagenicity data were used to determine the oncogenic potential of alachlor by the Agency. The Agency is still not convinced that the monkey is a better model for man than rodents when studying the metabolism of alachlor.

Page II-2 ¶ 2: A chronic rat study on acetochlor has been recently submitted to the Agency for review. This study has not been officially reviewed by the Agency; however, from a quick review of the study, it appears that alachlor induces adenomas of the nasal mucosa in rats and possibly thyroid adenomas. The Agency has not concluded that metalochlor induces nasal turbinate tumors.

Page II-3: Monsanto is correct in saying that the half-life of alachlor should read 8.2 and not 0.2.

Monsanto is also objecting to our statement that "a relatively high level of radioactivity was found in the eyes, brain, stomach and ovaries" of rats. By this statement we did not mean to imply that the levels of radioactivity in these tissues were very high, only that they were higher relative to other tissues/organs.

Page II-4, ¶ 8: The Agency concurs with the first sentence of this comment, that is that the inhalation study was conducted in rats and the LC_{50} was > 5.1 mg/l.

Page II-6 to II-8: Monsanto is correct in saying that the in vivo bone marrow cytogenetics assay is scientifically valid and was acceptable to Toxicology Branch.

The Agency feels that the results of the Ames Salmonella assays conducted on the urine obtained from alachlor treated rats are difficult to interpret. We would agree that definitive positive and/or negative results were not obtained. More importantly to be considered is that two urinary metabolites of alachlor have been found to be mutagenic in this assay system.

Page II-9: The Agency agrees that thyroid tumors are not induced by alachlor in Long-Evans, female rats.

Liver and brain tumors were not considered in the weight-of-the-evidence considerations on the classification of alachlor as a B_2 oncogen.

Page II-10, Table 4, Points i-vi: The registrant is correct on all of these points and the table should be corrected accordingly.

Page II-11, ¶ 1: The Agency has no problem with the registrant's suggested changes in the first two paragraphs of this comment.

The Agency's conclusion that a partial lifetime exposure can result in a similar tumor incidence as a lifetime exposure is accurate when considering the incidence of nasal turbinate tumors. The incidence of this tumor was the

basis for the SAP's conclusion that alachlor was a B_2 oncogen.

The registrant's argument about the direct correlation of total dosage administered and the incidence of nasal turbinate tumors will be discussed later in this memorandum under our comments to Appendix D of Monsanto's rebuttal.

Page II-11, ¶ 3: Monsanto is correct.

Page II-12, ¶ 1,2: The Agency accepts Monsanto's conclusion that the tumors originally diagnosed as brain tumors are in actuality extensions of nasal turbinate adenocarcinomas.

Page II-12, ¶ 3: No comment.

Page II-12, ¶ 4: The Agency cannot comment on the use of [redacted] as a stabilizer and the occurrence of nasal turbinate tumors without seeing the results of the studies the registrant is referring to and without knowing the structural identity of the acetanilides studied.

Page II-13, ¶ 1: The Agency could not accept this study as an adequate epidemiological study indicating that there is no association of cancer with alachlor manufacture.

Page II-13, ¶ 2-5:
¶ 1 of comment, see Peer Review Reports.
¶ 2 of comment, we agree with this statement.
¶ 3 of comment, we have not concluded that metolachlor induces nasal turbinate tumors.

Page II-14, ¶ 3: See Peer Review Reports.

Page II-14, ¶ 4: Accepted.

Page II-44: Oncogenicity in Mice - See attached Peer Review Reports.

Page II-46: Rebuttal Comment 2 (see Agency's Technical Support Document) - The Peer Review Committee agrees (see report of 4/15/97 Peer Review Meeting) that the weight-of-the-evidence indicates that alachlor, itself, is not a mutagen.

Page II-47: Rebuttal Comment 3 (see Agency's Technical Support Document)
The Agency is considering the results of genotoxicity tests in their weight-of-the-evidence determination (WOE) (see Peer Review Reports) on the oncogenic potential of alachlor. We agree that the WOE indicates that alachlor, itself, is not a mutagen. However, two metabolites of alachlor have been shown to be mutagenic in the Ames Salmonella assay. The mutagenic potential of these two metabolites has not been tested in other assays. The Agency would strongly recommend that these metabolites, as well as other HED's metabolites be subjected to a battery of mutagenic assays with different genetic endpoints as described in our Subdivision F Guidelines.

Pages II-48 to II-52:

See comment under Page I-1, ¶ 4 above. Also the Agency would like to note that in their rebuttal to the PD-1, Monsanto presented summaries of

metabolism studies. The complete reports of these studies were requested by Tox. Br. This request was forwarded to Monsanto; however, they have not been recieved to date.

Page II-50: Response to Comments Pertaining to the Blood Binding of Alachlor

No comment necessary.

2. Appendix A - Oncogenic Potential in Mice
Appendix B - Weight of Evidence Consideration

See attached Peer Review Reports.

3. Appendix D - Oncogenicity Following Five Months Exposure

The Agency agrees that carcinogens can produce tumors in less than lifetime exposure as was demonstrated with alachlor for the induction of nasal turbinate tumors. We also agree that the rate of tumor formation is usually dose-dependent for carcinogens.

4. Appendix E - Stomach Tumors

Monsanto is arguing that the stomach adenocarcinomas seen at 2.5 mg/kg/day in Long-Evans rats are not related to alachlor treatment. The Agency had erroneously listed this tumor as an undifferentiated sarcoma. On this basis we stated that the undifferentiated sarcoma could be related to alachlor treatment since it is an unusual tumor type and was seen at a higher dosage level of alachlor.

Adenocarcinoma of the stomach was seen at a dosage level of 126 mg/kg/day alachlor in two separate studies. It did not occur in the control groups in any of the studies conducted on alachlor in Long-Evans rats. If Monsanto wishes to pursue their argument that the adenocarcinoma seen at 2.5 mg/kg/day is not related to alachlor treatment, historical control data should be submitted on this tumor type in this strain of rat from contemporaneous studies conducted by the performing laboratories.

5. Appendix F. Brain Tumors

The Agency agrees with Monsanto after receipt of the information in this Appendix that the brain tumors originally diagnosed in rats at a dosage level of 126 mg/kg/day are actually extensions of nasal turbinate adenocarcinomas seen in these animals. All discrepencies noted by Tox. Branch in their comments on Monsanto's rebuttal to the Agency's PD 1 on alahclor (dated April 3, 1986) have been resolved.

National Network to Prevent Birth Defects

This group had two points to make on the toxicity or possible toxicity of alachlor in relation its use and health effects in man.

1. "A chemical that caused thyroid tumors and deterioration of the eye is certainly a candidate for neurological injury in adults and children".

The toxicity to the eye related to alachlor administration was uveal degeneration. Since the uvea refers to the vascular middle coat of the eye

we do not feel that uveal degeneration could be considered a neurotoxic related effect.

Thyroid tumors were induced by alachlor at relatively high dosage levels of alachlor when compared to nasal turbinate tumors. We are not aware of a direct link between the occurrence of thyroid tumors and neurotoxicity.

2. "The liver and kidney damage at rather low dosages is worrisome, and it does not appear that the chemical has been adequately tested for birth defects".

This group is correct in saying that alachlor has not "been adequately tested for birth defects" in rabbits. Two studies have been submitted to the Agency in rabbits but were found to be inadequate. A third teratology study in this species should be/has been requested. Alachlor has been adequately tested in the rat and found not to be teratogenic in this species.

National Audubon Society

The National Audubon Society is probably correct in saying that the full impact of alachlor exposure in the farming community has not yet been felt.

Toxicology Branch has no other comments on their response.

Natural Resources Defense Council (NRDC)

The NRDC is concerned about the SAP's comments (November 19, 1986 meeting) on the EPS's Cancer Guidelines for classifying the oncogenicity of chemicals. Although the SAP states that they are "not comfortable with the implied conclusion of the EPA Guideline" on alachlor they nevertheless classified it as a B_2 (probable human) carcinogen. The Toxicology Branch Peer Review Committee met after the SAP meeting and upheld the B_2 classification and, in addition, concluded the mouse study on alachlor was positive for oncogenicity (Monsanto and the SAP felt that it was negative).

The NRDC also "objects to the SAP suggestion that 'metabolic data from the monkey be used to scale the interpretation of risk from the rat data'". The Agency did not take such an approach and does not feel that it is justified.

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Reviewer's Peer Review Package for 1st Meeting

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FILE COPY

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

MAR 17 1986

OFFICE OF
PESTICIDES AND TOXIC SUBSTANCES

MEMORANDUM

SUBJECT: Peer Review on Alachlor

FROM: Ke Engler, Chief
Mission Support Staff
Toxicology Branch/HED (TS-769)

TO: Addressees

There will be a Peer Review of the weight-of-the-evidence on Alachlor on Tuesday, March 25, 1986 at 9:00 AM in Dr. Farber's office (Room 821 CM-2).

Attached for your review a comprehensive package prepared by Dr. Hauswirth. By reference the PD-1 (not attached) on Alachlor should also be considered by the panel members.

Attachment

ADDRESSEES

T. Farber
W. Burnam
J. Quest
J. Hauswirth
L. Chitlik
S. Johnson
R. Hill
A. Barton
L. Kasza
E. Rinde
D. Beal
E. Gray
W. Markus

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

OFFICE OF
PESTICIDES AND TOXIC SUBSTANCES

MEMORANDUM

Subject: Weight-of-the-Evidence and Oncogenic Properties of Alachlor

From: Judith W. Hauswirth, Ph. D. JWHauswirth 3/7/86
Mission Support Staff
Toxicology Branch/HED

Through: Reto Engler, Ph.D., Chief
Mission Support Staff
Toxicology Branch/HED

To: The Peer Review Committee
Toxicology Branch/HED

Contents

Appendices

A. DER: A chronic Feeding study of Alachlor in Rats. Bio/dynamics
B. DER: A Chronic Study of Alachlor Administered in Feed to Long-Evans Rats. Monsanto Environmental Health Laboratory.
C. DER: A Special Chronic Feeding Study with Alachlor in Long-Evans Rats. Monsanto.
D. Tumor incidence table for the three rat studies combined: also DER on Monsanto's reevaluation of submucosal gland hyperplasia seen in study reviewed under Part 5.b. of this report.

E. DER: An 18 Month Oncogenic Study in Mice. Bio/dynamics.
F. Sher, S. P., R. D. Jensen and D. L. Bokelman. Spontaneous Tumors in Control F344 and Charles River CD Rats and Charles River CD-1 and B6C3HF1 Mice. Toxicology Letters 11: 103-110, 1982.
G. Homburger, F., A. B. Russfield, J. H.Weisburger, S. Lim, S. P. Chak and E. K. Weisburger. Aging Changes in CD-1 HaM/ICR Mice Reared Under Standard Laboratory Conditions. J. Natl. Cancer Inst. 55: 37-45, 1975.
H. Historical Control Data from Bio/dynamics on Lung Tumors and Liver Tumors in CD-1 Mice.
G. Table: Q_1^* Potency Estimates for Alachlor Based on Rat Tumor Data (from the PD-1).

Data Evaluation Report on Alachlor for the Peer Review Committee

1. Background

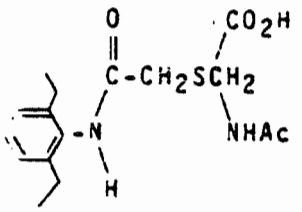
Alachlor is registered for use as a selective herbacide for control of many preemergent broadleaf weeds and grasses. It is structurally similar to another herbacide metalochlor.

A Special Review Position Document 1 was issued on alachlor in December of 1984. In this document the Agency concluded that "the weight of the evidence demonstrates that alachlor is oncogenic to laboratory animals and, in the absence of data on humans, it is prudent to treat alachlor as a probable human carcinogen". The Agency is presently preparing a Special Review Position Document 2,3 (PD 2,3) on alachlor. Since alachlor has not undergone the peer review process in Toxicology Branch, HED, it was felt that it would be beneficial at this time to put it through the process prior to issuing the PD 2,3.

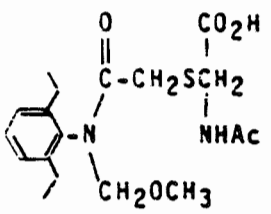
2. Metabolism of Alachlor

In Sprague-Dawley rats, fourteen metabolites of alachlor have been identified in the urine and 13 in feces. Only three of these metabolites were common to both urine and feces (see attached Figures 1 and 2 for alachlor metabolites identified in rat excreta). Approximately 89% of the radioactivity was eliminated within the first four days after dosing. The ratio of radioactivity found in urine versus feces was about 1:1. The rate of alachlor elimination was biphasic with the half life of the first phase being 8.2 to 10.6 hours and of the second phase 5 to 6 days. Mercapturic acid, glucuronic acid, sulfate conjugation and side chain hydroxylation were important metabolic pathways for alachlor metabolism in the rat. Metabolite XII, N-[2-ethyl-6-(1-hydroxyethyl)-phenyl]-N-(methoxymethyl)-2-(methylsulfonyl)acetamide, found in rat urine has been reported to be mutagenic in the Ames salmonella assay both with and without metabolic activation.

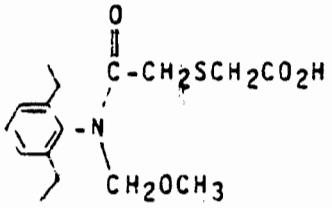
Metabolism studies on alachlor have also been done in Rhesus monkeys by three different routes of administration. In an intravenous injection study 92 to 94% of the total radioactivity in the urine was excreted during the first 24 hours and 91-94% of the radioactivity in the feces was excreted during the first 48 hours. The ratio of radioactivity found in urine versus feces was 9-10:1. Metabolites were identified in urine only and consisted of the following five conjugates of alachlor:



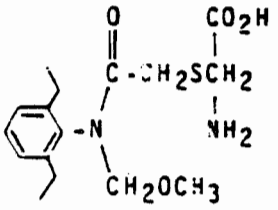
Secondary Mercapturate (4)



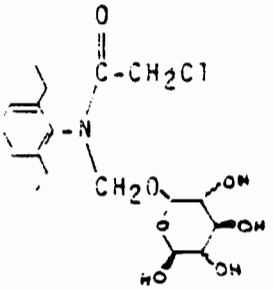
Tertiary Mercapturate (5)



Thioacetic Acid Conjugate (6)



Cysteine Conjugate (7)



Glucuronide Conjugate (8)

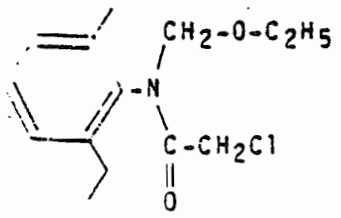
In intramuscular injection and topical administration metabolism studies only three urinary metabolites were identified, numbers 4, 5 and 6 illustrated above. These two studies were not considered aceptable because of the inhumane treatment of the animals and/or the length of time urine samples were frozen away prior to analysis (about 4 years).

In human biomonitoring studies metabolites which contained the DEA and HEEA (see below) moieties of alachlor were identified in the urine. There were questions about the adequacy of the analytical prodecures used in this study (i.e. poor recovery data for HEEA).

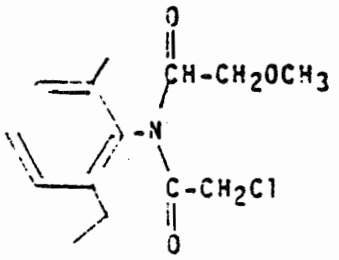
DEA HEEA

3. Structure Activity Relationship

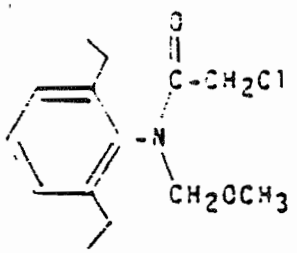
Alachlor is structurally related to metalochlor and acetochlor, structures of which are shown below.



Acetachlor



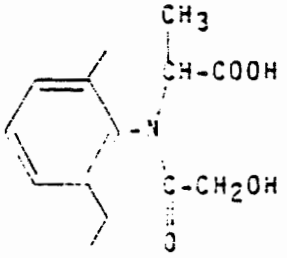
Metalochlor



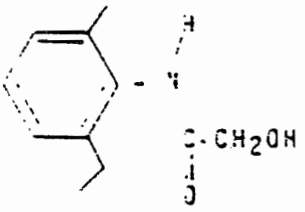
Alachlor

[redacted]

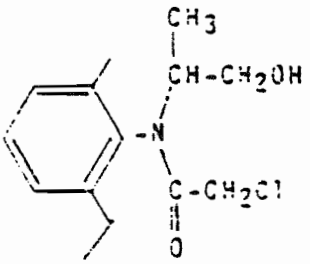
Limited mutagenicity data is available on metalochlor. It has been reported to be negative in the Ames salmonella assay and did not have any effects on fertility, zygote or embryo survival in the in vivo developing sperm mouse assay. Metalochlor, when fed to CD rats at levels of 30, 300 and 3000 ppm caused an increase in proliferative liver lesions (neoplastic nodules) in the high dose female rats. In this study nasal turbinate tumors were seen in two high dose males and one high dose female. Metalochlor was negative for oncogenicity in the mouse. Identified metabolites of metalochlor are as follows:



urine and feces



urine only



feces only

4. Non-Oncogenic Toxicological Effects

The acute oral LD_{50}'s of alachlor(90%) and technical alachlor are 2.3 g/kg and 0.93-1.2 g/kg, respectively. In mice the acute oral LD_{50} of technical alachlor is 2.1 g/kg.

In a 3-generation reproduction study in Charles River Sprague-Dawley CD rats the NOEL was 10 mg/kg based upon kidney effects (chronic nephritis, hydronephrosis) seen in F_2 adult males and F_{3b} male pups.

In a one year beagle dog study the NOEL was 1 mg/kg/day based upon hemosiderosis seen in liver, kidney and spleen of dogs in the 3 and 10 mg/kg/day groups.

Alachlor was not teratogenic to rats at 400 mg/kg/day, the highest dose tested.

A NOEL for non-neoplastic toxicity was established for alchlor in a 2-year chronic feeding/oncogenicity study in Long-Evans rats. The NOEL was 2.5 mg/kg/day based upon molting of retinal pigmentaion and increased mortality rate in the females and abnormal disseminated foci in male liver.

5. Summary of Relevant Chronic or Lifetime Studies

a. A Chronic Feeding Study of Alachlor in Rats. Bio/dynamics Inc., Project # 77-2065 (BD-77-421), 11/13/81; submitted on 1/5/82. Accession # 070586 to 070590. (DER for this study is Appendix A).

Long-Evans rats were maintained on diets containing 0, 100, 300, or 1000 ppm alachlor (Lasso technical) for 812 to 813 days for males and 741 to 744 days for females. This was equivalent to 0, 14, 42, and 126 mg/kg/day alachlor. Fifty male and 50 female rats were placed in each group.

Two different lots of technical alachlor were used during the study. Lot #XHI-167, stabilized with 0.5% epichlorohydrin, was used for the first 11 months of the study and Lot #MHK-6, stabilized with [redacted] was used for the remainder of the study. Epichlorohydrin is carcinogenic to male, wistar rats when given in drinking water causing forestomach tumors (squamous cell papillomas and carcinomas) (Konishi et al. Gann 71:922-923, 1980) and to Sprague-Dawley rats by inhalation exposure causing 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.

An NOEL for alachlor chronic toxicity could not be demonstrated in this study at 14 mg/kg/day. Ocular lesions (uveal degeneration syndrome) were seen in both males and females; however, the females were more sensitive to this effect with 2/50 affected at 14 mg/kg/day. Central lobular hepatocyte necrosis was also seen in males and females at all dosage levels.

For males, the percentages surviving to scheduled termination for 0, 14, 42 and 126 mg/kg/day groups were 52%, 34%, 36% and 38%, respectively and for females these percentages for the same dosage groups were 66%, 54%, 64% and 40%, respectively.

The following tumor types were found to increase as a result of alachlor:

- o nasal turbinate tumors (mainly benign) increased in both males ($p<0.001$) and females ($p<0.02$) at the mid dose level (42 mg/kg/day) and above
- o stomach malignant tumors increased significantly ($p<0.001$) in both sexes at the high dose level
- o thyroid folicular tumors (adenomas and carcinomas) significantly increased in males at the high dose level ($p<0.001$).

The incidences of these and other pertinent tumors is given in the following table.

	Males				Females			
	Control	Low	Mid	High	Control	Low	Mid	High
Stomach (N)	49	50	50	50	50	50	50	49
leiomyosarcoma	0	0	0	1	0	0	0	1
osteosarcoma	0	0	0	3	0	0	0	4
gastric adenocarcinoma	0	0	0	2	0	0	0	1
malignant mixed gastric tumor	0	0	0	11	0	0	1	17
Thyroid (N)	48	50	49	50	49	44	46	48
follicular ademoma	1	0	1	11	0	0	2	2
follicular carcinoma	0	0	0	2	0	0	0	2
Nasal Turbinates (N)	46	46	41	42	49	47	42	48
respiratory epithelium ademomas	0	0	10	23	0	0	4	13
carcinomas	0	0	1	0	0	0	1	2

b. A Chronic Study of Alachlor Administered in Feed to Long-Evans Rats. Monsanto Environmental Health Laboratory, R.D. #520, Special Report #MSL-3382, Project #ML-80-186, 2/27/84; submitted on 2/28/84. Accession # 252496-252498. (DER is Appendix B).

Long-Evans rats were maintained on diets containing alachlor equivalent to 0, 0.5, 2.5 and 15.0 mg/kg/day for 25 to 26 months. Fifty rats per sex were placed in each group. Technical alachlor (94.13%) was stabilized with [redacted] Epichlorohydrin was not used as a stabilizer.

An NOEL for alachlor chronic toxicity was determined in the study to be 2.5 mg/kg/day. This was based upon molting of the retinal pigmentation seen in female rats and upon abnormal disseminated foci of the liver seen in male rats at higher levels of alachlor.

Alachlor did not cause any increase in the mortality rate of the treated rats as compared to the control except in the high dose female group (16% increase in mortality). No statistically significant differences in body weight gain were seen between groups.

The following tumor types were found to increase as a result of alachlor treatment:

- o nasal turbinate tumors significantly increased ($p<0.01$) in both males and females at 15 mg/kg/day. One female rat in the mid dose group also had this tumor and one male in this group had a submucosal gland adenoma
- o thymus lymphosarcoma and adrenal phenochromacytoma significantly increased ($p<0.05$) in the high dose females
- o thyroid folicular cell tumors increased but not significantly in the high dose male group.

The incidence of these and other pertinent tumors/lesions is given in the following table.

	Males				Females			
	Control	Low	Mid	High	Control	Low	Mid	High
Thyroid (N)	49	50	49	49	49	49	49	47
follicular								
adenoma	2	4	3	4	1	1	0	2
carcinoma	1	0	1	2	3	1	1	1
Thymus (N)	49	50	46	50	48	50	48	43
lymphosarcoma	0	0	1	0	0	1	2	3

Adrenal (N)	50	50	50	50	49	50	50	49
pheochromocytoma								
benign	8	7	2	6	1	1	3	5
malignant	2	2	0	2	1	0	0	0
Nose/Turbinates (N)	45	48	4.	45	42	44	47	48
respiratory epithelium								
adenoma	0	0	0	11	0	0	1	9
neurofibroma	0	1	0	0	0	0	0	0
submucosal gland								
adenoma	0	0	1	0	0	0	0	0
epith. hyperplasia/								
metaplasia	1	1	1	1	0	0	0	2
submucosal gland								
Hyperplasia	2	1	3	21	2	5	5	11

The agency requested that Monsanto do a reevaluation of the submucosal gland hyperplasia seen in both males and females. A histological reevaluation of tissues of the nasal cavity was performed by Experimental Pathology Laboratories, Inc. (EPL) at the Research Triangle Park facility. Their report indicated that the submucosal nasal lesions (hyperplasia) were not neoplastic. However, their analysis reflected a slightly higher incidence of adenomas of the nasal cavity. A comparison of EPL's and Monsanto's diagnoses is given in the table below.

	Nasal turbinate adenomas			
Group	EPL's data		Monsanto's data	
(mg/kg/day)	Males	Females	Males	Females
0	0/44	0/42	0/45	0/42
0.5	0/47	0/42	0/48	0/44
2.5	0/44	1/47	0/45	1/47
15.0	15/45	14/48	11/45	9/48

c. A Special Chronic Feeding Study with Alachlor in Long-Evans Rats, R.D. #533, Special Report #MSL-3492, 4/16/84; Accession # 253306 and 253307. (DER is Appendix C).

This study was designed to determine the nature of the ocular lesions seen in the Bio/dynamics chronic feeding study described in Part 5. a. of this report. Treated animals (125 mg/kg/day in the diet) were divided into three groups after a period of exposure sufficient to induce ocular lesions.

The grouping process was performed as the ocular lesions were confirmed by the consulting ophthalmologist. Group I consisted of the animals that were to remain on the treated diet until the end of the two-year study period; group II consisted of the animals that were selected for interim sacrifice based on their ocular lesion status; and group III consisted of the animals that were selected for potential recovery from ocular lesions by being placed on untreated diets for the remainder of the study period. Additional animals that apparently did not show any ocular lesions were also placed in group II and III.

The distribution of animals in group III occurred after 5 months of exposure for females and 6 months for males. The assignment of animals to group II occurred after 6 months of exposure for the 10 males selected for interim sacrifice and after 5 months for 10/18 selected females; after 6 months for an additional 4 females; and at the 8th month for the remaining 4 females of this group. Only 6 animals/sex served as the control group for this part of the study. The control group from the study discussed above under Part 5. b. can also be considered here since the two studies were run concurrently and were considered by Monsanto as one study with two parts.

The incidence of neoplastic lesions in this study using the control incidence data from the study run concurrently is as outlined in the table below. Note that nasal turbinate adenomas developed in rats exposed to alachlor for only 5-6 months at the beginning of the study (Group III).

	Males			Females		
	Control	Grp I	Grp III	Control	Grp I	Grp III
Nasal turbinates (N)	45	61	17	42	25	46
respiratory epithelium adenoma	0	42	10	0	11	19
carcinoma	0	7	0	0	2	1
Thymus (N)	49	68	16	48	25	43
lymphosarcoma	0	1	1	0	0	1
Adrenal (N)	50	70	20	49	31	48
pheochromcytoma benign	3	8	2	1	0	2
malignant	2	2	1	0	0	0
Thyroid (N)	49	69	20	49	31	49
follicular adenoma	2	8	1	1	4	3
carcinoma	1	10	1	3	0	1

	Males			Females		
	Control	Grp I	Grp III	Control	Grp I	Grp III
Stomach (N)	50	68	20	50	31	49
mixed carcino-sarcoma	0	3	0	0	19	0
anaplastic sarcoma	0	1	0	0	3	0
adenocarcinoma	0	0	0	0	10	0
leiomyosarcoma	0	0	0	0	10	0
undiff. sarcoma	0	0	0	0	16	2
undiff. carcinoma	0	0	0	0	3	0
Brain (N)	50	70	20	50	31	49
neuroepithelioma	0	1	0	0	1	1
Liver (N)	50	70	20	50	31	49
hepatoma	1	3	0	0	1	0
neoplastic nodule	0	0	0	0	1	1
hepatocellular carcinoma	2	2	0	0	2	1

Monsanto has submitted a reevalation of the brain tumors (neuroepitheliomas) seen in this study (An addendum to a Special Chronic Feeding Study with alachlor in Long-Evans Rats, RD #533, MSL #3492, 2/12/85; Accession #256735). Electron microscopy was done on the tumor from one of these animals with a neuroepithelioma. It had "intermediate fiber typical of keratin" and from this Monsanto concluded that the tumor was epithelial, not neural. C.I.I.T. also reevaluated all three brain tumor and concluded that they were extensions of nasal adenocarcinomas and not brain tumors. However, a discrepency in animal numbers and diagnoses remains to be resolved prior to accepting Monsanto and C.I.I.T. 's conclusion.

In Appendix D, a table can be found that compares the tumor incidences derived from the three rat feeding studies. The changes in diagnoses made for the brain tumors by Monsanto is not reflected in this table.

d. An 18 Month Oncogenic Study in Mice, Bio/dynamics, Inc. Project #77-2064 (80-77-423) 5/5/81; Accession #070168 and 070169. (DER is Appendix E).

Groups of fifty CD-1 albino mice per sex were administered alachlor in the diet for 18 months at dosages corresponding to the following levels: 0, 26, 78 and 260 mg/kg/day. Alachlor (Lasso technical) was supplied in two batches. Lot XHI-167 used during the first 11 month of the study was stabilized wit 0.5% epichlorohydrin; lot MHK-6 used during the last 7 months of the study was stabilized with [redacted]

Decreased survivability was seen in the high dose female mice as well as a statistically significant decrease in body weight gain when compared to the control group.

The incidence of pertinent non-neoplastic and neoplastic changes are tabulated below.

	Male				Female			
	Control	Low	Mid	High	Control	Low	Mid	High
Lung (N)	50	50	50	50	50	50	50	50
bronchiolar-alveolar adenoma	6	1	4	10	2	4	7	10
carcinoma	3	5	7	2	1	1	1	1
fibrosarcoma	0	0	0	0	0	0	0	1
congestion	1	13	13	12	5	5	12	16
Liver (N)	50	50	50	50	50	50	50	50
adenoma	5	1	4	7	0	0	0	1
carcinoma	0	3	1	4	0	0	1	0
Uterus (N)					50	50	50	50
leiomyoma					0	2	0	0
leiomyosarcoma					1	0	2	3
endometrial carcinoma					0	1	0	0
endometrial polyp					1	3	0	3
granular cell myoblastoma					0	0	0	1

Lung was the major target organ for oncogenicity. The incidence of lung bronchioalveolar tumors was significantly increased in the high dose females ($p<0.05$) and was also significant ($p<0.01$) for the high dose females which died in extremis during the study. The incidence of lung tumors in females which died during the study was:

Control	0/30
Low	1/17
Mid	3/27
High	7/35

Monsanto submitted an addendum to this study on 2/25/85. The report contains an evaluation done by Bio/dynamics on the nasal turbinates of mice in the control and high dose group. Originally examination was done on only 10 mice/sex/group. Tissues from all remaining animals were examined. No nasal turbinate tumors were found.

6. Historical Control Information

Historical control data on lung tumors in CD-1 mice could be found in the open literature. Sher et al. (Toxicology Letters 11:103-110, 1982; Appendix F) at Merk, Sharp and Dohme Research Laboratories and Homberger et al. (J. Natl. Cancer Inst. 35:37-43, 1975; Appendix G) reported the following incidence of lung tumors in CD-1 and CD-1 HaM/ICR mice, respectively.

	MSD Studies	
Lung	Male	Female
N - animals	1232	1240
N - groups	24	24
Age	81-105 weeks	
adenoma	0 - 38%	0 - 41%
adenocarcinoma	0 - 16%	0 - 12%

	Homberger Data			
N - animals	99		102	
	Total*	18 mos.	Total*	18 mos.
adenoma	19	2	24	4
adeno-carcinoma	5	-	8	1

*Total refers to total tumors seen at 21.8 months for males with adenomas, 22.8 months for males with adenocarcinomas, 22.6 months for females with adenomas and 22.4 months for females with adenocarcinomas.

Additionally, historical control data was obtained from Bio/dynamics on the incidence of lung and liver tumors in CD-1 mice for concurrently run studies. This information can be found in Appendix H. Note that the control data given in these tables is from studies of longer than 18 month duration.

7. Mutagenicity

The results of mutagenicity testing conducted on alachlor are summarized in the following table.

Test	Core Classification	Result	Comments
Ames Assay	acceptable	negative	a positive response was seen at 5000 ug/plate in TA 1535 but the response was not repeated for consecutive doses.
Gene mutation in CHO cells HGPRT locus	acceptable	negative	
In-vivo bone marrow chromosome aberration assay	acceptable	negative	no structural or numerical chromosomal aberrations
In-vivo - in vitro hepatocyte DNA repair assay	acceptable	positive	positive at highest dose tested (1.0g/kg/day) - "weakly genotoxic"
DNA damage in B. subtilis M45 and H17	acceptable	negative	did not cause DNA damage. (20 - 20,000 ug/plate)

As noted in the metabolism section of this report one metabolite of alachlor was found to be positive in the Ames assay. N[2-ethyl-6(1-hydroxyethyl)-phenyl]-N-(methoxymethyl)-2-(methylsulfonyl)acetamide was positive in TA 100 both with and without metabolic activation over six test doses.

8. Summary

Administration of alachlor via the diet to Long-Evans rats is associated with the development of nasal turbinate tumors in both sexes. Thyroid follicular tumors and malignant stomach tumors are also significantly increased over controls in male rats and male and female rats, respectively receiving

Appendix B

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

APR 17 1984

MEMORANDUM

OFFICE OF PESTICIDES AND TOXIC SUBSTANCES

SUBJECT: Alachlor, EPA Reg. #524-316. Review of a New Chronic Feeding/Oncogenicity Study in Rats by Monsanto, R.D. #520, Special Report #MSL-3382, February 27, 1984; Report compiled by Robert W. Street, Volumes 1, 2 and 3. Accession Nos.: 252496, -7 and 8. CASWELL#11

FROM: Amal Mahfouz, Ph.D.
Toxicologist, Section V
Toxicology Branch/HED (TS-769)

TO: Robert Taylor, PM#21
Registration Division (TS-769)

THRU: Laurence D. Chitlik, DABT
Section Head, Section V
Toxicology Branch/HED (TS-769)
and
William L. Burnam, Chief
Toxicology Branch
Hazard Evaluation Division (TS-769)

Registrant: Monsanto Agricultural Products Company
800 N. Lindberg Blv.
St. Louis, Missouri 63167

Recommendations:

The 2-year chronic feeding/oncogenic study in the Long-Evans rats with Alachlor at 0.5, 2.5 and 15.0 mg/kg bw/day dosage levels indicated the following:

*A NOEL for Alachlor non-neoplastic toxicity can be established at 2.5 mg/kg/day. The LEL is 15 mg/kg/day (molting of retinal pigmentation and increased mortality rate in females; and abnormal disseminated foci in the male liver).

*Alachlor is oncogenic in rats in this new study. Toxicology Branch will base its risk assessment for Alachlor on the increased incidence of nasal turbinate tumors observed in this study. An increased incidence of this tumor type was evident at doses as low as 2.5 mg/kg/day. Additional neoplastic changes were also observed as noted in the following discussion.

Stomach malignant tumors increased significantly ($p < 0.001$) in both sexes at the high-dose level, 126 mg/kg/day (see description of these tumors in the review page 19).

Thyroid follicular tumors (adenoma + carcinoma) appeared to increase in both sexes at the high-dosage level. However the increase was significant ($p < 0.001$) only in males.

Incidence of other tumors in other organs was also noted to increase at the mid and high-dose levels, potentially as a result of Alachlor administration, i.e. liver tumors (adenoma + hyperplastic nodules) in both sexes and brain tumors (ependymoma). Incidence of animals bearing these tumors was not statistically significant at the mid-dose level. However at the high-dose level, when animals were combined (males + females), the incidence of liver tumors was significant ($p < 0.05$) as well as the incidence of brain tumors ($p < 0.05$).

Epichlorohidrin (ECH), used as a stabilizer in the technical Alachlor Lot#XHI-167, is an oncogen which causes nasal turbinate tumors in rats through inhalation exposure (JNCI: 65 #4, 1980) and also cause stomach tumors in rats through dietary exposure (Gann, 71, 921-923), December, 1980). ECH's nasal turbinate tumors appear to be similar in nature to the ones noted in this study. However ECH's stomach tumors are not malignant in nature as the above described Alachlor stomach tumors.

Risk assesment associated with Alachlor oncogenicity will be performed by Dynamac within the next 3 weeks. Thus we shall retain Accessions 070985, 070991 and 070992 until the Alachlor's risk assessment is completed. A decision will be made at that time relative to the requested Alachlor tolerances.

Study Classification: Core-Minimum

WRD for CDC

Amal Manfour 6/14/89

Amal Manfour, Ph.D
Toxicology Branch
Hazard Evaluation Division (TS-769)

TS-769:th:TOX,HED:AMAnfour:6-14-89:card3

•Incidence of brain ependymoma increased in the mid- and high-dose male and female groups. The total number of animals bearing these tumors (males and females combined) was dose-related: 0, 0, 2 and 3 animals in the control, low, mid and high-dose groups respectively. Only the incidence at the high-dose level (both sexes combined) was statistically significant ($p < 0.05$, Chi square test).

•Incidence of mammary gland tumors (mostly adenoma) appeared to increase in treated females, i.e. 8, 14, 12 and 14 animals with tumors in the control, low-, mid- and high-dose groups respectively. However this increases were not statistically significant.

NOTE: Statistical significances reported in the above discussion were calculated using the one sided Fisher exact test with the exception of liver and brain where the significances reported above were calculated using the Chi square test.

Conclusions:

Lifetime dietary exposure of Long-Evans rats to Alachlor at 14, 42 and 126 mg/kg/day dosage levels indicated the following:

•A NOEL for Alachlor chronic toxicity in rats could not be demonstrated in this study at the lowest dosage tested, 14 mg/kg/day. Degenerative ocular and hepatic changes as well as other pathological gross and microscopic findings (see review, i.e. thyroid, kidneys, brain, spleen, heart, prostate and ovaries) were noted at this low dosage level and above.

Ocular lesions were further confirmed in a new study on Long-Evans rats at 15 mg/kg/day (Personal communication with the registrant 6/7/82).

•Alachlor is oncogenic in rats at 42 mg/kg/day and above.

Nasal turbinate tumors (mainly benign) increased in both males ($p < 0.001$) and females ($p < 0.02$) at the mid-dose level (42 mg/kg/day) and above in a dose-related fashion (0/50, 11/50 and 23/50 in males and 0/50, 5/50 and 10/50 in females for the control group 42 mg/kg/day group and 126 mg/kg/day group respectively); see description of this kind of tumors on p. 19.

The above table reflects the following findings:

Incidence of stomach malignant tumors was noted in the high-dose male and female groups, and was statistically significant in both sexes, ($p < 0.001$). This kind of tumor was not noted in any other group in this study with the exception of one mid-dose female.

The stomach tumors were described in the study as follows "The neoplasm was pluripotent in its ability to form a mixed carcinoma-sarcoma type of tumor. Some of the neoplasms appeared to have been leiomyosarcomas, others formed osteoid and bone (osteosarcoma), some were pure adenocarcinomas while the bulk of the tumors were mixed carcinoma sarcoma cell types. The sarcomatous element frequently had the propensity to form osteoid and bone. Secondary spread of the tumors were recorded in the pancreas (rats 836, 843), liver (rat 843), mesenteric lymph nodes (rat 836), and lungs (rat 711). The small and large intestines were not involved".

Incidence of nasal turbinate adenomas was noted in animals of the mid- and high-dose groups and was dose-related. Turbinate carcinomas were noted only at the mid-dose level in one male and one female rats. The incidence of these tumors (primarily benign) was statistically significant in mid- and high-dose female groups ($p < 0.02$ - $p < 0.001$, respectively) and in mid- and high-dose male groups ($p < 0.001$).

The study describes this kind of tumors as follows "The tumors developed from the respiratory epithelium primarily in the mid region of the dorsal turbinate. They were characterized by rows and swirls of crowded but typical appearing columnar epithelial cells often crowned with cilia. The cell masses grew inward and when large tended to conform to the shape of the turbinate lumen. Some contained well vascularized supporting stroma while others were more densely cellular with little supporting stroma".

*Incidence of thyroid follicular tumors (adenoma - carcinoma appeared to increase in both males and females of the high-dose group as compared to the control group. However the increase was only significant ($p < 0.001$) in males.

*Incidence of liver tumors (adenoma - hyperplastic nodules increased in both sexes in the mid- and high-dose groups. The total number of animals bearing these tumors (males and females combined) was dose-related: 3, 6 and 9 animals in the control, mid and high-dose groups respectively. Only the incidence at the high-dose level (both sexes combined) was statistically significant ($p < 0.05$, Chi square test).

*Note: Epichloronidrin (ECH), used as a stabilizer in the technical Alachlor's Lot#KHI-167, is an oncogen which causes nasal turbinate tumors in rats through inhalation exposure (JNCI.65 #4, 4, 1980) and also causes stomach tumors in rats through dietary exposure (Gann, 71, 933-938, December, 1980). However ECH's stomach tumors are not malignant in nature as described above.

Liver	50	50	50	50	50	50	50	50
adenoma	1	0	2	1	0	0	1	3
Nodular heperplasia	0	0	1	4	2	1	2	1
Thyroid	48	50	49	50	49	44	46	49
C-cell adenoma	4	5	2	7	2	3	4	3
carcinoma	1	0	0	0	0	0	1	0
Follicular adenoma	1	0	1	11***	0	0	2	2
carcinoma	0	0	0	2	0	0	0	2
Mammary gland	35	31	31	35	40	46	50	42
adenoma	0	0	0	0	7	14	12	14
carcinoma	0	0	0	0	1	0	0	0

*p < 0.02
***p < 0.001

As noted in the above table the number of animals with multiple tumors of different histogenic origin was generally higher in all treated male groups and in the high-dose female group than the respective control groups. It was reported that animals of the high-dose group were commonly found with 3 to 4 tumor types and occasionally 5 or 6. One high dose rat (#744) had seven different histogenic tumor types. This noted multiplicity of tumors is compound related. Also increased mortality rates in treated animals during the study is compound (and neoplasia) related.

The following table summarizes the kind and location of observed tumors:

Organs Examined

	MALES				FEMALES			
	Control	Low	Mid	High	Control	Low	Mid	High
Stomach	49	50	50	50	50	50	50	50
Malignant tumor (see page 19)	0	0	0	17***	0	0	1	13***
Nasal Turbinates	46	46	41	42	49	47	45	48
Adenoma	0	0	10***	23***	0	0	4*	10***
Carcinoma	0	0	1	0	0	0	1	0
Brain	50	50	50	50	50	50	50	50
Ependymoma	0	1	0	2	0	0	0	1
Oligodendroglioma	0	0	0	1	0	0	0	0
Astrocytoma	0	0	0	0	1	1	0	0
Granular cell tumor	0	0	1	0	0	0	0	0

ANIMALS WITH ONE OR MORE MALIGNANT TUMORS

Group	D		T		Total[1]	
Males	No.	%	No.	%	No.	%
Control	14/24	58.3	11/26	42.3	25/50	50
Low	12/33	36.4	5/17	29.4	17/50	34
Mid	13/32	40.6	4/18	22.2	17/50	34
High	20/31	64.5	11/19	57.9	31/50	62
Females						
Control	6/17	35.3	7/33	21.2	13/50	26
Low	11/23	47.8	8/27	29.6	19/50	38
Mid	5/18	27.8	6/32	18.8	11/50	22
High	22/30	73.3	12/20	60	34/50***	68

***: $p < 0.001$

The table above reflects a higher incidence of animals (male and female) bearing malignant tumors at the high-dose level and in the low-dose level in the female group (especially for the rats that died in extremis). However the increase was only significant ($p < 0.001$) in the high-dose female group.

ANIMALS WITH MULTIPLE TUMORS OF DIFFERENT HISTOGENIC ORIGIN

Group	D		T		Total[1]	
Males	No.	%	No.	%	No.	%
Control	10/24	41.6	12/26	46.1	22/50	44.0
Low	16/33	48.3	11/17	64.7	27/50	54.0
Mid	16/32	50.0	10/18	55.5	26/50	52.0
High	19/31	61.2	14/19	73.6	33/50	66.0
Females						
Control	6/17	35.2	16/33	48.4	22/50	40.0
Low	8/23	34.7	11/27	40.7	19/50	38.0
Mid	8/18	44.4	13/32	40.6	21/50	42.0
High	23/30	76.6	13/20	65.0	36/50	72.0

D - died or sacrificed during study
T - sacrificed at termination of study
1 - based on total number of animals in study

Neoplastic Lesions

The incidence of tumor bearing rats in all treatment groups was similar to the incidence noted in the control group with the exception of a slightly higher incidence in the high-dose male group. However the number of those rats that died before the scheduled termination was higher in all treatment groups with the exception of the mid-dose females (the incidence of tumors in this group was similar to the control animals).

The total number of tumors was markedly higher in the high-dose group than in the control, low, and mid-dose groups.

The table below reflects the above discussed data:

ANIMALS WITH ONE OR MORE TUMORS OF ANY KIND

Group	D		T		Total[1]		Total No. of Tumors Per Total No. of Animals with Tumors
Males	No.	%	No.	%	No.	%	
Control	17/24	70.8	24/26	92.3	41/50	82.0	78/41
Low	29/33	87.8	15/17	88.2	44/50	88.0	89/44
Mid	24/32	75.0	14/18	77.7	38/50	76.0	86/38
High	29/31*	93.5	18/19	94.7	47/50	94.0	127/47
Females							
Control	14/17	82.3	31/33	93.9	45/50	90	79/45
Low	22/23	95.6	24/27	88.8	46/50	92	76/46
Mid	13/18	72.2	30/32	93.7	43/50	86	75/43
High	27/30	90.0	18/20	90.0	45/50	90	109/45

D - died or sacrificed during study
T - sacrificed at termination of study
1 - based on total number of animals in study

*: $p < 0.05$

	Control		Low		Mid		High	
	M	F	M	F	M	F	M	F
Thyroid:	49	49	50	44	49	46	50	49
*Squamous cyst	3	10	10	8	6	8	10	11
*Follicular atrophy	1	0	-	0	1	0	5	13
Spleen:	49	50	49	49	50	50	49	49
*Extramedullary hematopoiesis	8	10	10	12	15	8	16	30
Kidneys:	50	50	50	50	50	50	50	50
*Interstitial lymphocytic infiltrate	5	3	1	14	4	9	2	14
Urinary Bladder:	48	47	50	48	45	48	49	49
*Transitional cell hyperplasia	5	0	3	1	1	4	14	6
Prostate:	47	-	50	-	47	-	50	-
*Atrophy	6	-	15	-	16	-	16	-
Ovaries:	-	47	-	49	-	49	-	50
*Atrophy	-	9	-	19	-	13	-	14
Eyes:								
*Cataracts	7 46	7 47	1 47	0 50	5 45	2 47	35 46	48 50
*Retinal degeneration	0 46	1 47	0 47	0 50	0 45	1 48	16 46	38 44
*Iris Atrophy	0 46	0 47	0 47	0 50	0 45	0 47	14 46	18 40
Liver:	50	50	50	50	50	50	50	50
*Periportal hepatocyte hypertrophy	2	5	4	9	12	29	13	15
*Ground glass cytoplasmic change	0	0	1	0	7	21	1	4
*Cytoplasmic laminated bodies	1	0	0	0	6	4	9	5
*Central lobular hepatocyte necrosis	0	0	5	5	9	1	11	11
*Dimpling of liver surface	0	0	1	0	0	1	0	1

- : Unremarkable difference from control.

Organ weight changes noted at the low-dose level were not always statistically significant. However they may be considered significant biologically: i.e. 21% increase in thyroid relative organ/brain weight, in males; 11-19% increase in kidney weight values in females; 12-15% increase in heart weight values in females; and 28-35% increase in spleen weight values in females.

Gross observations which correlated with microscopic tissue findings and were considered to have been compound related, included degenerative liver changes at all dosage levels; cataracts and tumors of the glandular stomach in the high dose rats.

Chronic renal disease and neoplasia were the major causative factors of animal deaths during the study period.

Histopathology

Non-Neoplastic lesions:

The following microscopic lesions were noted in this study. These lesions appear to be compound related but not always dose-dependent. The following table describes these lesions and the incidence of their occurrence relative to the control group:

(Number of animals examined is listed across from the designated organ):

	Control		Low		Mid		High	
Organ	M	F	M	F	M	F	M	F
Brain:	50	50	0	50	50	50	50	50
Compression atrophy	0	0	0	4	2	10	0	0
Mediastinal	41	40	38	42	39	44	39	42
Lymph nodes:								
Plasma cell hyperplasia	1	1	1	0	3	3	1	10
Heart:	50	50	50	50	50	50	49	50
Myofiber hypertrophy atria	1	0	6	1	1	0	5	5
Lung:	48	50	50	50	50	49	49	49
*Paabronchial Lymphoid hyperplasia	25	32	34	30	35	33	30	37
*Alveoli filled with foamy macrophages	3	5	8	4	3	-	-	-
Mesenteric Lymph Nodes:	48	50	49	49	47	49	48	48
*Plasma cell hyperplasia	-	3	-		-		-	8

Organ weights for animals killed at termination of the study reflected an increase in thyroid weights (absolute and relative) for both males and females of all groups, increase in liver weight of the high-dose males and females, and increase in relative kidney, heart and brain weights of both high-dose males and females. The spleen of the high dose females also significantly increased in weight while the ovaries in the same animal group decreased. Changes in spleen weight in males were inconsistent. (See table below):

% of Control

		Thyroid		Liver		Kidney		Heart	
		Female	Male	Female	Male	Female	Male	Female	Male
Low	Absolute	114.2	119.4	115.7*	-	119.9*	-	114.8*	-
	Rel. to bw	111.2	126.5	110.0	-	111.4	-	112.3	-
	Rel. to brain	112.2	121.7	113.6	-	113.5	-	112.9*	-
Mid	Absolute	128.4**	151.9	108.6	-	-	-	-	-
	Rel. to bw	128.9**	174.0*	108.0	-	-	-	-	-
	Rel. to brain	129.9**	158.1*	169.6	-	-	-	-	-
High	Absolute	115.6	123.7	122.7**	113.1*	-	108.9	108.9	107.0
	Rel. to bw	138.8**	159.3	148.4**	147.0**	125.1*	128.8**	130.2**	139.7**
	Rel. to brain	117.8	126.4	124.6**	115.7*	108.0	10[illegible]9*	110.0	109.5*

		Brain		Spleen		Ovaries
		Female	Male	Female	Male	Female
Low	Absolute	-	-	135.2	82.3	84.9
	Rel. to bw	-	-	128.0	87.7	81.0
	Rel. to brain		-	133.2	87.2	84.0
Mid	Absolute			-	83.6	-
	Rel. to bw	-	111.8	-	91.1	-
	Rel. to brain		-	-	10.20	-
High	Absolute	-	-	174.0**	102.0	73.6*
	Rel. to bw	117.9**	127.0**	205.7**	131.5**	86.7
	Rel. to brain	-	-	166.4**	105.1	76.1*

*p < 0.05 **p < 0.01; -: no change noted

NOTE: Organ weight calculations: $\frac{W \text{ for treatment group}}{W \text{ for control group}} \times 100$

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At week 86 examination 2/43 low-dose male rats unilaterally exhibited this syndrome. However, these rats died prior to subsequent examinations, and no additional low-dose rats developed this syndrome during the study period. The mid-dose group exhibited this syndrome in 24% of the males and 53% of the females on week 88 of study; the incidence of this finding increased in this group to 67% and 62% in males and females respectively at termination. All animals of the high-dose group were affected from week 86 throughout the remainder of the study period. None of the control animals were affected.

It is important to note that this syndrome was not noted in previously tested rats of different strains and that subsequent microscopic examination of the eyes of these animals did not demonstrate the presence of this syndrome. However it is also important to note that in life slit lamp evaluation of the eye is superior to microscopic evaluation of eye sections.

Hematology, Blood Chemistry and Urinalysis

No consistent dose related variations in these parameters were reported. Occasional statistically significant deviations were reported. For example in females SGOT values statistically decreased ($p < 0.05$) in all treated group on month 8 of study and SGPT decreased ($p < 0.01$) only at the high-dose level in this determination interval while decreased ($p < 0.05$) in both the mid- and high-dose rats at the 12-month determination interval. These decreases were not noted later in the study.

Alkaline phosphatase increased in females of all treated groups at the 12-month determination interval ($p < 0.05$ for low and mid-dose) and at the 18 and 24-month intervals for the high-dose group (but not statistically significant). Reticulocyte values decreased ($p < 0.05$) in females of the mid and high dose groups at the 12-month determination.

In males SGOT values were significantly decreased ($p < 0.05$ for both mid- and high-dose rats of the 12-month interval and for high-dose males on the 18-month interval. SGPT significantly decreased ($p < 0.05 - 0.01$) in all male dosage groups at the 12-month interval and in the high-dosage group ($p < 0.01$) at the 18-month interval. These decreases did not persist until termination at the low dose but continued to decrease at the mid and high dose levels for SGOT values for month 24 and terminal determinations, and for SGPT values for both dosage levels at termination.

Mean water consumption (mg/kg/day) for the high-dose females was statistically reduced ($p < 0.01$) in both the 3-day period of determinations at month 12 and 18; data at termination appear to be erratic due to excessive water spillage in female controls.

Mean water consumption was slightly reduced, (but not significantly) at the 12 and 18 month intervals for the low- and mid-dose females and all treated male groups.

Ophthalmoscopy

Alachlor caused damage to the uveal tissue in a progressive and dose-related fashion in this Long-Evans strain of rats. This uveal degeneration syndrome was first identified in the study when opnthalmoscopic examinations were performed on animals exhibiting eye opacities (described then as corneal) during the second year of study. This syndrome does not resemble the usual spontaneous (and generally transient) iritis or uveitis in the rat.

A clinical description of this uveal degeneration syndrome is presented on pages 20 to 24 of the Bio-dynamics final report (Section II, Vol. 2, Acc.#070586): "In its mildest form the syndrome was characterized by free floating iridial and choroidal pigment in the ocular chambers and pigment deposition on the cornea and lens. In its most severe form, the syndrome was characterized by bilateral degeneration of the iris and diminution of the size of the ocular globe with secondary total cataract formation".

The table below (pages 22 & 298 of Bio/dynamics report reflects the number of rats with the treatment-related uveal syndrome:

Dosage Group mg/kg/day		Weeks 86 & 88[a] No.[b]	%	Week 106 No.	%	Week 115 No.	%
0	Males	0/44	0	0/37	0	0/28	0
	Females	0/46	0	0/33	0	-	-
14	Males	2/43[c]	5	0/31	0	0/20	0
	Females	0/43	0	0/27	0	-	-
42	Males	11/45	24	22/23	67	14/21	67
	Females	23/43	53	21/34	62	-	-
126	Males	41/41	100	27/27	100	20/20	100
	Females	38/38	100	20/20	100	-	-

[a]Slit lamp exam conducted Week 88, 106 and 115; Opnthalmoscopic exam conducted Week 86, 106 and 115.

[b]Number of animals with treatment-treatment uveal syndrome total number of animals examined.

[c]Both rats #314 & 307, affected unilaterally with the mildest form of uveal syndrome died prior to Week 106 examination.

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For males, the percentages surviving to scheduled termination for 0, 14, 42 and 126 mg/kg/day groups were 52%, 34%, 36% and 38% respectively; and for females these percentages for the same dosage groups were 66%, 54%, 64% and 40% respectively.

Body Weight, Food and Water Consumption

Mean body weight of treated animal groups was unremarkable from the control group during the first year of study. However statistically significant decreases were noted in the mid- and high-dose male groups and in the high-dose female group throughout the second year of the study. The greatest decrease in the mean body weight values was noted at week 106, i.e. 12% and 20% decrease in the mid and high-dose male groups respectively and 16% decrease in the high-dose female group as compared to the control rats.

The table below reflects the mean body weight data at week 106:

Mean Group Body Weights (No. of rats in group)

MALES

Week	Control	Low	Mid	High
0	181.8(50)	180.2(50)	179.9(50)	180.0(50)
52	611.3(40)	613.3(39)	616.0(40)	585.2(37)
106	622.8(37)	607.3(31)	547.0**(33)	497.8**(26)

FEMALES

Week	Control	Low	Mid	High
0	147.1(50)	146.9(50)	146.9(50)	147.4(50)
52	332.6(40)	340.7(39)	340.1(38)	335.4(39)
106	359.9(28)	381.4(22)	361.4(28)	302.2*(15)

*p < 0.05 **p < 0.01

Mean food consumption and feed efficiency values were unremarkable from the control group for all treated males and females. However feed efficiency was evaluated for the first 13 months only.

Results:

Alachlor Concentration in Diet

Based on food consumption and body weight data the calculated compound consumption was found to be as follows:

Group	Dosage (ppm)	Dosage Level (mg/kg/day) Weeks 2 to 4 M	F	Weeks 5 to Termination M & F
I	0	0.00	0.00	00
II	100	11.67	14.09	14
III	300	35.59	40.90	42
IV	1000	118.34	138.38	126

Chemical analysis of the treated diets indicated that at the beginning of the week of preparation the diets contained an average of 86% to 102% of the theoretical dosage levels. Test diets sampled at the end of the week of preparation contained an average of 84% to 95% of the target level.

Chemical analysis of the technical grade Alachlor used to prepare the diets demonstrated a mean percent active ingredient of 89.6%.

Observations and Mortality

The report states that corneal opacity was routinely observed in many of the high-dose females during the second year of study, and that no other physical observations were noted in any of the treated animals which were considered to be related to the administration of Alachlor (Clinical observations data for individual animals were not submitted).

Survival at termination, males at 27 months and females at 25 months, is noted below:

Dose Group mg/kg day	Survivors M	F
0	26	33
14	27	27
42	18	32
126	19	30

lungs with mainstem bronchi and trachea
lymph node (mesenteric, mediastinal)
ovary (2)
pituitary
prostate/seminal vesicles
salivary gland (mandibular)
skeletal muscle/sciatic nerve (right biceps femoris)
skin and mammary gland (right inguinal)
spinal cord (cervical)
spleen
stomach
testis (2)
thymus
urinary bladder
uterus
gross lesions (including a section of normal-appearing portion of same tissue)
tissue masses or suspect tumors and regional lymph nodes

Histological Examinations

Eyes with Harderian glands, testes and epididymides were preserved in Bouin's solution for 48 to 72 hours followed by 10% neutral buffered formalin.

All other tissues were preserved in 10% neutral buffered formalin. Tissues were stained with hematoxylin and eosin.

Slides of all tissues listed in the above section (including 2 sections of spinal cord and 3 coronal sections through the head) were prepared for all animals by American Histolabs, Inc., Rockville, Maryland and evaluated microscopically by Dr. Robert F. McConnell, Flemington, New Jersey.

Statistical Analysis

Statistical analyses of data was performed by using various statistical methods. F-test and Student's t-test were used for analysis of the hematology and clinical chemistry data. Dunnett's test was used for analysis of data on body weight, food consumption, feed efficiency, water consumption, organ weights, organ/body and organ/brain weight ratios. Chi square and Fisher exact tests were used for analysis of oncogenic data.

Statistically significant differences from the control group were indicated at $p < 0.05$.

Necropsy:

All animals were subject to necropsy. Complete postmortem examinations were performed on animals that died during the study or at scheduled termination. Animals were sacrificed by exsanguination under ether anesthesia.

Brain (with entire brain stem), liver, kidneys, heart, spleen, thyroid, pituitary, adrenals, testes and ovaries were weighted at necropsy for animals sacrificed at termination and organ to body weight and organ to brain weight were calculated.

The following tissues were preserved (and histopathologically examined) for all animals:

Tissues Preserved:[2]

adrenals (2)
aorta (abdominal)
blood smear
bone and bone marrow (costochondral junction)
brain with entire brain stem
epididymis (2)
esophagus, trachea/thyroids parathyroids
eye (2) with optic nerve and Harderian gland
head with entire skull cap
heart with coronary vessels
intestine
cecum
colon
duodenum/pancreas
ileum
jejunum
kidney (2)
liver

---

[2]Numbers in parentheses indicate number of organs section preserved.

Laboratory Studies

Blood was collected from 10 rats/sex/group. The animals were selected randomly and used at all intervals when feasible. Rats were fasted overnight prior to blood collections (via venipuncture of the orbital sinus under light ether anesthesia. Analyses were performed at the following intervals:

Parameter Evaluated

Hematology (performed at months 4, 8, 12, 18 and 24).

- hemoglobin
- hematocrit
- erythrocytes
- reticulocytes (if anemia was indicated)
- platelets
- total and differential leukocytes
- erythrocyte morphology

Blood Biochemistry (performed at months 4, 8, 12, 18 and 24):

- serum glutamic oxalacetic transaminase
- serum glutamic pyruvic transaminase
- alkaline phosphatase
- lactic acid dehydrogenase
- blood urea nitrogen
- fasting glucose
- cholesterol
- total protein
- albumin
- globulin
- A/G ratio
- total bilirubin
- potassium
- calcium

Urinalysis (performed at months 4, 12, 18 and 24):

- gross appearance
- specific gravity
- pH
- protein
- glucose
- ketones
- bilirubin
- occult blood
- microscopic analysis

Preparation of Test Diet:

Crystalline technical Lasso was melted to 45°C and appropriate amounts were mixed with 100 ml acetone and incorporated into the standard laboratory diet weekly. The amount of test substance was adjusted weekly based on the most recent weekly body weight and food consumption data.

Diet analyses were performed on 4 oz. samples of the treated and control feed at the following intervals: weeks 1, 2, 3, 4, 6, 7, 8, 9, 12, 24, 36, 48, 49, 50, 51, 53, 55, 59, 71, 81, 96.

Technical grade Alachlor was also assayed at 12 intervals to determine its stability during storage.

Observations:

The animals were observed twice daily for toxicologic effects. Physical examination and palpation for tissue masses were performed weekly.

Ophthalmoscopic examination were performed by Dr. Lionel F. Rubin (D.V.M.) on weeks 86, 106 and 116 (for males only) a slit lamp was used for these examinations (except on week 86).

Body weights and food consumptions were determined at pretest, weekly through 13 weeks and biweekly from week 14 to termination.

Compound intake and food efficieny were calculated from body weights and food consumptions data.

Water intake was also determined for 10 animals/sex/group for two 3-day periods at 12 and 18 months and for one 2-day period at termination.

Study Design

Male and female rats were randomly divided into groups and fed Alachlor continously in the diet at the following nominal concentrations for the entire duration of the study:

Group	Dosage ppm M/F*	Dosage mg/kg/day M/F	Number of Animals/Sex/Group Initial M/F	Clinical Lab. Studies M/F	Histopathology
I	0**	0	50	10	All Animals
II	100	14	50	10	All Animals
III	300	41	50	10	All Animals
IV	1000	126	50	10	All Animals

M/F: represents males and females
**Vehicle (Acetone) was administered to feed.

Test Animals:

Two hundred sixty nine male (mean body weight 135g) and 264 female (mean body weight 120g) rats, Long-Evans strain, were initiated in this study. The rats were obtained from Blue Spruce farms (Altamont, New York 12009) when 37 day-old and acclimatized for 12 days before treatment at age 50 days. Animals were given a physical examination and assigned to groups after ear tagging for identification.

The rats were individually housed in elevated stainless steel cages and maintained on a 12-hour light/dark cycle and temperature controlled environment. Control and test diet (untreated treated Purina Lab Chow, R-5001) and water were available ad libitum.

•Stomach malignant tumors increased significantly ($p < 0.001$) in both sexes at the high-dose level, 126 mg/kg/day (see page 19).

•Thyroid follicular tumors (adenoma + carcinoma) appeared to increase in both sexes at the high-dosage level. However the increase was significant ($p < 0.001$) only in males.

•Incidence of other tumors in other organs was also noted to increase at the mid and high-dose levels, potentially as a result of Alachlor administration, i.e. liver tumors (adenoma - hyperplastic nodules) in both sexes and brain tumors (ependymoma oligodendroglioma). Incidence of animals bearing these tumors was not statistically significant at the mid-dose level. However at the high-dose level, when animals were combined (males + females), the incidence of liver tumors was significant ($p < 0.05$) as well as the incidence of brain tumors ($p < 0.05$).

•A risk assessment associated with Alachlor oncogenicity will be performed by Dynamac Corporation within the next 3 weeks. A decision will be made at that time relative to the requested Alachlor tolerances.

REVIEW

Study Identification

A Chronic Feeding Study of Alachlor in Rats. Bio/dynamics Inc., Project#77-2065 (BD-77-421), 11/13/81; submitted on 1/5/82. Accessions#070586 to 070590.

In life phase of study was from 4/12/78 through 7/1 to 2/80 (612 to 613 days) for males and from 4/12/78 through 4/21 to 04/80 (741 to 744 days) for females.

Materials and Methods

Test Substance

Alachlor (Lasso® Technical), a clear brown, slightly viscous liquid, was supplied in two batches by Monsanto. Lot#XMI-16 (92.6% a.i.), stabilized with 0.8% epichlorohydrin, was used from 4/12/78 to 3/6/79; and Lot#MHX-6 (92.19% a.i.), stabilized with [redacted] was used from 3/7/79 to termination.

Appendix A

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

MEMORANDUM JUN 16 1982

OFFICE OF
PESTICIDES AND TOXIC SUBSTANCES

TO: Robert Taylor (12)
Registration Division (TS-769)

THRU: Orville E. Paynter, Chief
Toxicology Branch
Hazard Evaluation Division (TS-769)

SUBJECT: EPA Reg.#524-316; Alachlor; Review of Monsanto Chronic Feeding Oncogenicity Study of Alachlor in Rats. R.D.#396, Special Report MSL#1983; Section II, Volumes 2 to 6. Accessions#070586, -87, -88, -89 and -90. CASWELL#11

Action Requested:

A review is requested for a chronic feeding, oncogenic study in rats submitted by Monsanto Company as a part of the requirement to support registrations and tolerances for Alachlor (2-chloro-2',6' diethyl-N-(methoxymethyl)-acetanilide), a herbicide.

Conclusions:

*This study is classified as Core-Minimum.

1) A NOEL for Alachlor chronic toxicity in rats could not be demonstrated in this study at 14 mg/kg/day (LDT). Ocular lesions (uveal degeneration syndrome) and hepatotoxicity are among the most noted findings associated with Alachlor administration at all dosage levels tested. (See Conclusions page 20).

2) Alachlor is oncogenic in rats at 42 mg/kg day and above.

*Nasal turbinate tumors (mainly benign) increased in both males ($p < 0.001$) and females ($p < 0.02$) at the mid-dose level (42 mg/kg/day) and above (see page 19).

LOT

Page_____ is not included in this copy.

Pages 129 through 134 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.

____ Description of quality control procedures.

____ Identity of the source of product ingredients.

____ Sales or other commercial/financial information.

____ A draft product label.

____ The product confidential statement of formula.

____ Information about a pending registration action.

✓ FIFRA registration data.

____ The document is a duplicate of page(s) _____.

____ The document is not responsive to the request.

The information not included is generally considered confidential by product registrants. If you have any questions, please contact the individual who prepared the response to your request.

alachlor. In female, CD-1 mice alachlor administration (dietary) is associated with statistically significant increase in lung tumors (bronchioalveolar adenomas + carcinomas) over control mice.

Alachlor has been tested in several *in vitro* and *in vivo* assays for mutagenicity and/or DNA damage. It was found to be "weakly genotoxic" in an *in vivo* - *in vitro* rat hepatocyte DNA repair assay. All other assays were judged negative. However, a metabolite (see metabolite XII in Figure 1) of alachlor has been found to be positive in the Ames almonella assay (strain TA 100) both with and without metabolic activation.

Oncogenicity data is available on two other herbacides which are structurally related to alachlor. [redacted] Metalochlor when fed to CD rats caused an increase in proliferative liver lesions (neoplasitc nodules) in the high dose female rats. In this same study, nasal turbinate tumors were seen in two high dose males and one high dose female.

Nasal turbinate tumors significantly ($p < 0.01$) increased in both males and females at 15 mg/kg/day. This kind of tumor was also noted in one mid-dose female. A different kind of nasal tumor (submucosal gland adenoma) was noted in one mid dose male.

Nasal submucosal gland hyperplasia significantly increased ($p < 0.01$) in both sexes at the high dose level. According to Dr. L. Kasza (pathologist), the picture of this lesion (picture 9) cannot lead to a definitive conclusion as to whether this lesion is hyperplasia or neoplasia. It is advisable for the registrant to ask a second opinion from an independent pathologist. The Agency is willing to further discuss this matter with Monsanto representatives.

Two additional kinds of tumors, thymus lymphosarcoma and adrenal phenochromocytoma significantly increased ($p < 0.05$) in the high dose females.

An increase was also noted in the incidence of thyroid follicular cell tumors in the high dose male group (13.3% incidence in the high dose as compared to 6.7% in the control). Although this increase was not statistically significant, it is considered biologically significant.

Some increase in the total incidence of malignant tumors was noted in all treated female groups especially at the high dose level (3%, 10% and 21% above the control group in the low, mid and high dose, respectively).

A significant decrease in lactate dehydrogenase activity was noted in this study in all treatment groups. The decrease was dose-related in both sexes. The author stated that this finding may be related to 'some chemical interference with the analytical procedure' in this testing facility. However, this reviewer notes that this explanation does not preclude a compound related-effect. In addition, the nature of this chemical inteference and its relation to the test compound needs to be explained by the registrant.

A high incidence of brain compression was noted in this study in the female groups including the control group. The incidence of this finding was remarkable in the high dose male as compared to the male control group. However, the brain weight was not reported in this study. The registrant should submit these data for review.

A new risk assessment associated with alachlor oncogenicity will be performed by the Toxicology Branch based on the new incidence of nasal turbinate tumors.

This study is classified as Core-Minimum. However, this study must be considered in conjunction with a previous study (BD-77-421) where higher dosage levels were tested (0, 14, 42 and 126 mg/kg/day) so that the non-neplastic lesions (ocular lesions and hepatotoxicity) and neoplastic lesions (nasal turbinate tumors, thyroid tumors, etc.) can be adequately assessed. Study ML-80-224 which will be submitted in the near future and where higher dosage, 126 mg/kg/day, was concurrently tested with the dosages used in the present study (ML-80-186) should be also considered in conjunction with this evaluation for the same reasons stated above.

Background:

On January 5, 1982, Monsanto submitted for review a chronic feeding/oncogenic study of Alachlor in the Long-Evans strain of rats (RD#396, Special Report MSL #1983; Accession Nos.: 070586 - 070590). The study was performed by Bio/dynamics Inc. (BD-77-421), reviewed on 6/16/82 and classified Core-Minimum.

During the in-life stage of the above study, April 1978 to July 1980, the animals treated with Alachlor developed a unique ocular lesion, namely, the uveal degeneration syndrome. Monsanto decided to further study this lesion and to establish a NOEL for this effect. Thus, in August 1980, the registrant initiated a new study in the Long-Evans rats, Study #ML-80-186/224 .

However, after the initiation of the new study, Monsanto decided to modify the protocol in 1981 in order to re-investigate the apparent oncogenicity of this chemical which was noted in the histopathology of the animals examined in the previous study.

Epichlorohydrin, a known carcinogen, was used as a stabilizer in the Alachlor sample used during the first year of study #BD-77-421. The registrant apparently suspected that the oncogenicity observed in this study was triggered by epichlorohydrin. Thus, Monsanto replaced epichlorohydrin by [redacted] in Alachlor products. The Alachlor samples used in the new study were epichlorohydrin-free.

According to a letter by Monsanto dated 11/10/83, the registrant apparently divided the data obtained from the new study (ML-80-186/224) into two studies:

(1) Study #ML-80-186 which contains data for the lower dosage groups, i.e. 0.5, 2.5 and 15 mg/kg/day, was submitted for review on 2/28/84.

(2) Study #ML-80-224 which contains data for the highest dosage group, 126 mg/kg/day, will be submitted at a later date. Only a summary of the nasal turbinate tumors in this study was submitted on 3/9/84 (see Appendix 1 to the following review) and an unaudited summary of the other neoplastic lesions in this study was submitted on 3/28/84.

Review

Study Identification:

Study Title: A Chronic Study of Alachlor Administered in Feed to Long-Evans Rats.

Accession Numbers: 252496, -7 and -8

Sponsor: Monsanto Company

Testing Laboratory: Monsanto Environmental Health Laboratory (EHL), St. Louis, Missouri 63110.

Study No.: EHL #800218, Project #ML-80-186, Report #MSL-3284

Study Director and Author: L.D. Stout

Quality Assurance Manager: Arther Gelmer

Dates: In life stage: From 8/20/80 to 10/14/82
Report date: 2/12/83
Study was submitted to EPA on 2/28/84

Note: The study director, L.D. Stout, referred to this report as MSL-3284. However, this report was submitted to EPA as report MSL-3282, compiled by Robert W. Street.

Test Substance: Technical Alachlor 94.13% a.i.; stabilized with [redacted] Lot #MULT 0417B. The compound, an orange/amber solid with a low melting point, was received from Monsanto Company on 4/25/80.

CERT INGREDIENT INFORMATION IS NOT INCLUDED

Dosage Tested: 0, 0.5, 1.5 and 15 mg/kg/day.

Diet Preparations: The Alachlor sample was melted at 55 or 60°C, then mixed with an equal amount of acetone and added to Ralston Purina Rodent Chow® 5002 to obtain a premix containing 1,000 ppm alachlor. The appropriate amounts of premix were added to the animal diet in order to obtain the target dosages listed in the above section. The control diet was treated with acetone at a level similar to the amount of acetone used in the premix preparation.

The diets were prepared weekly and were periodically analyzed for homogenicity and stability of the test substance. The diet analyses were performed weekly for the first seven weeks of the study and less often thereafter (see the study's report, Appendix III. table #4). The technical alachlor sample was also analyzed periodically to determine its stability during storage.

Test Animals:

Male and female Long-Evans rats were obtained from Charles River Laboratory (Schoolcraft, Michigan) on 8/6/80 and quarantined for two weeks before treatment at age 7 weeks. The males weighed 210.8 to 264.7 grams and the females weighed 151.1 to 185.1 grams on the first day of treatment. Fifty animals/sex/group were randomly assigned to 4 groups: one control and 3 treatment groups. (One hundred animals per sex were utilized in an additional 125 mg/kg/day treatment group; however, data for this group will be submitted later as a separate study).

During the quarantine period, animals were selected for weight determination, serology tests and histopathological examinations. All animals on test were also ear tagged during this period.

The rats were individually housed in suspended stainless steel cages and maintained on a 12-hour light/dark cycle and in a controlled temperature (70-74°F) and humidity (30 to 60% in environment. Control and test diets and tap water were available ad libitum.

Observations:

The animals were observed twice daily for clinical signs of toxicity and mortality. Physical examination and palpation for tissue masses were performed weekly.

Opnthalmic examinations were performed approximately
ery three months using slit-lamp biomicroscopy. The
ithalmoscopic examinations on month 13 and 24 of the study
18/81 and 8/20/82) were performed by Dr. Lionel F. Rubin
.V.M.).

Body weights and food consumptions were determined weekly
the first 13-weeks of the study and biweekly thereafter
:il the end of the study period.

Compound intake and food efficiency were calculated from
y weights and food consumption. Food efficiency calculations
e only performed for the first 13 weeks of the study.

oratory Studies:

The Laboratory tests were not performed at regular intervals
:ng the study as usually required, because the initial
ective of this study was to investigate the ocular lesions
:h were seen in this strain of rats in a previous chronic
ding/oncogenicity study with alachlor (#BD-77-421, reviewed
3/82). However, a decision was later made in 1981 during
performance of the study to also examine the animals for
:genic effects. Thus, blood chemistry tests and hematological
uations in this study were only performed at termination.

Ten animals/dose/sex were randomly selected, anesthetised
chloroform, and blood was collected from the posterior
cava before sacrifice for gross necropsy. The animals
fasted overnight before blood collection/necropsy. The
:wing hematological and clinical chemistry parameters were
:red:

tology	Clinical Chemistry
rocytes (RBC)	Albumin
cytes (WBC)	Total Protein
lets	Blood Urea Nitrogen
ocrit	Total Bilirubin
lobin	Direct Bilirubin
Corpuscular Volume	Glucose
Corpuscular Hemoglobin	Glutamate Pyruvate Transaminase
Corpuscular Hemoglobin centration	Alkaline Phosphatase
	Glutamate Oxaloacetate Transaminase
	Lactate Dehydrogenase
	Creatinine
	Cholesterol
	Calcium
	Phosphorus
	Chloride
	Sodium
	Potassium*

ecropsy:

All animals were subject to necropsy. Complete postmortem examinations were performed on animals that died during the study or at scheduled termination (a different necropsy schedule for some animals of the 126 mg/kg/day dosage group was adopted, see appendix 1 to this review). The animals were fasted overnight and sacrificed by exsanguination under chloroform anesthesia.

The liver, heart, kidneys, adrenals, spleen, thyroid with parathyroid and ovaries/testes with epididymides were weighed at necropsy for animals sacrificed at termination and organ to body weight ratio was calculated. Note that the brain weight was not included in this protocol.

The following organs/tissues were preserved for all animals and histopathologically examined):

orta	lung
drenals (2)	mammary gland
emur (with marrow)	salivary gland
esenteric and submandibular lymph nodes	pancreas
sciatic nerve	spinal cord
pituitary	trachea
stomach	heart
hyroid with parathyroid	brain
sophagus	nose
varies (2)	eyes with optic nerve
estes with epididymides	duodenum
minal vesicles	jejunum
ctus femoris muscle	ileum
kin	large intestine
pleen	thymus (when present)
rinary bladder	growths
ternis	masses or tumors
kidney	abnormal lesions"
iver	

The eyes were preserved in buffered 2% glutaraldehyde and formalin. Some adrenals with tumors were preserved in 5% glutaraldehyde then 'mordanted with potassium dichromate to stain chromaffin granules'. The remaining tissues/organs were preserved in 10% formalin. Tissues were stained with hematoxylin and eosin for microscopic examinations.

Statistical Analyses:

Statistical analyses of data were performed using the following various statistical methods:

The Generalized Savage[5] and Generalized Wilcoxon[6] techniques were used to analyze the difference in survival of animals between control and treatment groups.

The analysis of variance and Dunnett's test for comparing multiple treatments with a control[1] were used to evaluate the compound-related effects on terminal body weights and the differences in the absolute organ weights.

The Dunnett's test was also used in the analysis of the hematology and blood chemistry data.

The Mann-Whitney test[2] using the Bonferoni Inequality procedure for the comparison of unpaired samples[3,4] was used to analyze the relative organ weight data.

The Fisher Exact Test[3] with the Bonferoni Inequality Procedure[3,4] for comparing unequal groups was used to determine the significances of differences between mean frequencies of microscopic lesions in control and treated groups.

The Peto procedure[7] was used to analyze the significance of some tumors. 'This method calculates, without biases due to differences in longevity, the observed and expected numbers of animals with particular tumor types in each treatment group, and derives from these the p-value for positive trend with respect to dose.'

Note: The above references: 1, 2, 3, 4, 5, 6 and 7 are listed at the end of this review.

Results:

Alachlor Concentration in Diet

Based on food consumption the nominal compound intake was as follows:

"Cumulative Average Test Material Dosage (mg/kg/day)

Month of Study	Level:	Males Low	Mid	High	Females Low	Mid	High
3		0.46	2.29	13.86	0.48	2.38	14.18
6		0.47	2.34	14.13	0.48	2.40	14.37
12		0.48	2.40	14.47	0.49	2.44	14.64
18		0.49	2.43	14.63	0.49	2.46	14.73
25		0.49	2.45	14.74	0.50	2.47	14.80*

As noted above the calculated values for alachlor intake were comparable to the values for the target dosages of 0.5, 2.5 and 15 mg/kg/day.

The prepared diets apparently were homogeneous on the day of preparation. Samples taken from the top, middle and bottom of the low and high dosage diets, and chemically analyzed for alachlor concentrations, reflected a maximum difference of 18% between any two samples in the same location and a maximum difference of 11% between any two samples of different locations (i.e. - top and bottom of container) for the same dosage level. These large differences were only noted in the low dose diet although the analytical concentration in any low dose sample was within +10% to -34% of the target dosage. In the high dosage diet the difference between any two samples of the same location did not exceed 6%; the difference between any two samples of different location, did not exceed 6%; and the difference between any analytical value and the target value in any high dose sample was +21% to +28%. No chemical analysis for homogenicity of the diet was performed on the mid dose diet preparations.

The overall results of the chemical analysis of the treated diets as presented by the author on page 10 of the study indicated that the alachlor mean concentrations were within -11% of the target concentrations. Appendix III (table 4) in the summitted study often reflected much larger variations in the analytical values of alachlor in the diets, see table below:

Period in Weeks	% Difference* between target & analytical Values		
	Low Dose	Mid Dose	High Dose
1 to 7	16.7 to -25.9	6.5 to -20.0	5.3 to -11.5
8 to 52	25 to - 30.1	4.5 to -31.8	8.6 to -32.2
53 to 111	39.8 to -30.0	19.6 to -38.9	9.5 to -21.8
Study Mean	-4.2	-11.5	-8.3

*% Difference = $\frac{\text{analytical value - target value}}{\text{target value}} \times 100$

The test substance apparently was stable in the diets for a 17-day period at room temperature in both the low and high dosage groups, the mid-dose level was not tested (up to 20% decrease in the target concentration was noted in the low dose diet and a 14% decrease was noted in the high dose diet during this period).

The values obtained from the chemical analysis of the technical grade Alachlor sample which was used to prepare the diets demonstrated that alachlor was stable under storage (92.6% to 94.8% a.i.).

Observations and Mortality

The clinical observations of the animals in this study reflected the following symptoms in both the control and treatment groups: hair loss, skin edema and ulceration or abrasions, scabs, overgrown teeth, and teary eyes. However the skin lesions were more noted in the treatment groups than the control group. Occasional findings of animals with apparent misuse or disuse of limbs were also noted in all animal groups including the control group. Also, a few animals with chromadacryorrhea, urogenital discharge, discolored urine, and blood encrustration around eyes were noted in all groups including the control groups. The most significant finding was noted in the animals of the control and treated groups that died during the study; most of these animals exhibited one or more of the following symptoms: piloerection, hypoactivity, paleness, ataxia, salivation and emaciation. In addition to these symptoms some dying animals in the treatment groups were denydrated, had swollen mammary gland or mouth, also blood incrustation around nose was seen in few of these rats.

This reviewer also notes that several animals in the control and treatment groups had missing ear tags.

Alachlor did not cause any increase in the mortality rate of the treated rats as compared to the control rats except in the high dose female group, i.e. a 16% increase was noted in this group as compared to the control female rats (6% increase when compared to the control male rats), see the cumulative mortality rate in the table below:

Cumulative Mortality*

Dose Level (mg/kg/day)	MALES			FEMALES		
	18-mo.	21-mo.	Term.	18-mo.	21-mo.	Term.
0	5	13	33	6	10	28
0.5	3	9	21**	7	11	24
2.5	8	11	21**	7	7	27
15.0	5	8	27	8	16	36

*Based on 50/rats/sex/group
**Statistically significant ($p \leq 0.01$) using the Generalized Savage Test

Although the author indicated that the rate of animal mortality was significantly reduced in males of the low and mid dose groups ($p < 0.01$), this reviewer notes that the apparent longevity in the male treatment groups may be due to an exceptionally high mortality rate in the male control group in this study.

In-Life Palpable Masses

Palpable masses were reported in many animals in the control and treatment groups. They were mostly located on the abdominal area and sometimes on the thorax. A few animals had masses located on the rear limbs, eye, head or neck.

The following table reflects the number of animals which had palpable masses at one time or another during this study:

Number of Animals With Palpable Masses*

Dosage Group	Control	Low	Mid	High
Male	29	21	28	28
Female	30	34	30	37

Total no. of animals/sex/group is 50.

As noted above, the incidence of animals with palpable masses appeared to be slightly higher in both the high-dose female group (14% above control) and the low dose female group (8%); no difference was noted in the mid dose female group.

Ophthalmoscopic Examinations

In-life ophthalmoscopic examinations did not reflect any significant incidences of ocular lesions associated with the uveal degeneration syndrome, a lesion which was significant in a previous chronic study in this strain of rats (study #BD-77-421). The most common finding in this study was "pigment hypertrophy at the pupillary border" in all animal groups. This finding occurred almost with the same frequency in the treated and control groups and was not considered compound-related.

Only 2 females (#6 and #47) of the high dose group (15 mg/kg/day) exhibited an initial stage of the uveal degeneration syndrome (molting of retinal pigmentation) upon the 9/18/81 ophthalomoscopic examination. However these 2 animals died before a second examination on 8/20/82 by Dr. Rubin. It was interesting to note that a year later in this second examination, 1 control male and 2 control females were affected as compared to one male and one female in the high dose group. No effect was seen at any time in males or females of the 0.5 or 2.5 mg/kg/day dosage groups. It would have been helpful if historical data for this lesion were made available to further assess the significance of this finding in this study.

From the above two examinations in this new study, Dr. Lionel Rubin concluded that 'There is no evidence of dose relationship, and, in my opinion, administration of the test compound had failed to produce ocular abnormality'.

This reviewer notes that such a conclusion must also consider: 1) the new study at high dose level, 126 mg/kg/day (ML-80-224), confirming target effects; and 2) the finding in the previous study (#BD-77-421) where two males treated with 14 mg/kg/day also exhibited the above mentioned effect but died before further examination. This effect was dose-related in this study because higher dosages (42 and 126 mg/kg/day) were also tested and demonstrated finding to be followed at lower doses. Hence, it may be concluded that the NOEL for this effect is 2.5 mg/kg/day.

Body Weight and Food Consumption

No statistically significant differences were noted in the mean body weight or the mean food consumption in the treated animal groups as compared to the control group. The table below reflects the mean body weights at the study initiation and after one and two years on study. The final in-life mean body weight determinations are also listed below as well as the mean terminal body weight at necropsy.

Mean Group Body Weights (No. of Rats in Group)

MALES

	Control	Low	Mid	High
8/20/80 (Pretest)	239(50) ±15	239(50 ±15	239(50) ±15	239(50) ±15
8/26/81 1-year	711(49) ±85	719(49) ±92	710(48) ±76	706(48) ±90
8/24/82 2-years	703(25) ±118	748(32) ±113	759(32) ±122	723(25) ±134
9/21/82 (Final*)	703(20) ±105	723(29) ±104	726(30) ±124	696(23) ±136
Terminal**	685(17) ±23	694(29) ±20	692(29) ±23	665(23) ±29

FEMALES

	Control	Low	Mid	High
8/20/80 (Pretest)	170(50) ±8	170(50) ±8	170(50) ±8	170(50) ±8
8/27/81 1-year	410(48) ±68	404(47) ±60	395(50) ±48	416(49) ±61
8/25/82 2-years	490(26) ±110	469(29) ±107	459(29) ±91	464(19) ±92
9/22/82 (Final*)	487(21) ±93	463(28) ±102	449(23) ±80	455(15) ±97
Terminal**	456(22) ±20	446(26) ±20	489(23) ±18	421(14) ±23

*Final: Final in-life mean body weight determinations.
**Terminal: Mean body weights after sacrifice at necropsy. Terminal sacrifices were performed from 9/28/82 to 10/12/82.

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Mean food consumption and feed efficiency values were unremarkable from the control group for all treated male and female groups.

Hematology and Blood Chemistry

The mean hemoglobin and hematocrit values were slightly higher in all treated female groups as compared to the control group. In males, the mean hematocrit values were slightly higher in the low and high dose group than the control group; and the mean hemoglobin values were similar to the control group except for a slight decrease in the high dose value.

Variations in the red blood cell counts in the high dose female group contributed to statistically significant ($p < 0.05$) lower values for the calculated mean corpuscular hemoglobin (MCH) and mean corpuscular hemoglobin concentration (MCHC). The mean red blood cell counts were slightly higher than the control group in all treated animal groups of both sexes.

The mean white blood cell counts were slightly lower than the control in all treated female groups, and slightly higher than the control in the low and high dose male groups.

The mean BUN values were also higher than the control groups in both the low dose male and the high dose female groups.

The mean LDH values were much lower than the control group in both sexes in all dosage groups. The decrease was statistically significant ($p < 0.01$) in all treated female groups. The author indicated that the decrease in LDH values were noted before in his testing facility and that it may be associated with 'some chemical interference with the analytical procedure'. However this explanation does not preclude an actual effect on this parameter. Also the registrant needs to explain the nature of this chemical interference and the role played by the test compound in this interference.

The mean SGOT values decreased in all treated female groups and in the low and high dose male groups; these changes were not statistically significant, but reflected the same trend noted in a previous study (BD-77-421). Also the mean alkaline phosphatase values slightly increased in the high dose female group and in all treated male groups as compared to the respective control group; and a slight decrease was noted in both the low and mid dose female groups. None of these changes were statistically significant.

The mean glucose values increased in all treated animal groups of both sexes. However the increase was only statistically significant ($p < 0.05$) in the high dose female group.

Increases were noted in all treated male groups in the mean potassium values(statistically significant at the mid and high dose levels, $p<0.05$) and in the mean phosphorus values (statistically significant at the mid dose, $p<0.05$). These effects may be of questionable biological significance, although increases in phosphorus values may be associated with thyroid lesions, and increases in potassium values may be associated with adrenal lesions. Additional variations in electrolytes (not statistically significant) were noted in the male groups. The electrolyte variations in the female groups were not statistically significant and were less remarkable than in the male groups.

Necropsy

The mean organ weights (absolute and relative to the body weight) for animals killed at termination did not reflect any significant differences between the treatment groups and the control group. No significant gross lesions were noted at necropsy.

Few relevant findings were noted in the thyroid, pituitary, thymus and liver as described below:

The thyroid absolute weight appeared to increase in all treated male groups but no effect was noted in the female groups. However, the relative thyroid weight to body weight increased in all treated female groups, but only increased at the mid and high dose levels in male groups.

Additional effects were noted in the thyroid upon gross examination:

1) In animals that died during the study, enlargement of the thyroid was observed in all male groups (including the control) and in the mid and high dose female groups (2/27 and 3/36 respectively as compared to 0/28 in the control group). However, at termination, incidences of enlarged thyroid were only noted in males at the high dose level (6/23 animals were affected in this group as compared to 0/17 in the control group).

2) Visible masses in this organ were only noted in males at the end of the study (1/23 mid dose males and 1/23 high dose males were affected).

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Enlarged and congested pituitary glands were especially noted in 14/27 high dose males that died before termination as compared to 8/33 in the control group.

The thymus in all treated female groups had visible masses with 1/50, 3/50 and 3/50 animals affected in the low, mid and high dose groups as compared to 0/50 in the control group.

The livers of 10/23 high dose males that were sacrificed at the end of the study appeared to have abnormal disseminated foci as compared to 4/17 in the control group.

No effects other than those mentioned above were noted at gross examination. However, this reviewer notes that the brain weight was not reported in this study, although significant changes in this organ weight were noted in a previous study (BD-77-421, at dosages higher than 14 mg/kg/day, i.e., 42 and 126 mg/kg/day). These data should be submitted by the registrant as soon as possible.

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Number of Animals Bearing Tumors (%)

Dosage (Group) mg/kg/day	D		T		Total[1]	
Males	No.	(%)	No.	(%)	No.	(%)
0.0 (Control)	29/33	(87.9)	14/17	(82.4)	43/50	(86.0)
0.5 (Low)	16/21	(76.2)	20/29	(69.0)	36/50	(72.0)
2.5 (Mid)	14/21	(66.7)	22/29	(75.9)	36/50	(72.0)
15.0 (High)	25/27	(92.6)	19/23	(82.6)	44/50	(88.0)
Females						
0.0 (Control)	26/28	(92.9)	20/22	(90.9)	46/50	(92.0)
0.5 (Low)	22/24	(91.2)	24/26	(92.3)	46/50	(92.0)
2.5 (Mid)	25/27	(92.6)	22/23	(95.7)	47/50	(94.0)
15.0 (High)	32/35	(92.4)	14/14	(100)	46/49	(93.4)

D: Died or sacrificed moribund during study.

T: Sacrificed at termination of study.

1: Based on total number of animals examined.

No remarkable differences were noted in the number of treated males bearing malignant tumor or having more than one type of tumor as compared to the control group. In females, the incidence of animals with malignant tumors slightly increased in a dose-related response when compared to the control group. The incidence of females with more than one type of tumor also slightly increased in both the low and high dose groups. However, these increases were not statistically significant, see the table below:

Dosage Group	Total Number of Malignant Tumor Bearing Rats (%)	Total Number of Rats with more than one type of tumor (%)
Males		
Control	23/50 (46)	22/50 (44)
Low	17/50 (34)	20/50 (40)
Mid	15/50 (30)	14/50 (28)
High	16/50 (32)	20/50 (40)
Females		
Control	16/50 (32)	25/50 (50)
Low	20/50 (40)	33/50 (66)
Mid	21/50 (42)	25/50 (50)
High	26/49 (53)	30/49 (61)

Number of Animals Bearing Tumors (%)

Dosage (Group) mg/kg/day	D		T		Total[1]	
Males	No.	(%)	No.	(%)	No.	(%)
0.0 (Control)	29/33	(87.9)	14/17	(82.4)	43/50	(86.0)
0.5 (Low)	16/21	(76.2)	20/29	(69.0)	36/50	(72.0)
2.5 (Mid)	14/21	(66.7)	22/29	(75.9)	36/50	(72.0)
15.0 (High)	25/27	(92.6)	19/23	(82.6)	44/50	(88.0)
Females						
0.0 (Control)	26/28	(92.9)	20/22	(90.9)	46/50	(92.0)
0.5 (Low)	22/24	(91.2)	24/26	(92.3)	46/50	(92.0)
2.5 (Mid)	25/27	(92.6)	22/23	(95.7)	47/50	(94.0)
15.0 (High)	32/35	(92.4)	14/14	(100)	46/49	(93.4)

D: Died or sacrificed moribund during study.

T: Sacrificed at termination of study.

1: Based on total number of animals examined.

No remarkable differences were noted in the number of treated males bearing malignant tumor or having more than one type of tumor as compared to the control group. In females, the incidence of animals with malignant tumors slightly increased in a dose-related response when compared to the control group. The incidence of females with more than one type of tumor also slightly increased in both the low and high dose groups. However, these increases were not statistically significant, see the table below:

Dosage Group	Total Number of Malignant Tumor Bearing Rats (%)	Total Number of Rats with more than one type of tumor (%)
Males		
Control	23/50 (46)	22/50 (44)
Low	17/50 (34)	20/50 (40)
Mid	15/50 (30)	14/50 (28)
High	16/50 (32)	20/50 (40)
Females		
Control	16/50 (32)	25/50 (50)
Low	20/50 (40)	33/50 (66)
Mid	21/50 (42)	25/50 (50)
High	26/49 (53)	30/49 (61)

17)

As noted above, the most statistically significant tumor
:cide nces in this study are the nasal turbinate tumors in both
:les and females of the high dose group; the thymus tumors and
:e adrenal tumors in the high dose females.

The stomach tumor in the mid dose male 's a rare tumor and
: considered biologically significant in this study because in
older study (#BD-77-421), this kind of tumor occurred at a
atistically significant rate at much higher dosage levels.

scussion:

In a previous study (#BD-77-421, reviewed on 6/16/82),
a specific type of tumor, nasal turbinate tumors, was noted
occur only in the alachlor treated rats. In the study
der review at the present time (ML-80-186) this kind of
:or appeared to be most significant in the Long-Evans
:ain of rats based upon daily exposure to alachlor in feed.

The incidence of the nasal turbinate tumors in this study
(L-80-186) is much higher than in the previous study (#BD-77-421).
:s, a summary of the incidence of nasal turbinate tumors at a
:her dosage level, 126 mg/kg/day (study #ML-80-224), was
:o forwarded for consideration in this review in order to
:vide a comprehensive comparison with the previous data in
dy #BD-77-421.

The following table reflects the incidence of nasal turbinate
ors in the above mentiond 2 studies:

As noted above, the most statistically significant tumor :cid:nces in this study are the nasal turbinate tumors in both :les and females of the high dose group; the thymus tumors and :e adrenal tumors in the high dose females.

The stomach tumor in the mid dose male 's a rare tumor and : considered biologically significant in this study because in older study (#BD-77-421), this kind of tumor occurred at a atistically significant rate at much higher dosage levels.

scussion:

In a previous study (#BD-77-421, reviewed on 6/16/82), specific type of tumor, nasal turbinate tumors, was noted occur only in the alachlor treated rats. In the study der review at the present time (ML-80-186) this kind of mor appeared to be most significant in the Long-Evans rain of rats based upon daily exposure to alachlor in feed.

The incidence of the nasal turbinate tumors in this study (L-80-186) is much higher than in the previous study (#BD-77-421). :s, a summary of the incidence of nasal turbinate tumors at a :her dosage level, 126 mg/kg/day (study #ML-80-224), was :o forwarded for consideration in this review in order to :vide a comprehensive comparison with the previous data in dy #BD-77-421.

The following table reflects the incidence of nasal turbinate ors in the above mentiond 2 studies:

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2. In addition to the above listed neoplastic lesion in the nasal respiratory epithelium, alachlor appears to have the potential to induce additional proliferative changes in the nasal submucosal gland. The author referred to this lesion as submucosal gland hyperplasia. A picture of this lesion (#9) was provided with the submitted report in appendix #VIII. This lesion occurred at a statistically significant rate in the 15 mg/kg/day dosage group in both sexes as compared to the control group. Also it occurred at a slightly higher incidence rate at the two lower dosage groups in the female rats as compared to the control group, see the table below:

Dosage mg/kg/day	No. of Animals Affected/No. of Animals Examined							
	Males				Females			
	0.0	0.5	2.5	15.0	0.0	0.5	2.5	15.0
Nasal submucosal gland hyperplasia:	2/45	1/48	3/45	21**/45	2/42	5/44	5/47	11**/48

**P < 0.01

Note that only one animal in this study, a mid dose male (#38), had both submucosal gland adenoma and hyperplasia. Also note that in the high dose group 7/11 of the above affected males and 5/11 of the affected females also had the nasal turbinate tumors previously described on the previous page as respiratory epithelium adenoma.

Due to the noted high incidence of this lesion in this study, and due to the fact that picture #9 of this lesion cannot lead to a definitive conclusion as to whether this lesion is hyperplasia or neoplasia, it is advisable for the registrant to ask a second opinion from an independent pathologist. The Agency is willing to further discuss this matter with Monsanto representatives.

3. The only stomach tumor noted in this study in a mid-dose male should be considered significant because of its previous occurrence at a high incidence rate in the old study (#BD-77-421) and its presence in a new study (#ML-80-224) at 126 mg/kg/day.

4. Compression of the brain due to enlarged pituitary and due to pituitary tumors appeared to increase in the high dose male group. The incidence of this finding was much higher in all female groups including the control group and did not appear to be compound-related. However, the brain weight was not reported; thus, additional evaluation cannot be made at the present time.

5. In the high dose group, the noted ocular lesion in females (melting of the retinal pigmentation in two females early in the study) and the gross lesion in the male livers (gross finding of disseminated abnormal foci in 10/23 animal survivors), would not have been considered as significant compound-related effects. In the case of the eye lesion, the same incidence was noted in the control at termination; and in the case of the liver gross lesion, no further significant effects were noted microscopically. However, similar ocular lesions and hepatotoxicity occurred in a dose-related fashion at higher dosages in the previous study #3D-77-421.

6. The significance of the noted decrease in the lactic dehydrogenase (LDH) in all dosage group in this study remains to be explained.

7. The high mortality rate in the male control group (66%) as compared to the mortality rate in the high dose group (54%) and to the lower dosage groups (21%) needs to be addressed by the registrant. This reviewer also notes that the incidence of palpable masses and the incidence of malignant tumors were highest in this male group than in any of the male treatment groups.

Conclusions

The 2-year chronic feeding/oncogenic study in the Long-Evans rats with Alachlor at 0.5, 2.5 and 15.0 mg/kg bw/day dosage levels indicated the following:

- A NOEL for Alachlor non-neoplastic toxicity can be established at 2.5 mg/kg/day. The LEL is 15 mg/kg/day (melting of retinal pigmentation and increased mortality rate in females; and abnormal disseminated foci in the male liver).

- Alachlor is oncogenic in rats at 2.5 mg/kg/day. The tumor of interest at this dosage level and above is the nasal turbinate tumor.

Nasal turbinate tumors significantly ($p < 0.01$) increased in both males and females at 15 mg/kg/day. This kind of tumor was also noted in one mid-dose female. A different kind of nasal tumor (submucosal gland adenoma) was noted in one mid dose male.

Nasal submucosal gland hyperplasia significantly increased ($p < 0.01$) in both sexes at the high dose level. According to Dr. L. Kasza (pathologist), the picture of this lesion (picture #9) cannot lead to a definitive conclusion as to whether this lesion is hyperplasia or neoplasia. It is advisable for the registrant to ask a second opinion from an independent pathologist. The Agency is willing to further discuss this matter with Monsanto representatives.

18.)

Two additional kinds of tumors, thymus lymphosarcoma and adrenal phenochromocytoma significantly increased ($p < 0.05$) in the high dose females.

An increase was also noted in the incidence of thyroid follicular cell tumors in the high dose male group (13.3% incidence in the high dose as compared to 6.7% in the control). Although this increase was not statistically significant, it is considered biologically significant.

Some increase in the total incidence of malignant tumors was noted in all treated female groups especially at the high dose level (8% 10% and 21% above the control group in the low, mid and high dose, respectively).

A significant decrease in lactate dehydrogenase activity was noted in this study in all treatment groups. The decrease was dose-related in both sexes. The author stated that this finding may be related to 'some chemical interference with the analytical procedure' in this testing facility. However, this reviewer notes that this explanation does not preclude a compound related-effect. In addition, the nature of this chemical inteference and its relation to the test compound needs to be explained by the registrant.

A high incidence of brain compression was noted in this study in the female groups including the control group. The incidence of this finding was remarkable in the high dose male as compared to the male control group. However, the brain weight was not reported in this study. The registrant should submit these data for review.

A new risk assessment associated with alachlor oncogenicity will be performed by the Toxicology Branch based on the new incidence of nasal turbinate tumors.

This study is classified as <u>Core-Minimum</u>. However, this study must be considered in conjunction with a previous study (3D-77-421) where higher dosage levels were tested (0, 14, 42 and 126 mg/kg/day) so that the non-neplastic lesions (ocular lesions and hepatotoxicity) and neoplastic lesions (nasal turbinate tumors, thyroid tumors, etc.) can be adequately assessed. Study #ML-80-224 which will be submitted in the near future and where a higher dosage, 126 mg/kg/day, was concurrently tested with the dosages used in the present study (ML-80-186) should be also considered in conjunction with this evaluation for the same reasons stated above.

TS-769:th:TOX/HED:AManfour:4-3-84:card #9

*References:

1. Dunnett, C.W. Multiple comparison procedure for comparing several treatments with a control. Jour. Am. Stats. Assoc. 1096-1121 ((1955).

2. Mann, H.B. and Whitney, D.R. On a test of whether one of two variables is stochastically larger than the other. Ann. Math. Stat. 18:50 (1947).

3. Ryan, T.A. Multiple comparisons in psychological research. Psychol. Bull. 56: 25-47 (1959).

4. Miller, R.G. Simultaneous Statistical Inferences. McGraw Hill Co. NY (1966).

5. Mantel, N. Evaluation of survival data in two new rank-order statistics arising in its consideration. Cancer Chemother. Rpts. 50: 163-170 (1966).

6. Breslow, N. A generalized Kruskal-Wallis test for comparing K-samples subject to unequal patterns of censorship. Biometrika. 57: 579-594 (1970).

7. Peto, R., et al. Long Term and Short Term Screening Assays for Carcinogenicity: A Critical Appraisal. I.A.R.C. Monograph, Supplement 2. The World Health Organization (Lyon) (1980).

8. Fisher, R.A. Statistical methods for research workers. Oliver and Boyd, Publisneds. Edinburgh (1946).*

Page 183 is not included in this copy.

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.

____ Description of quality control procedures.

____ Identity of the source of product ingredients.

____ Sales or other commercial/financial information.

____ A draft product label.

____ The product confidential statement of formula.

____ Information about a pending registration action.

✓ FIFRA registration data.

____ The document is a duplicate of page(s) _____.

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The information not included is generally considered confidential by product registrants. If you have any questions, please contact the individual who prepared the response to your request.

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____ Identity of product inert ingredients.

____ Identity of product impurities.

____ Description of the product manufacturing process.

____ Description of quality control procedures.

____ Identity of the source of product ingredients.

__✓__ Sales or other commercial/financial information.

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____ FIFRA registration data.

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The information not included is generally considered confidential by product registrants. If you have any questions, please contact the individual who prepared the response to your request.

Appendix C.

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

NOV 2 1984

MEMORANDUM

OFFICE OF PESTICIDES AND TOXIC SUBSTANCES

SUBJECT: Alachlor (Lasso), EPA Reg. #524-316. Review of Additional Studies: A Special Chronic Study in Rat and Two New Mutagenicity Studies. CASWELL#11

TO: Robert Taylor, PM#25
Registration Division (TS-767C)

FROM: Amal Mahfouz, Ph.D.
Toxicologist, Section V
Toxicology Branch/HED (TS-769C)

THRU: Laurence D. Chitlik, DABT
Section Head, Section V & VI
Toxicology Branch/HED (TS-769C)
and
William L. Burnam, Chief
Toxicology Branch/HED (TS-769C)

Action Requested:

Monsanto Company submitted the following studies in support of the registration of Alachlor:

A. A Special Chronic Feeding Study with Alachlor in the Long-Evans Rats, R.D.#533, Special Report #MSL-3492, 4/16/84: Accession #253306 and 253307. This study was reviewed by Dr. A. Mahfouz.

B. Two Mutagenicity Studies: R.D.#534, Special Report #MSL-3508: 4/26/84: Accession #253308. These 2 studies, reviewed by Dr. I. Mauer, are listed below:

1. UDS/HPC-rat repair assay: An Evaluation of the Potential of Alachlor to Induce Unscheduled DNA Synthesis in the *In Vivo* - *In Vitro* Hepatocyte DNA Repair Assay.

2. *In Vivo* Bone Marrow Chromosome Aberration Study in Rats.

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Recommendations:

A. Special Chronic Study

1. This study is an acceptable study and should be considered as an addendum to the two previous studies in the same strain of rats (Study #ML-80-186, 3/12/84 and #BD-77-421, 11/13/81).

2. This study successfully achieved its objective in determining the nature of the ocular lesions. It is clear that the females are more sensitive than the male Long-Evans rats to Alachlor. Once initiated, the uveal degeneration syndrome (UDS) is irreversible (as demonstrated by the group of animals that were removed from treated to untreated diets after 5 to 6 months of exposure).

3. The neoplastic and non-neoplastic lesions noted in this study are similar to the ones noted at the lower dosages in study #ML-80-186. The major neoplasms are also similar to the ones found in study #BD-77-421 by Bio/dynamics, 11/13/81. These neoplasms are listed below in order of importance (see also the incidences and description of these neoplasms on pages 23 and 24 of this review, and the pathology report, attachment #3):

1) Nasal turbinates tumors, both sexes
2) Stomach malignant tumors, both sexes with a higher response in females
3) Thyroid tumors, both sexes (with a considerable increase in follicular cell carcinoma in males).

In addition to the above tumors, Neuroepithelioma, a rare tumor, was reported in the new high dose study in one male and 2 females. No historical data were provided for comparison. This reviewer has considered, but is not aware of, any kind of direct or indirect relationship between UDS and the above noted neuroepithelioma (a malignant tumor which arises in the eye from precursors of the neuro-epithelial receptor cells of the retina). It should be noted that nearly all of the animals in this study were affected by UDS while only 3/200 animals had this kind of tumor.

Furthermore, this reviewer is not aware of any kind of direct relationship between the UDS and the other tumors noted above, i.e. nasal turbinate tumors. Alachlor is not a volatile compound and available data does not demonstrate that it is either a primary dermal or ocular irritant. However, it has been shown to be a skin sensitizer in the guinea pig.

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Also, it is noted that the incidence of liver tumors (hepatoma + hepatocarcinoma) appears to increase in females at the 126 mg kg/day dosage level in both the old and new studies as compared to the control (10% and 6% as compared to 0% in the control in the new study and old study respectively). No remarkable increase was noted in the male group although hepatotoxicity was noted in both males and females of this group in the new and the old studies (see study review and attachment #5 for the review of the old study).

4. It appears that the nasal turbinate tumors had a shorter latent period than the stomach tumors. Thus, unlike the stomach tumors which were not present in most of the animals exposed to Alachlor for only 5 to 6 months, the nasal turbinate tumors were present at a high incidence rate in this group of rats (59% in males and 42% in females of this group as compared to 81% in males and 52% in females of the group of animals that were exposed to alachlor for two years, and as compared to no incidence of this kind of tumor in the control group .

5. The assignment of animals to groups in this study (which occurred after 5 to 6 months of treatment with alachlor) was by design a selective process based on the susceptability of the animals to ocular lesions. Thus, caution should be used (due to a potential bias) if the oncogenic data from this study are used for a quantitative risk assessment (see more complete discussion, pgs. 5 and 6 of this review).

B. Mutagenicity Studies

1. UDS HPC - rat repair assay - ACCEPTABLE. The study is positive at the highest dose tested, 1000 mg/kg in the Fischer 344 strain of rat.

2. In Vivo bone marrow chromosome aberration assay - rat - UNACCEPTABLE. Evidence of systemic absorption and or transport of effective concentration at target tissue should be provided; or repeat study should be performed employing i.p. administration of the test compound.

TOXICOLOGY BRANCH:A. DATA REVIEW

CHEMICAL: Alachlor

Caswell No.: 11
EPA Cem. No.: 090501

Study Type: Chronic Feeding Study in Rat. This study should be considered as an Addendum to the previously submitted chronic studies in the Long-Evans Rats: #BD-77-421 by Bio/dynamics, submitted in 1/5/82: and #ML-80-186 by Monsanto's EHL, submitted in 2/28/84.

Study Identification:

Study Title: A Special Chronic Feeding Study with Alachlor in Long-Evans Rats.

Accession Numbers: 253306 and 253307

Sponsor: Monsanto Company

Testing Laboratory: Monsanto Environmental Health Laboratory
St. Louis, Missouri 63110

Study No.: ELH-800219, Project #ML-80-224, Special Report #ML-3492

Study Director and Author: L.D. Scott

Dates: In life stage: from 8.20.80 to 10/5/82
Report date: 4/16/84
Study was submitted to EPA on 4/23/84

Test Substance: Technical Alachlor, 94.1% a.i.: stabilized with [redacted] Lot #MULT-04178. The compound, an orange/amber solid with a low melting point, was received from the sponsor on 4/28/80.

Experimental Design & Methods: A copy of the testing laboratory experimental design and methods is attached to this review (attachment #1). It is clear from this protocol that although this study is titled as a chronic feeding study, it was designed to investigate the ocular lesion (retinal degeneration syndrome) which was noted in an earlier Alachlor chronic study, BD-77-421 (with epichlorohydrin). Only the highest dose tested in that earlier study, 126 mg/kg/day, was reinvestigated in this study: however, the test material was epichlorohydrin-free in this new study. The protocol of this new study was modified

to allow for more animals to remain on the treated diet until the end of the two-year study period so that the apparent oncogenic potential noted in study #BD-77-421 with epichloronydrin could be further examined.

In the evaluation of the results of this study, this reviewer took into consideration the results of study #ML-80-186 (especially data from the control group) which was performed concurrently with the present study, #ML-80-224. In fact, both studies were considered as one study with two parts, part I had a control group of 50 animals/sex and three low dosage groups, 0.5, 2.5 and 15 mg/kg/day; and part II with a smaller control group (6 animals/sex) and one high dose group 126 mg/kg/day. The animals in the high dose group, 126 mg/kg/day, followed a different regimen of treatment than the animals treated in study #ML-80-186 in order to investigate the nature and reversability of the uveal degeneration syndrome (UDS).

The registrant indicated that the treated animals (100 animals sex treated with 126 mg/kg/day dosage of Alachlor) were divided into three groups after a period of exposure sufficient to induce ocular lesions. The grouping process was performed as the ocular lesions were confirmed by the consulting Ophthalmologist. Group I consisted of the animals that were to remain on the treated diet until the end of the two-year study period; group II consisted of the animals that were selected for interim sacrifice based on their ocular lesion status; and group III consisted of the animals that were selected for potential recovery from ocular lesions by being placed on untreated diets for the remainder of the study period. Additional animals that apparently did not show any ocular lesions were also placed in group II and III.

The distribution of animals in group III occurred after months of exposure for females and 4 months for males. The assignment of animals to group II occurred after 6 months of exposure for the 10 males selected for interim sacrifice and after 5 months for 10/18 selected females; after 6 months for an additional 4 females; and at the 8th month for the remaining 4 females of this group (see table on page 6).

Thus the grouping process was by design a selective process based on the susceptibility of the animals to ocular lesions. Although some unaffected animals were also placed in these groups, it is obvious that they were not randomly distributed. On the other hand, 99% (79/80 animals) of the females were affected by the uveal degeneration syndrome at month 13 of the study period and afterward until the end of the study (100%). It can therefore be assumed that all animals were sensitive to UDS although the time of onset varied. Therefore, deliberate selection on the basis of development of UDS into subgroups alone versus random selection may result in little meaningful bias in the subgroups later assessed for oncogenic potential. It should be noted that although it is unknown whether there is a relationship between the sensitivity to UDS and the oncogenic response observed in this study, no such relationship is visibly apparent.

This reviewer also noted a discrepancy in the reported number of females in groups I and II. However, upon examination of the individual animal data in tables 1, 4, 6 and 7 in Appendix II, the animals involved in this difference were identified, see table below:

Source	Number of animals per group (126 mg/kg/day)					
	Group I		Group II		Group III	
	M	F	M	F	M	F
registrant						47
reviewer noted						49

As noted above, only 18 females were noted in group II. The two additional animals that were erroneously reported in this group by the registrant, #43 and #85, appeared to belong to group I, see table #1, appendix II. Animal #43 died spontaneously and was reported as auto, but 111 hours later at necropsy. Animal #85 was reported with a [illegible] ear tag; it was sacrificed in extremis.

Hematology and blood chemistry parameters were only determined in males (10/18 survivors on treated diet) at the end of the study period. These data cannot be fully representative of the effects of this chemical since no analyses were performed during the study.

RESULTS

Test Substance Concentration in Feed

Analytical determinations of Alachlor in random samples of feed which were taken during the study indicated that the actual concentrations of Alachlor in feed were generally within an acceptable range (±15%) of the nominal concentration.

As previously discussed in the review of study #ML-80-186 (attachment #4), the test substance (Alachlor, Lot #MULT-0417B), was also stable on storage and in diet during the one week feeding period.

Clinical Observations

The clinical observations of the animals in this study included the following symptoms in both the control and treatment groups: hair loss, edema and ulceration, teary eyes and overgrown teeth. Scabs, piloerection, miscellaneous breathing difficulties, and misuse or disuse of limbs were also noted in treated animals in this study. However, some of these symptoms were also noted but to a lesser extent in the control group for study #ML-80-186 (this study used lower dosage levels of Alachlor and a larger number of animals as control: 50 animals/sex instead of the 6 control animals/sex used in the high dose study).

Hypoactivity, paleness and emaciation were also noted in animals that died in both groups. Dehydration, nasal and urogenital blood discharge were noted in dying animals in the treated groups.

Mortality

Mortality data were not summarized in the submitted report; only individual animal data were listed in Appendix II, table 7. The authors of the report did not attempt to list the total mortality rate for: group I, and group III. This reviewer had to sort and summarize this information from several tables (tables #1, 4 and 7, all in Appendix II). Also comparison of these data with the limited number of control animals (6 animals/sex) in this study and the larger number of control animals (50 animal/sex) study #ML-80-186 was performed by this reviewer as noted in the following table:

Cumulative Mortality

Groups	Males			Females		
	18-mo.	21-mo.	Term.	18-mo.	21-mo.	Term.
Control* (Study #ML-80-186)	5/50 10%	13/50 26%	33/50 66%	6/50 12%	10/50 20%	28/50 56%
Treatment 126 mg/kg/day (study #ML-80-224)						
Group I	11/70 16%	19/70 27%	52/70 74%	8/33 24%	18/33 55%	29/33 88%
Group III	2/20 10%	5/20 25%	14/20 70%	8/49 16%	18/49 37%	33/49 67%

*Due to the small number of animals in the control group of study #ML-80-224, data for this control were not listed in the above table. However, mortality in this group at the end of the study was 2/4 males (50%) and 1/4 females (25%).

The above data reflect an increased mortality rate in females. No effect on the survivability of males was noted in either group I or III as compared to the control groups.

Group I females appeared to be affected early in the study with a 2 fold increase in the mortality rate as compared to the control group at both 18 and 21 months (12% and 35% respectively above control values) and remained high until termination (32% above the controls). Group III females, which were removed from the treated diet after 5 months of exposure, reflected a significant increase above the control group (17%) only after 21 months of the study initiation. By the end of the study period, this difference was only 11%.

In-Life Masses

As noted in the concurrent study performed at the lower dosage levels (ML-80-186), this study also reflected incidences of palpable masses located on the abdominal area and sometimes the thorax. A few animals had masses located on the rear limbs, jaw, head or neck. The following table reflects the incidence of palpable masses in this study:

Number of Animals with Palpable Masses/Number of Animals in Group (%)

			Treatment Groups (126 mg/kg/day)					
	Control*		Group I		Group II (Interim)		Group III	
	M	F	M	F	M	F	M	F
Incidence in animals later found dead (D)	0/2 0%	0/1 0%	30/52 58%	17/29 59%	- -	- -	6/14 43%	19/33 58%
Incidence in terminal or interim sacrifice animals (T)	2/2 100%	3/3 100%	13/18 72%	1/4 25%	2/10 20%	1/18 5%	6/6 100%	14/16 88%
Total incidence per group	2/4 50%	3/4 75%	43/70 64%	18/23 55%	2/10 20%	1/18 5%	12/20 60%	33/49 67%

*Note: The number of animals in this control group is very small for adequate comparison of data; however, the total incidence in the created groups in this study may be compared to the total incidence in the control group of the concurrent study with the lower alachlor dosages (ML-80-186). This incidence was 29/50 males (58%) and 30/50 females (60%). These incidences are within the same range of the incidences noted in the above table for the total number of animals with masses.

Body Weight & Food Consumption

Summary data were not available. Body weight data were compared by this reviewer to the larger control group which was used in the concurrent study with the lower dosage groups (ML-80-186). Cursory review of the individual animal data reflected only a limited increase in the body weight of animals maintained on treated diet (especially in the female group) as compared to the animals removed from the treated diet. Also animals that died during the study generally showed a marked decrease in body weight before death.

The food consumption in both males and females apparently was not affected by treatment.

Hematology and Blood Chemistry

Ten males from group I were examined for these parameters at the end of the study period. No effect was noted except in lactic dehydrogenase values where 5/10 animals had levels higher than the maximum normal range and 1/10 animals with a lower value than the minimum normal range. These data were not compared to the study's control animals because they were not examined. However, when these data were compared to the control animals in study #ML-80-186 it appeared that no remarkable effect was noted except for the one animal with a low LDH value.

This reviewer notes that females in the lower dosage groups (0.5, 2.5 and 15 mg/kg/day) which were tested in study #ML-80-186 reflected a statistically significant reduction in the LDH values as compared to the control group; also the males of these groups appeared to reflect a remarkable decrease in this parameter (see attachment #4, page 14). These conflicting data have not been explained.

The table below describes these findings:

Normal[a] range	LDH (IU) 100-600	
Dosage mg/kg/day)	Male	Female
0.0	664 ±297[b]	675 ±199
0.5	438 ±327	349** ±202
2.5	587 ±445	322** ±272
15.0	327 ±216	236** ±180
126.0	636 ±353	- -

**: $p < 0.01$ (two-tailed Dunnett's test)

a: Reference: Review of Data Standards related to Laboratory Animals Data Bank (Interim Report for HEW, March 1980).

b: Standard deviation.

Ophthalmoscopic Examinations

The animals were examined at the following intervals by Dr. Lionel F. Rubin:

Dates:	1/12/81	3/18/81	9/18/81	8/20/82
Months on Study:	5	7	13	24

A copy of the description of ocular lesions is attached to this review (attachment #2, page 19, codes a to w). Although, Dr. Rubin did not clearly state that these lesions reflected different stages of the uveal degeneration syndrome (UDS), he specified in his letter of 5/15/81 that the following 8 lesions (listed under codes f to m, in the attached copy) are abnormalities of toxicological significance. These abnormalities are as follows:

- Pigment mottling in retina
- Posterior synechiae
- Pigment dispersion onto lens
- Retina not clearly visible
- Loss of iris architecture
- Pigment hypertrophy at pupillary border
- Hyphema
- Fibrin in anterior chamber

In the above mentioned letter, Dr. Rubin summarized his findings of the 1/12/81 examination which indicated that 39/100 females were affected by this syndrome (moderate to severe) while none of the males had been affected. The affected animals are described below:

- 23 females (19 bilateral and 4 unilateral) had the mildest (earliest) symptoms, i.e. distinct mottling of pigment throughout much of the retina. In all the unilateral cases, the retina in the other eye was not visible due to other abnormalities.

- 14 females (10 bilateral and 4 unilateral) had the most severe symptoms, i.e. posterior synechiae and pigment dispersion onto the lens surface which often interfered with visualization of the retina. In all the unilateral cases, the other eye had different abnormalities.

- 2 females were also affected with other lesions in addition the ones described in the above two groups: one of these two animals had intraocular hemorrhage bilaterally; and the other had extensive fibrin deposits bilaterally.

The abnormalities noted in 6 male rats at this point of the study period were considered as 'relatively common ones in the pigmented rats' they usually occurred in treated and untreated rats. These are as follows:

- Superficial corneal scarring in rats #4 and #75
- Focal depigmentation spots in the retina in rat #13
- Hyaloid cataract in rat #25
- Myelination of retinal nerve in rat #31
- Focal pigment deficiency lateral to optic dish in rat #42

It is clear from the Rubin's letter that Alachlor causes eye lesions, namely the uveal degeneration syndrome, as early as five months after exposure to 126 mg/kg/day Alachlor in feed. It is also clear from this letter that females are more sensitive to this effect than males as they were the only sex affected by this syndrome (39%) at the first examination 1/15/81. The syndrome (UDS) was observed in these animals at different stages of its development (from mild to severe).

Dr. Rubin did not identify the animals affected in his letter of 5/15/81, thus I attempted to identify them from the individual animal data in table #6 appendix II. I noted that additional animals had manifestations of ocular alterations that appeared to be associated with UDS or other serious ocular lesions. Approximately 71% of the females and 13% of the males were affected as early as month 5 to 6. These animals were distributed in the study groups as follows:

	No. animals affected/No. in group (%)	
Group I	Females	Males
maintained on treated diets for 2 years (examined for 5 to 6 months)	27/33 (81%)	7/70 (10%)
Group III		
removed from treated diet on month 5 to 6	31/49 (63%)	3/20 (15%)
Group II		
Interim Sacrifice on month 5 to 6	13/18 (72%)	3/10 (30%)
Total	71/100 (71%)	13/100 (13%)
Total by Dr. Rubin (examination of 1/12/81)	39%	0%

In table #6 appendix II, only affected animals were listed; which means that 29% of females and 87% of males were not affected. However this reviewer also noted that some of the animals were affected as early as September 1980 (the second month of the study period). None of the control animals in study #ML-80-186 or ML-80-224 reflected any effect during that period. "Pigment hypertrophy at pupillary border" appeared in the majority of the control animals in both sexes at month 13 of the study. However, this lesion in the control animals appeared to be associated with aging since no further significant deterioration of the eyes were observed at month 24.

The affected animals in group III did not recover even after removal from the treated diets. However the syndrome severity increased in group I animals and in a few of group III animals. Additional animals were reported with these lesions in both groups on months 7, 13 and 24 examinations.

The fact that additional animals in group III were affected by this syndrome even after removal from the treated diets indicates that the UDS is an irreversable process once initiated in the animal system. The full manifestation of this syndrome may be slower and less severe in these animals (group III) than in the animals exposed for lifetime. This fact is demonstrated in the registrant's summary tables for males and females respectively (see copies on next two pages) where 90% of the male survivors in group I, 30% of the male survivors in group III, 94% of the female survivors in group I and 65% of the female survivors in group III were affected at month 13. Also, copies of Dr. Rubin's reports to Monsanto on May 15 and 28, 1981 and September 23, 1982 are attached to this review (see attachment #2).

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____ The product confidential statement of formula.

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Necropsy and Histopathology

The mean absolute organ weights for survivors in group I (18/70 males and 4/33 females) were determined as indicated in the procedure. The relative organ/body weight was not determined. The available data indicated that the absolute liver, thyroid and adrenal weights increased in males. The increase appeared to be biologically significant in males although for the adrenals, apparently the large increase in the mean weight was due to only 2/18 animals. In the females, this reviewer noted an increase in the absolute liver weight. Considering the number of females examined (only four animals), no adequate conclusion could be made from the female organ weight data.

The organ weights for the few animals used as controls in this study were not determined. Thus, this reviewer compared the above data with the control data from study #ML-80-186 (concurrently performed in the same testing facility). The table below provides a comparison between this control group and the data in this study.

Mean absolute organ weight in g

Group	MALES Liver	Thyroid	Adrenal
126 mg/kg/day, GI (study #ML-80-224)	22.894 ±0.702	0.195 ±0.046	0.138 ±0.043
N	18	18	18
Control (study #ML-80-186)	19.206 ±0.675	0.058 ±0.003	0.078 0.005
N	17	17	17
	FEMALES Liver	Thyroid	Adrenal
126 mg/kg/day, GI (study #ML-80-224)	19.206 ±0.208	0.058 ±0.017	0.070 ±0.016
N	4	4	4
Control (study #ML-80-186)	14.658 ±0.922	0.048 ±0.003	0.089 ±0.006
N	14	19	22

N: Number of animals examined.

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N*: Number of animals examined.

Several lesions were grossly observed and confirmed by microscopic examinations. The following three tables were compiled by this reviewer from the summary tables and the individual animal data in the submitted study. These 3 tables reflect this reviewer's observation of apparent lesions in one or both sexes in group I and group III.

Group II, the interim sacrifice animals, did not reflect any additional information on the toxicity of Alachlor except for what already has been indicated in the ophthalmoscopic report. Only one of 18 females in this group had a malignant tumor (keratinized cystic carcinoma) in the peritoneal cavity. Thus this group will not be included in the following 3 tables.

These tables reflect the gross necropsy findings, the non-neoplastic lesions, and the neoplastic findings for groups I and III in this study. A copy of the registrant's summary table on these lesions follows these 3 tables. Also a copy of the pathology report is attached to this review (see attachment #3) as a reference for the description of lesions and neoplasms.

Incidences of the tumors of interest in this study, namely, the nasal turbinate tumors, stomach tumors and thyroid tumors are summarized in the conclusion section of this review and compared to the incidences noted in the previously submitted two chronic feeding studies in the Long-Evans strain of rats (incidences of animals with brain tumors and hepatic neoplasms were also included for comparison), see pages 23 and 24.

Gross Necropsy Findings

No. of Animals affected/No. of Animals Examined

	Males			Females		
	C	GI	GIII	C	GI	GIII
Adrenals	50	70	20	50	31	49
°enlarged	3	7	2	3	1	4
°masses	0	1	1	0	0	1
Nose	50	70	20	50	31	49
°masses	0	1	1	0	0	1
Brain	50	70	20	50	31	49
°growth/masses	0	2	0	0	0	0
°abnormal ventricule fluid	0	1	0	0	0	1
°abnormal color	0	0	0	0	3	0
°hemorrhage	0	0	0	0	1	0
Kidney	50	70	20	50	31	49
°abnormal color	9	11	3	4	6	3
Heart	50	70	20	50	31	49
°thickened Myocardium	1	2	0	0	0	1
°abnormal color	0	0	0	0	2	0
°enlarged	0	0	1	0	2	0
°cyst/mass	0	1	0	0	0	0
Liver	50	70	20	50	31	49
°growth/mass/cyst	3	6	0	1	2	1
Stomach	50	70	20	50	31	49
°growth/masses	1	4	0	1	17	0
°ulcer	0	0	1	0	1	3
Spleen	50	70	20	50	31	49
°abnormal color	1	3	0	1	2	2
Thyroid	50	70	20	50	31	49
°enlarged	2	21	2	2	1	1
°masses	0	2	2	0	17	0
Urinary bladder	50	70	20	50	31	49
°masses	0	4	0	0	0	1

Non-Neoplastic Lesions

No. Organ	No. of Animals affected/No. of Animals Examined					
	Males			Females		
	Control	GI	GIII	Control	GI	GIII
Brain	50	70	20	49	31	49
°Compression atrophy	6	9	6	23	6	18
Nose	45	61	17	42	25	46
°Submucosal gland hyperplasia	2	6	5	2	0	13
°Inflammation of nasal passage	4	10	6	3	6	9
Heart	50	70	20	50	31	49
°Myocardial Fibrosis/Scar	1	21	11	0	4	9
Adrenal	50	70	20	49	31	48
°Cortical Telangiectasis	0	11	1	29	14	24
°Cortical hypertrophy/hyperplasia	9	13	5	11	4	17
Bone Marrow	49	66	20	47	31	48
°Myelocytic hyperplasia	4	14	4	13	13	10
Liver	50	70	20	50	31	49
°Hepatocytic necrosis/lysis	6	10	1	3	1	6
°Foci of cellular alterations	7	25	6	13	7	8
Urinary Bladder	45	68	20	48	30	45
Epithelial hyperplasia	4	9	1	0	4	0

NOTE: Control animals from study #ML-80-186 were used for comparison when possible.

Neoplastic Lesions

Organ/Tissue	No. of Animals affected/No. of Animals Examined					
	Males			Females		
	Control	GI	GIII	Control	GI	GIII
Nasal turbinate*	45	61	17	42	25	46
°Respiratory epithelial adenoma or papillary adenoma	0	42	10	0	11	19
°Adenocarcinoma	0	7	0	0	2	1
°Fibrosarcoma	0	1	0	0	0	0
Skin	50	69	19	50	30	49
°Benign tumors[a]	9	10	1	4	1	-
°Malignant tumors[a]	6	6	3	1	-	1
Thymus	19	68	16	48	25	43
°Lymphosarcoma #M	0	1	1	0	0	1
Adrenal	50	70	20	49	31	48
°Pheochromocytoma						
#B	8	8	2	1	0	2
#M	2	2	1	0	0	0
°Cortical adenoma	1	2	1	0	3	4
°Cortical carcinoma	1	1	0	0	0	0
Thyroid*	49	69	20	49	31	49
°C-cell adenoma	5	4	1	2	3	2
carcinoma	0	0	0	0	0	1
°Follicular adenoma	2	8	1	1	4	3
carcinoma	1	10	1	3	0	1
Uterus	-	-	-	50	31	48
°Benign musocal polyps & other benign tumors	-	-	-	4	2	5
°Malignant tumors	-	-	-	1°°	0	2°°
Mammary Gland	-	-	-	50	25	40
°Adenoma	-	-	-	16	12	20
°Carcinoma	-	-	-	3	1	2
Stomach*	50	68	20	50	31	49
°Malignant tumors[a]	0	3	0	0	19[b]	1
Brain*	50	70	20	50	31	49
°Neuroepithelioma	0	1	0	0	1	1

°°: One control female had a malignant mucosal polyp, one high dose female had fibrosarcoma and the other had a malignant stromal tumor.

(chart continues on next page)

(chart continued)

Organ/Tissue	No. of Animals affected/No. of Animals Examined					
	Males			Females		
	Control	GI	GIII	Control	GI	GIII
Liver*	50	70	20	50	31	49
*Hepatoma	1	3	0	0	1	0
*Neoplastic nodules	0	0	0	0	1	1
*Hepatocellular carcinoma	2	2	0	0	2	1
Urinary Bladder	50	68	20	50	30	45
*Papilloma	0	1	0	0	0	0
*Carcinoma	0	1	0	0	0	1
*Sarcoma	0	0	0	0	1	0

NOTE: Control animals from study #ML-80-186 were used for comparison when possible

*See table pg. 24 for % affected.

[a]Full description of these tumors is provided in the pathology report (copy attached). #B: benign, #M: malignant.

[b]8/20 animals affected with stomach tumors were also affected with nasal turbinate tumors. Also another digestive tract tumor was found in one female #29 of group III in the duodenum (adenocarcinoma). However, no stomach tumors were reported in this group.

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A final comment concerning the necropsy data and histopathology data of the brain in this study: the brain appears to be directly and indirectly affected by Alachlor. Neuroepithelioma, a rare tumor that arise in the eye retina was reported in 1/70 males of group I, 1/31 in females of group I and 1/49 females of group III. In addition to this primary tumor, four males of group I were reported to have extension of the nasal turbinate tumors into the brain, and 2 females of this group apparently had extensions of the pituitary tumors in the brain.

Brain compression was noted in several animals of both sexes in this study, the registrant indicated that this effect was indirectly related to the pituitary tumors. However, this reviewer notes that 8/24 females with brain congestion did not have pituitary tumors. In addition, this reviewer questions the fact that the registrant did not weigh this organ (brain), although it is a major organ which appeared to be target of several direct or indirect pathological events in this study.

Conclusions:

1. This study successfully achieved its objective in determining the nature of the ocular lesions. It is clear that the females are more sensitive than the male Long-Evans rats to Alachlor. Once initiated, The uveal degeneration syndrome (UDS) is an irreversible process as demonstrated by the group of animals that were removed from treated to untreated diets after 5 to 6 months of exposure.

2. It is clear that the neoplastic and non-neoplastic lesions noted in this study are similar to the ones noted at the lower dosages in study #ML-80-186 with the exception of the thymus and adrenal tumors which do not appear to be significant in this study. The major neoplasms are also similar to the ones found in a previous study, study #BD-77-421 by Bio/dynamics, 11/13/81. These neoplasms are listed below in order of importance.

1) Nasal turbinates tumors, both sexes
2) Stomach malignant tumors, both sexes with a higher response in females
3) Thyroid tumors, both sexes (with a considerable increase in follicular cell carcinoma in males)

Comparison of the incidence of the above mentioned tumors in the submitted 3 studies in the Long-Evans rats are presented on pages 23 and 24.

In addition to the above tumors, Neuroepithelioma was reported in the new high dose study in one male and 2 females (a total of 3/200 treated animals were affected in this study). Although this tumor is a rare tumor, an accurate evaluation of its presence in this study cannot be performed because no historical data were provided for comparison.

Also, this tumor was reported in the submitted data as a benign tumor in the brain, however, according to the Pathology Handbook by Smith, Jones and Hunt (1972 edition) this tumor is listed as a tumor of the nervous system which is identified as 'Retinoblastoma or Neuroepithelioma' a malignant tumor which arises in the eye from the precursors of the neuro-epithelial receptor cells of the retina. It is also indicated in this reference that although this kind of tumor is rare in animals, it occurs in children and has a startling high incidence in some human families. The only tumors noted in the eye in the old study #BD-77-421 was a melanoma in the iris of a high dose male (126 mg/kg/day) and a hardenian gland tumor, also in one high dose male.

In view of the fact that the eyes are one of the major target organs in this strain of rats (UDS), this reviewer questions if there is any kind of direct or indirect relationship between this effect and the noted tumors that arose in the eye at 126 mg/kg/day.

Dosage mg/kg/day	Study #ML-80-224				Study #ML-80-186								Study #DD-77-421							
	126				0		.5		2.5		15		0		14		42		126	
No. of tissues examined and incidence of animals with tumors:	GI*		GIII*																	
	M	F	M	F	M	F	M	F	M	F	M	F	M	F	M	F	M	F	M	F
Nasal turbinate tumors	61	25	17	46	45	42	48	44	45	47	45	48	46	49	46	47	41	45	42	48
*adenoma	42	11	10	19	0	0	0	0	0	1	11	9	0	0	0	0	10	4	23	10
*adenocarcinoma	7	2	0	1	0	0	0	0	0	0	0	0	0	0	0	0	1	1	0	0
Stomach	60	31	20	49	50	50	50	50	50	50	49	48	49	50	50	50	50	50	50	50
*malignant tumors	3	19	0	1	0	0	0	0	1	0	0	0	0	0	0	0	0	1	17	23
- mixed carcinosarcoma	2	6	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	1	11	17
- undifferentiated sarcoma	-	5	-	1	-	-	-	-	1	-	-	-	-	-	-	-	-	-	-	-
- undifferentiated carcinoma	-	1	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
- adenocarcinoma	-	3	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	2	1
- Leiomyosarcoma	-	3	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	1	1
- Anaplastic sarcoma	1	1	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
- Osteosarcoma	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	3	4
Thyroid	70	31	20	49	49	49	50	49	49	49	49	47	48	49	50	44	49	46	50	49
*C-cell adenoma	4	3	1	2	5	2	3	2	7	4	6	3	4	2	5	3	2	4	7	3
carcinoma	0	0	0	1	0	0	0	0	1	0	0	0	1	0	1	0	0	1	0	0
*Follicular adenoma	8	4	1	3	2	1	4	1	3	0	4	2	1	0	0	0	1	2	11	2
carcinoma	10	0	1	1	1	3	0	1	1	1	2	1	0	0	0	0	0	0	2	2
Brain	70	31	20	49	50	50	50	50	50	50	49	49	50	50	50	50	50	50	50	50
*Neuroepithelioma	1	1	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Liver	70	31	20	49	50	50	50	50	50	50	50	49	50	50	50	50	50	50	50	50
*Hepatoma	3	1	0	0	1	0	0	1	0	0	0	0	1	0	0	0	2	1	1	3
*Nodular hyperplasia	0	1	0	1	0	0	0	0	0	0	0	1	0	2	0	1	1	2	4	1
*Carcinoma	2	2	0	1	2	0	1	1	2	0	0	2	0	0	0	0	0	0	0	0

*GI: animals maintained on treated diets for 2-year; GIII: animals maintained on treated diets for only 5 to 6 months.

Dosage mg/kg/day	Study #ML-80-224				Study #ML-80-166								Study #BD-77-421							
	126				0		.5		2.5		15		0		14		42		126	
No.of tissues examined and % incidence of animals with tumors:	GI*		GIII*																	
	M	F	M	F	M	F	M	F	M	F	M	F	M	F	M	F	M	F	M	F
Nasal turbinate tumors	61	25	17	46	45	42	48	44	45	47	45	48	46	49	46	47	41	45	42	48
*adenoma	69	44	59	41	0	0	0	0	0	1	25	20	0	0	0	0	24	9	55	21
*carcinoma	12	8	0	2	0	0	0	0	0	0	0	0	0	0	0	0	2	2	0	0
Stomach	68	31	20	49	50	50	50	50	50	50	49	48	49	50	50	50	50	50	50	50
*malignant tumors	4	61	0	2	0	0	0	0	2	0	0	0	0	0	0	0	0	2	34	46
- mixed carcinosarcoma	3	19	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	2	22	34
- undifferentiated sarcoma	-	16	-	2	-	-	-	-	2	-	-	-	-	-	-	-	-	-	-	-
- undifferentiated carcinoma	-	3	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
- adenocarcinoma	-	10	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	4	2
- leiomyosarcoma	-	10	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	2	2
- Anaplastic sarcoma	1	3	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
- Osteosarcoma	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	6	8
Thyroid	70	31	20	49	49	49	50	49	49	49	49	47	48	49	50	44	49	46	50	49
*C-cell adenoma	6	10	5	4	10	4	6	4	14	8	12	6	8	4	10	7	4	9	14	6
carcinoma	0	0	0	2	0	0	0	0	2	0	0	0	2	0	2	0	0	2	0	0
*Follicular adenoma	11	13	5	6	4	2	8	2	6	0	8	4	2	0	0	0	2	4	22	4
carcinoma	14	0	5	2	2	6	0	2	2	2	4	2	0	0	0	0	0	0	4	4
Brain	70	31	20	49	50	50	50	50	50	50	49	49	50	50	50	50	50	50	50	50
Neuroepithelioma	1	3	0	2	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Liver	70	31	20	49	50	50	50	50	50	50	50	49	50	50	50	50	50	50	50	50
*Hepatoma	4	3	0	0	2	0	0	2	0	0	0	0	2	0	0	0	4	2	2	6
*Nodular hyperperplasia	0	3	0	2	0	0	0	0	0	0	0	2	0	4	0	2	2	4	8	2
*Carcinoma	3	7	0	2	4	0	2	2	2	0	0	4	0	0	0	0	0	0	0	0

*GI: animals maintained on treated diets for 2-years GIII: animals maintained on treated diets for only 5 to 6 months.

As noted in the above table, the nasal turbinate tumors increased significantly in the repeat studies, ML-80-186 and ML-80-224, as compared to the incidence in study #BD-77-421. In addition these repeat studies had a high incidence of animals with submucosal gland hyperplasia (see table pg. 18 of this review and pg. 17 of attachment #4) which was not noted in study #BD-77-421, these lesions are questionable as hyperplasia or neoplasia and a reading of the slides by a second pathologist was requested from the registrant in April 1984.

Also, the nasal turbinate tumors (adenoma + carcinoma) are the most important tumors in this study since they occurred at an incidence rate of 81% in males and 52% in females which were exposed to Alachlor for the duration of the study. In animals removed from the treated diet after 5 to 6 months of exposure, the incidence rate remained high at 59% in males and 42% in females. However, the incidence of nasal turbinate carcinoma was high in males (12%) and in females (8%) which were exposed to Alachlor for 24 months as compared to the incidence in males (0%) and in females (2%) which were exposed for only 5 to 6 months. Thus, unlike the stomach tumors (discussed below) which were not present in most of the animals exposed to Alachlor for only 5 to 6 months, these tumors appear to have a short latent period.

The stomach tumors (all malignant) were only noted in 4% of the males and 6% of the females which were exposed to the treated diet for 24-months. In animals exposed for 5 to 6 months, the incidence rate was 2% in females and 0% in males.

The table on page 24 also indicates that in addition to the significant increases noted in both sexes for the nasal turbinate tumors, the stomach tumors, and the thyroid tumors, remarkable increases in the following tumors are noted in the high dose females in this study: Liver tumors (10% in GI and 2% in GIII as compared to 0% in the control group), and C-cell thyroid tumors (10% in GI and 6% in GIII as compared to 4% in the control group). Also, follicular cell carcinomas are noted to increase only in males (14% in GI males as compared to 0% in females of this group and as compared to 2% and 6% in the control male and female groups respectively).

3. Based on the higher incidence of nasal turbinate tumors in the repeat studies, it appears that the new technical product (with 1.28% epoxidized soybean oil as stabilizer) is a more potent oncogen than the product discontinued from use which was tested in study #BD-77-421 (with 0.5% epichlorohydrin, as a stabilizer, a known carcinogen). The comparison between the potency of the new technical product and the discontinued old product as an oncogen should take in consideration the following differences:

		ML-80-186/ML-80-224	BD-77-421
1.	Different testing facilities	Monsanto's Environmental Health Laboratory	Bio/dynamics
2.	Nature of stabilizer	[redacted]	Epichlorohydrin (known carcinogen)
3.	Different Pathogists	W.E. Ribelin	Robert McKonnel

4. Finally, caution should be used for any risk assessment based on these data since due to the study design, there is some potential for bias relative to the subgroup assignments, see discussion on pages 4 and 5.

Study Classification:

This special chronic study successfully achieved its objective in determining the nature of the ocular lesions which was noted in the previous study #BD-77-421. This study is an Acceptable study which should be included as an addendum to the concurrent study #ML-80-186 where lower dosages were tested.

TOXICOLOGY BRANCH:A. DATA REVIEW

CHEMICAL: Alachlor

Caswell No.: 11
EPA Chem. No.: 090501

STUDY TYPE: Mutagenicity: in vivo UDS in rat HPC's

CITATION: An Evaluation of the Potential of Alachlor to Induce Unscheduled DNA Synthesis in the In Vivo - In Vitro Hepatocyte DNA Repair Assay.

ACCESSION NO./MRID NO.: 253308/NA

SPONSOR/CONTRACTING LAB.: Monsanto/SRI International
Menlo Park, CA

REPORT NO./DATE: SR-83-293/April 5, 1984

TEST MATERIAL: Technical (Lot #MDLT 1114D), purity = 95.2%.

PROCEDURES: NB: This is the newer, in vivo counterpart to the in vitro Williams UDS assay, the latter involving in vitro exposure of rat hepatocytes isolated from untreated animals. The methods for the present assay have not been standardized (see photocopy of Purpose and Methods, attached to this Review), but the end-point assayed (determination of radioactive-labelled unscheduled DNA synthesis) is the same as for the in vitro assay.

Fischer 344 male rats (presumably adults, but neither age nor body weight was stated) were given single oral doses of 0 (corn oil), 50, 100, 200 and 1,000 mg/kg in corn oil, 2 and 12 hr prior to sacrifice; the HDT is reportedly the approximate (oral ?) LD_{50} for alachlor in rats. 2-Acetylaminofluorene (2AF) served as the positive control. At sacrifice, hepatocytes were isolated, cultured with tritiated thymidine, and microscope slides prepared according to conventional radiolabelling techniques. A minimum of 50 cells per slide and 3 slides per animal per time point (3 animals per test group, and 2 per controls) were scored (i.e., a total of 450 cells/dose/time point) for net nuclear silver grain counts. A test result was considered "positive" if net counts were elevated over negative control by 5 counts or greater.

RESULTS: Compared to a significantly elevated net grain count of 18.7 ± 4.6 for 2AF-treated rat hepatocytes *in situ* (average % cells "in repair" = 82 ± 11), only the HDT test group which received 1000 mg/kg 12 hr prior to sacrifice showed increased repair over controls. (2.1 ± 2.4 *vs* - 6.0 ± 1.5, constituting 35 ± 12% of cells *vs* 0%).

Hence, the authors concluded that alachlor induced DNA damage in hepatocytes at the LD_{50} in this assay, i.e., was "weakly genotoxic" (positive), an assessment with which this reviewer concurs.

EVALUATION: The procedures employed were apparently adequate to generate valid (positive) results, and the study is thus ACCEPTABLE.

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Pages 219 through 219 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.

____ Description of quality control procedures.

____ Identity of the source of product ingredients.

____ Sales or other commercial/financial information.

____ A draft product label.

____ The product confidential statement of formula.

____ Information about a pending registration action.

✓ FIFRA registration data.

____ The document is a duplicate of page(s) _____.

____ The document is not responsive to the request.

The information not included is generally considered confidential by product registrants. If you have any questions, please contact the individual who prepared the response to your request.

007699

TOXICOLOGY BRANCH:A.DATA REVIEW

CHEMICAL: Alachlor

Caswell No.: 11
EPA Chem. No.: 090501

STUDY TYPE: Mutagenicity: in vivo cytogenetics in rat bone marrow (chromosome aberrations).

CITATION: In Vivo Bone Marrow Chromosome Study in Rats with Alachlor.

ACCESSION NO./MRID NO.: 253308/NA

SPONSOR/CONTRACTING LAB.: Monsanto/Hazleton Laboratories,

REPORT NO./DATE: HL-83-165/March 1, 1984

TEST MATERIAL: Technical (Lot #MDLT-08-02B), purity = 95.4%.

PROCEDURES: Alachlor was administered orally to Sprague-Dawley male and female rats at single dose levels of 0, 100, 333 and 1,000 mg/kg in corn oil, and bone marrow cells processed according to standard cytological procedures for chromosome aberration analysis (clastogenesis). (A photocopy of methods employed is attached to this review.) Cyclophosphamide (CP) served as the positive control substance.

RESULTS: In preliminary range-finding, no changes in mitotic index were apparent at dosages up to 1,300 mg/kg, but clinical effects were observed at doses of 1,000 mg/kg and above (weight loss, chromodacryorrhea, chromorhinorrhea). Compared to significant increases in both % aberrant cells and mean number of aberrations per cell for the CP-treated group, no level of alachlor induced structural or numerical chromosome aberrations when compared to corn oil controls. Hence, the authors concluded that alachlor was neither a clastogen nor an aneugen (altered chromosome modal number) at doses producing adverse clinical effects but no deaths (assuming the HDT approximates the LD_{50}). This reviewer does not concur in this evaluation, since no evidence has been presented that alachlor was absorbed from the gut, and transported to the bone marrow in effective concentrations.

DISCUSSION AND EVALUATION: This study is UNACCEPTABLE, since the following data are lacking:

Positive evidence of (1) absorption of test compound from the gastro-intestinal tract (eg, systemic effects); and/or (2) transport to target tissue (bone marrow). Toxicology Branch recommends repeating the study employing i.p. administration of test compound to assure effective concentration in bone marrow.

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Pages 121 through 122 are not included.

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____ Identity of the source of product ingredients.

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Appendix D.

004091

UNITEDSTATES ENVIRONMENTAL PROTECTION AGENCY
WASHINGTON, D.C. 20460

NOV 19 1984

OFFICE OF PESTICIDES AND TOXIC SUBSTANCES

Cancell # 11

MEMORANDUM

SUBJECT: Alachlor (Lasso), EPA Reg. #524-316, EPL's Re-Evaluation of Nasal cavity Lesions in a 2-year chronic study in rats # ML-80-186 (800218), by Monsanto.

TO: Robert Taylor
Product Manager (25)
Registration Division (TS-767C)

FROM: Anal Mahfouz, Ph.D. 11/10/84
Toxicology Branch
Hazard Evalaution Division (TS-769C)

THRU: Laurence D. Chitlik, DABT 11/13/84
Head, Review Section V
Toxicology Branch
Hazard Evaluation Division (TS-769C) 11/16/84

and

William L. Burnam, Chief
Toxicology Branch
Hazard Evaluation Division (TS-769C)

Submucosal nasal hyperplasia was noted in a recently submitted and reviewed low dose chronic feeding/oncogenic study in rats with Alachlor, see page 22 of my 4/17/84 review of this study (study # ML-80-186 by Monsanto, Environmental Health Laboratory, 2/12/84).

The Agency requested a histological re-evaluation of the above mentioned finding in order to determine if these lesions were hyperplasia or neoplasia. A histological re-evaluation of tissues of the nasal cavity was performed by Experimental Pathology Laboratories, Inc. (EPL) at the Research Triangle Park facility. The newly submitted EPL report dated 10/12/84 (which was forwarded to the Toxicology Branch on 11/1/84) indicated that the submucosal nasal lesions were not neoplastic lesions.

However, this report reflected a slight increase in the previously reported incidences of papillary adenoma at 15 mg/kg/day dosage level (papillary adenoma was also reported as respiratory epithelium adenoma in the original final report of study # ML-80-186). The updated incidences of this nasal tumor is presented in the table below:

Study # ML-80-186 (800218)

Incidences of Nasal Turbinate Tumors (adenoma)

Dosage mg/kg/day	EPL's data (10/12/84)		Monsanto's data (2/12/84)	
	Males	Females	Males	Females
0.0	0/44	0/42	0/45	0/42
0.5	0/47	0/42	0/48	0/44
2.5	0/44	1/47	0/45	1/47
15.0	15/45	14/48	11/45	9/48

Please note that the EPL data need to be included in the 2 tables on pages 23 and 24 of my 10/24/84 review of the special chronic study (high dose), # ML-80-224, by Monsanto. Copies of these 2 pages (with the updated indidences of nasal turbinate tumors in study # ML-80-186) are attached to this memo.

Attachment: 2

cc: R. Englar
B. Litt
G. Burin

Dosage mg/kg/day	Study #ML-80-224 126				Study #ML-80-186 ** 0		.5		2.5		15		Study #BD-77-421 0		14		42		126	
No.of tissues examined and incidence of animals with tumors:	GI* M	GI* F	GIII* M	GIII* F	M	F	M	F	M	F	M	F	M	F	M	F	M	F	M	F
Nasal turbinate tumors	61	25	17	46	44	42	47	42	44	47	45	48	46	49	46	47	41	45	42	48
*adenoma	42	11	10	19	0	0	0	0	0	1	15	14	0	0	0	0	10	4	23	10
*adenocarcinoma	7	2	0	1	0	0	0	0	0	0	0	0	0	0	0	0	1	1	0	0
Stomach	68	31	20	49	50	50	50	50	50	50	49	48	49	50	50	50	50	50	50	50
*malignant tumors	3	19	0	1	0	0	0	0	1	0	0	0	0	0	0	0	0	1	17	23
- mixed carcinosarcoma	2	6	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	1	11	17
- undifferentiated sarcoma	-	5	-	1	-	-	-	-	1	-	-	-	-	-	-	-	-	-	-	-
- undifferentiated carcinoma	-	1	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
- adenocarcinoma	-	3	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	2	1
- leiomyosarcoma	-	3	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	1	1
- Anaplastic sarcoma	1	1	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
- Osteosarcoma	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	3	4
Thyroid	70	31	20	49	49	49	50	49	49	49	49	47	48	49	50	44	49	46	50	49
*C-cell adenoma	4	3	1	2	5	2	3	2	7	4	6	3	4	2	5	3	2	4	7	3
carcinoma	0	0	0	1	0	0	0	0	1	0	0	0	1	0	1	0	0	1	0	0
*Follicular adenoma	8	4	1	3	2	1	4	1	3	0	4	2	1	0	0	0	1	2	11	2
carcinoma	10	0	1	1	1	3	0	1	1	1	2	1	0	0	0	0	0	0	2	2
Brain	70	31	20	49	50	50	50	50	50	50	49	49	50	50	50	50	50	50	50	50
*Neuroepithelioma	1	1	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Liver	70	31	20	49	50	50	50	50	50	50	50	49	50	50	50	50	50	50	50	50
*Hepatoma	3	1	0	0	1	0	0	1	0	0	0	0	1	0	0	0	2	1	1	3
*Nodular hyperplasia	0	1	0	1	0	0	0	0	0	0	0	1	0	2	0	1	1	2	4	1
*Carcinoma	2	2	0	1	2	0	1	1	2	0	0	2	0	0	0	0	0	0	0	0

*GI: animals maintained on treated diets for 2-year; GIII: animals maintained on treated diets for only 5 to 6 months.

** after the 11/17/84 EPL submission.

Dosage mg/kg/day	Study #ML-80-224				Study #ML-80-186 **								Study #BD-77-421							
	126				0		.5		2.5		15		0		14		42		126	
No. of tissues examined and % incidence of animals with tumors:	GI*		GIII*																	
	M	F	M	F	M	F	M	F	M	F	M	F	M	F	M	F	M	F	M	F
Nasal turbinate tumors	61	25	17	46	44	42	47	42	44	47	45	48	46	49	46	47	41	45	42	48
*adenoma:	69	44	59	41	0	0	0	0	0	2	33	29	0	0	0	0	24	9	55	21
*carcinoma	12	8	0	2	0	0	0	0	0	0	0	0	0	0	0	0	2	2	0	0
Stomach	68	31	20	49	50	50	50	50	50	50	49	48	49	50	50	50	50	50	50	50
*malignant tumors	4	61	0	2	0	0	0	0	2	0	0	0	0	0	0	0	0	2	34	46
- mixed carcinosarcoma	3	19	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	2	22	34
- undifferentiated sarcoma	-	16	-	2	-	-	-	-	2	-	-	-	-	-	-	-	-	-	-	-
- undifferentiated carcinoma	-	3	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
- adenocarcinoma	-	10	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	4	2
- Leiomyosarcoma	-	10	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	2	2
- Anaplastic sarcoma	1	3	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
- Osteosarcoma	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	6	8
Thyroid	70	31	20	49	49	49	50	49	49	49	49	47	48	49	50	44	49	46	50	49
*C-cell adenoma	6	10	5	4	10	4	6	4	14	8	12	6	8	4	10	7	4	9	14	6
carcinoma	0	0	0	2	0	0	0	0	2	0	0	0	2	0	2	0	0	2	0	0
*Follicular adenoma	11	13	5	6	4	2	8	2	6	0	8	4	2	0	0	0	2	4	22	4
carcinoma	14	0	5	2	2	6	0	2	2	1	4	2	0	0	0	0	0	0	4	4
Brain	70	31	20	49	50	50	50	50	50	50	49	49	50	50	50	50	50	50	50	50
Neurocpithelioma	1	3	0	2	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Liver	70	31	20	49	50	50	50	50	50	50	50	49	50	50	50	50	50	50	50	50
*Hepatoma	4	3	0	0	2	0	0	2	0	0	0	0	2	0	0	0	4	2	2	6
*Nodular hyperperplasia	0	3	0	2	0	0	0	0	0	0	0	2	0	4	0	2	2	4	8	2
*Carcinoma	3	7	0	2	4	0	2	2	2	0	0	4	0	0	0	0	0	0	0	0

*GI: animals maintained on treated diets for 2-year; GIII: animals maintained on treated diets for only 5 to 6 months.

** After EPL's corrections.

007699

Appendix E.

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

MEMORANDUM JUN 16 1982

TO: Robert Taylor (12)
Registration Division (TS-769)

OFFICE OF PESTICIDES AND TOXIC SUBSTANCES

THRU: Orville E. Paynter, Chief
Toxicology Branch
Hazard Evaluation Division (TS-769)

SUBJECT: EPA Reg.#524-316; Alachlor. Review of Monsanto 18-Month Oncogenic Study in Mice. R.D.#365, Special Report MSL#1649; Volumes 1 and 2; 6/18/81, Accessions#070168 and #070169. CASWELL#11

Action Requested:

A review is requested for an 18-month oncogenic study in mice submitted by Monsanto Company as a part of the requirement to support registrations and tolerances for Alachlor (2-chloro-2',6' diethyl-N-(methoxymethyl)-acetanilide), a herbicide.

Conclusion:

Classification: Core-Minimum

Alachlor is oncogenic in mice.

*Incidence of animals bearing tumors increased significantly at the high-dose level (260 mg/kg bw/day) in males, $p < 0.05$. The incidence for the combined high-dose male and female animals was also highly significant, $p < 0.01$.

*Lung is the major target organ for oncogenicity. Incidence of lung bronchioalveolar tumors(adenoma + carcinomas) was significant in the high-dose females, $p < 0.05$, and the combined high-dose animals (males and females), $p < 0.05$. The incidence of these tumors was also highly significant, $p < 0.01$, for the high-dose females which died in extremis during the study.

*Incidence of tumors in other organs was also noted to increase at the high-dose level, potentially as a result of Alachlor administration, i.e. liver tumors (adenoma + carcinoma) in the high-dose males and uterine tumors (see description on p. 18) in the high-dose females. The incidence of animals bearing these tumors was not statistically significant.

REVIEW

Study Identification

An 18-month chronic feeding study of Alachlor in mice, BD-77-423, 6/18/81. A final report compiled by R.W. Street, and submitted on 7/1/81 by Monsanto Company, St. Louis, MO. 63166 (Volumes 1 and 2; Accessions#070168 and 070169).

The Study was performed by Bio/dynamics Inc., project#77-2064 (BD-77-423). The final report was dated May 6, 1981 and signed by Ira W. Daly, Ph.D (Study Director) and Geoffrey H. Hogan, Ph.D (Vice President of Toxicology).

In life phase of study was from April 14, 1978 to October 14 to 18, 1979 (550 to 554 days).

Materials and Methods

Test Substance

Alachlor (Lasso® technical) material, a clear, brown, slightly viscous liquid, was supplied in two batches by Monsanto. Lot#XHI-167 (92.6% a.i.) was received on 1/5/78 and used from 4/12/78 to 3/6/79; Lot#MHK-6 (92.19% a.i.) was received on 2/8/79 and used from 3/7/79 to termination.

Lot XH7-167 used during the first 11 months of the study was stabilized with 0.5% epichlorohydrin; Lot MHK-6 used during the last 7 months of the study was stabilized with [redacted] (Ref.: Sec. I, Vol. 1, p. 1).

Study Design

Male and female mice were randomly divided into groups and fed Alachlor continously in the diet at the following nominal concentrations for the entire duration of the study:

			Number of Animals/Sex/Group			
			Hematology at 12-month and termination	Necropsy at termination		
Group	Dosage mg/kg/day M/F*	Initial M/F	M/F	M	F	Histology
I	0	50	10	24	20	All animals
II	26	50	10	16	33	All animals
III	78	50	10	24	23	All animals
IV	260	50	10	22	15	All animals

*M/F represents # males and # females

Test Animals

Two hundred male (mean body weight 25.65g) and 200 female (mean body weight 20.75 g) CD-1 albino mice were initiated in this 18-month feeding study. The mice were obtained from Charles River Laboratories (Wilmington, Mass.) when 35-days old and then acclimatized for a period of 17 days before treatment. Each mouse was toe-clipped for identification.

The mice were individually housed in elevated stainless steel wire mesh cages and maintained on a 12-hour light/dark cycle, and temperature controlled rooms (monitored twice daily). Control and test diet and tap water were available ad libitum throughout the study.

Preparation of Test Diet

The treated diet was prepared weekly based on body weight and food consumption data. Dietary levels of Alachlor were adjusted weekly for each dosage group so as to provide each group with the designated mg/kg/day intake of test material. Appropriate amounts of Alachlor were dissolved in 100 ml acetone and incorporated into 9 to 12 kg of feed (Purina R-5001 Lab. Chow). Acetone alone was added to the control diet.

Diet analyses were performed on weeks 0, 1, 2, 3, 4, 6, 7, 8, 9, 11, 12, 13, 24, 36, 48, 50, 51, 53, 55, 59, 71 and 72. Technical grade Alachlor was also analyzed to determine its stability during storage. The mice were offered the treated diet at age 53 days and continously thereafter for 550 to 554 days (from 4/14/78 to 10/14-18/79).

Observations

The animals were observed twice daily for signs of overt toxicity and mortality. Detailed physical examination was performed weekly for signs of local or systemic toxicity, pharmacologic effects and tissue masses.

Body weights and food consumption were measured at pretest, weekly through the first 4 weeks and biweekly from week 16 through week 78 (body weights were also measured terminally after fasting).

Compound intake and food efficiency were calculated; however, food efficiency was calculated weekly for weeks 1-14.

Absolute (ml/interval) and relative (ml/kg/day) water consumption values were determined for 10 mice/sex/group during two 3-day periods on week 75 (month 18) and two 3-day periods on week 78 (termination).

Laboratory Studies

Blood was collected for 10 mice/sex/group at 12 months and at termination (19 months). The mice were fasted overnight prior to blood collection and the blood was obtained via the orbital sinus technique. The same animals were used at both intervals for blood samples when feasible.

The hematological parameters evaluated in this study included hemoglobin concentration, hematocrit value, total erythrocyte counts, total and differential leucocyte counts, and erythrocyte morphology.

Necropsy

All animals were subject to necropsy. Complete gross postmortem examination was performed on all animals dying spontaneously or sacrificed *in extremis* during the study and on all surviving animals at termination (10/15 to 19/1979). All animals were sacrificed by exsanguination under ether anesthesia.

Brain, adrenal, testes, ovaries, spleen, heart, kidneys, liver and pituitary were weighed at necropsy for animals sacrificed at termination only; and organ to body weight and organ to brain weight ratios were calculated.

The following tissues were preserved (and histopathologically examined) for all animals dying spontaneously or sacrificed in extremis or at termination:

abdominal aorta
adrenals
blood smear[1]
bone and bone marrow (costochondral junction)
brain (3 sections)
epididymides
esophagus
eyes (with optic nerve and contiguous harderian glands; right eye processed for histopathology)
gall bladder
gonads
head (with entire skull cap)
heart
intestine
  cecum
  colon
  duodenum
  ileum
  jejunum
kidneys
liver (2 sections)
lungs (with mainstem bronchi and trachea)
lymph nodes (mediastinal and mesenteric)
nerve (right sciatic)
pancreas
parathyroid
pituitary
prostate
salivary gland (mandibular)
skeletal muscle (right biceps femoris)
skin (with mammary gland)
spinal cord[2]
stomach (3 sections)
thymus
thyroid
urinary bladder
uterus
gross lesions
tissue masses

---

[1]Taken from all animals examined only if anemia, enlarged thymus, lymphadenopathy or hepatosplenomegaly is present.

[2]Two sections of spinal cord and three coronal sections through the head were taken for 10 animals/sex/group sacrificed at termination. One section of spinal cord was taken for all other animals.

Histopathological Examinations

The eyes with contiguous Hardenian glands, testes and epididymides were preserved in Bouin's solution for 48-72 hours, followed by neutral buffered 10% formalin. All other tissues (listed in the above section) were preserved directly in the 10% formalin solution. Hematoxylin and eosin stained sections of these tissues were microscopically examined from all mice.

Statistical Analysis

Statistical analyses of data was performed by using various statistical methods. Statistically significant differences from control were indicated at $p \leq 0.05$.

RESULTS:

Alachlor Concentrations in Diet

Based on food consumption and body weight data, the calculated compound consumption was found to be as follows:

Group	Dose Level (ppm)	Dose level (mg/kg/day) Week 2 to 4 Male	Week 2 to 4 Female	Week 5 to Termination Male and Female
I	0	0.00	0.00	0.00
II	100	23.96	29.27	26.00
III	300	72.04	83.78	78.00
IV	1000	240.23	280.03	260.00

Chemical analysis of the treated diets indicated that Alachlor was found to be within 81% to 95% (89% average) of the target dosage levels (except for week 12 when the result was 150%).

Mortality and Observations

The report states that no changes considered to be related to the compound were observed in the general appearance of the animals (individual clinical observations data were not submitted). Periodic findings in some animals include alopecia, lacrimation, hypoactivity and ano-genital staining. In life tissue masses were unremarkable.

The fate of animals at the end of the study (third week of the 19th month) was as follows (initial number of animals: 50 animals/sex/group):

Dosage mg/kg/day	Survivors M	Survivors F	Spont. Death M	Spont. Death F	Moribund M	Moribund F	Accidental Death M	Accidental Death F
0	24	20	18	23	8	2	0	5
26	16	33	24	16	9	1	1	0
78	24	23	22	18	4	8	0	1
260	22	15	17	29	11	5	0	1

The above table reflects decreased survivability at the high-dose level in females at termination, but survival varied greatly among the test and control groups. However 50% of the animals in each group survived for 15 months of the study period.

Body Weight, Food and Water Consumption

High-dose females had a slightly reduced mean body weight (7%) than the control group during the second year of study (this decrease was statistically significant, $p < 0.05$-0.01, at most of the determination intervals). Mean body weights of all treated male groups and low- and mid-dose females were generally similar to the respective control groups.

Mean body weights at 78 weeks (termination) were slightly reduced in both treated males (2-10%) and females(3-10%) as compared to the respective control groups, see table below:

Dosage Level mg/kg/day	Group Mean Body Weights (grams) Males	Females
0	40.6	37.5
26 (~ reduction in bw)	36.4 (10)	36.3 (3)
78 (~ reduction in bw)	39.6 (2)	34.9 (7)
260 (~ reduction in bw)	37.7 (7)	33.8 (10)

Mean food consumption values were variable for the control and treated animals. However, although statistical differences were found between control and treated groups at some determination intervals, no consistent dose-response relationship was apparent. Food efficiency calculations (determined weekly from weeks 1 through 14) were also variable and inconsistent.

Mean water consumption for the low- and mid-dose animals were similar to the control mice; however the high dose animals (both males and females) reflected a significant increase ($p < 0.05$) in water consumption than the respective control groups.

Ophthalmology

No ophthalmoscopic examination was performed.

Hematology

The 12-month interim data and the terminal hematology data were unremarkable in all animal groups (Note: animal #821, a high-dose female, was removed from these calculations because it was reflecting an unusually low value for all hematology parameters).

Gross Necropsy

Animals killed at termination of study reflected statistically significant increases in mean liver weights (absolute, relative-organ/body and organ/brain) in males and females of the mid- and high-dose levels. Increases were also noted in mean kidney weights (absolute and relative organ/brain weight ratio) for mid- and high-dose males. However in females, mean kidney weights increased at the low-dose, slightly decreased at the high-dose, and were similar to the control group at the mid-dose level, see table below:

Group (ppm)	Measure	Liver		Kidney	
		Males	Females	Males	Females
Control	Absol.	1.53	1.47	0.66	0.49
	Rel./bw	4.51	4.90	1.94	1.65
	Rel./br	3.35	3.20	1.44	1.06
Low	Absol. (%)	1.56(2)	1.59(8)	0.68(3)	0.56*(15)
	Rel./bw (%)	4.81(7)	5.34 (9)	2.08(7)	1.89*(15)
	Rel./br (%)	3.40(2)	3.43 (7)	1.48(3)	1.21(14)
Mid	Absol. (%)	1.75(14)	1.68(14)	0.74*(12)	0.48(2)
	Rel./bw (%)	5.16(14)	6.00*(22)	2.17*(12)	1.70(3)
	Rel./br (%)	3.81(14)	3.75 (17)	1.59*(10)	1.07(1)
High	Absol. (%)	1.90*(24)	1.81*(24)	0.71(9)	0.45(8)
	Rel./bw (%)	6.18**(37)	6.12*(25)	2.23*(15)	1.55(6)
	Rel./br (%)	4.29**(28)	4.11*(28)	1.59*(10)	1.02(4)

*p < 0.05
**p < 0.01

Other Sporadic differences between the mean organ weights of control and treated mice were noted, however no dose-dependent pattern was evident, i.e. pituitary and spleen weights in both sexes, and ovaries weight in females. Also large sporadic differences between individual weights for these three organs were noted in treated animals.

Gross necropsy observations also reflected a variety of inflammatory, and non-inflammatory alterations, but no compound relationship was evident. However the cause of death in most animals was associated with glomerular amyloidosis (especially renal amyloidosis). Lymphoblastic lymphosarcoma was occasionally noted as a cause of death (i.e. 1, 3, 1 and 4 animals of the control, low, mid and high dose groups respectively were identified with this lesion as the cause of death).

Histopathology

Non-neoplastic lesions

At termination and for animals that died during the study, amyloidosis of different organs, especially the liver and kidney. was noted at a higher incidence in treated animals than the control-rats; this increase was not dose dependent. Other lesions were also noted at higher rates in treated groups. The following table reflects the increase in compound related lesions:

Organs affected	MALES				FEMALES			
	Control	Low	Mid	High	Control	Low	Mid	High
Thyroid	39	39	32	36	36	36	33	36
Amyloid	7	11	7	10	6	10	8	13
Follicular atrophy	1	1	5	6	4	0	5	10
Liver	50	50	50	50	50	50	50	50
Perivascular amyloid	25	28	27	29	19	30	30	36
Kidneys	50	50	50	50	50	50	50	50
Amyloid	29	39	36	33	32	40	42	40
Chronic interstitial fibrosis	1	1	4	6	1	-	5	13
Ovaries					43	47	46	46
Atrophy					10	29	31	31
Eyes	50	49	49	50	50	50	49	50
Retinal atrophy	1	2	2	6	4	6	6	6
Bone marrow	44	46	47	48	45	45	43	46
Hyperplasia	10	25	28	26	-	-	-	-
Lungs	50	50	50	50	50	50	50	49
Amyloid	7	5	20	13	2	10	-	-
Congestion	1	13	13	12	5	5	12	16

-: no difference in female groups

From the above table it is also noted that incidence of thyroid follicular atrophy increased in the mid- and high-dose males and high-dose females.

Incidence of chronic interstitial fibrosis of the kidneys increased in all treated groups except in the low-dose male group. It is noted that the mean kidney weight values significantly increased ($p < 0.05$) in the mid- and high-dose males and in the low-dose females. The mid-dose female values were similar to the control group. However the high-dose female group (which experienced the highest incidence of kidney fibrosis in this study, see organ weight values, p. 9) reflected a slight decrease in these weight values.

Atrophy of the ovaries was noted in all treatment groups.

The incidence of Retinal atrophy was slightly increased in the treated groups as compared to the control group. This increase was most evident in the high-dose males (6 vs 1 in controls).

The incidence of bone marrow hyperplasia was 2.5 to 2.8 times higher in treated males of any group as compared to controls. No difference was noted in female groups.

Lung congestion increased in all treated male groups and mid- and high-dose female groups. Twenty-five percent of the males and 28% of the females were affected as compared to the control mice (2% M, 10% F).

High incidence of amyloidosis of liver, kidneys and lungs was noted in all animal groups but somewhat greater in treatment groups. The exact relationship between Alachlor administration and amyloidosis in this study is unclear considering that the CD-1 strain of mice (supplier Charles River Laboratories of Wilmington, Mass.) is known to have a high incidence of amyloidosis (JNCI:55 #1, 1975). Thus it is unlikely amyloidosis is compound related in this study.

Neoplastic lesions

The total number of animals bearing tumors increased (almost doubled) in the high-dose group as compared to the control group. The increase was statistically significant for the high-dose males ($p < .05$) and the combined high dose animals, males and females ($p < 0.01$). Increases were also noted in the low- and mid-dose female groups but were not statistically significant. In addition to these findings, the total number of tumors was noted to increase significantly at the high-dose level in both males ($p = 0.05$) and females ($p = 0.009$). However the number of malignant tumors in all treated male and female groups almost doubled.

The following table reflects the tumor incidence in both sexes:

	MALES			
	Control	Low	Mid	High
#of animals examined	50	50	50	50
#malignant tumors (%)	5 (10)	11 (22)	9 (18)	10 (20)
#of tumor bearing animals (%)	14 (28)	14 (28)	14 (28)	25 (50)
total # of tumors	19	17	17	28

	FEMALES			
	Control	Low	Mid	High
#of animals examined	50	50	50	50
#malignant tumors (%)	4 (8)	8 (16)	6 (12)	7 (14)
#of tumor bearing animals (%)	9 (18)	14 (28)	13 (26)	16 (32)
total # of tumors	10	18	14	22

Lungs, liver and uterus appear to be the most affected organs:

•Lung tumor bearing animals increased in all treated female groups (dose-dependent response), and very slightly in mid- and high-dose males. The increase was significant ($p < 0.05$) only at the high-dose level for females and for the combined number of high-dose animals, males and females ($p < 0.01$). Incidences of lung tumors (bronchioalveolar adenoma+carcinoma) in males and females are demonstrated in the table below:

ANIMALS WITH LUNG TUMORS (Bronchiolar-Alveolar, adenoma + carcinoma)

Group				D	T	Total
Females	D	T	Total[1]	No. %	No. %	No. %
Control	30	20	50	0/30(0)	3/20(15)	3/50(6)
Low	17	33	50	1/17(6)	4/33(12)	5/50(10)
Mid	27	23	50	3/27(11)	5/23(21)	8/50(16)
High	35	15	50	7/35(23)**	4/15(27)	11/50(22)*
Males						
Control	26	24	50	3/26(12)	6/24(25)	9/50(18)
Low	34	16	50	1/34(3)	5/16(31)	6/50(31)
Mid	26	24	50	1/26(4)	10/24(42)	11/50(22)
High	28	22	50	5/23(18)	7/22(32)	12/50(24)

*: $p < 0.05$ **: $p < 0.01$
D: died or sacrificed during study.
T: sacrificed at termination of study.
1: total is based on total number of animals in study.

It is also apparent from the above table that the incidence of lung tumors increased in treated females which died in extremis during the study. This increase was only statistically significant ($p < 0.01$) for the high-dose females.

This reviewer also notes that the incidence of lung tumors in the male control group is unusually high for this strain of mice (JNCI:46 #5, 1971). Consequently the increased incidence of this tumor in treated males may potentially be reduced when compared to the control males.

*The number of liver tumor bearing animals increased in the high-dose male group. However this increase was only significant ($p < 0.05$) for the combined number of affected high-dose males and females, though only 1/50 females was affected.

The table below reflects the incidences of liver tumors in males:

INCIDENCE OF LIVER TUMORS (ADENOMA & ADENOCARCINOMA) IN MALES

Group	D	T	Total	D No.	%	T No.	%	Total No.	%	Malignant No.	(%)
Control	26	24	50	2/26	(8)	3/24	(13)	5/50	(10)	0/50	(0)
Low	34	16	50	2/34	(6)	2/16	(12)	4/50	(8)	3/50	(6)
Mid	26	24	50	0/26	(0)	5/24	(21)	5/50	(10)	1/50	(2)
High	28	22	50	3/28	(11)	8/22	(36)*	11/50	(22)	4/50	(8)

*: $p = 0.06$

The above table indicates that the incidence of liver tumors in the high-dose males is almost statistically significant ($p = 0.06$) in animals sacrificed at the end of the study. The table also reflects an increase in the incidence of adenocarcinoma in all treated males; this increase is not statistically significant.

*Animals bearing uterine tumors increased by a factor of two in the low- and high-dose females. The increase was only significant ($p < 0.05$) for the high-dose females which were sacrificed at the end of the study, see table below:

ANIMALS WITH UTERINE TUMORS[a]

Group	D	T	Total[1]	D No.	%	T No.	%	Total No.	%
Control	30	20	50	2/30	(7)	1/20	(5)	3/50	(6)
Low	17	33	50	2/17	(12)	6/33	(18)	8/50	(16)
Mid	27	23	50	1/27	(4)	1/23	(4)	2/50	(4)
High	35	15	50	2/35	(6)	5/15	(33)*	7/50	(14)

*: p = 0.04
D: died or sacrificed during study.
T: sacrificed at termination.
1: total is based on total number of animals in study.
a: kind of tumors described on p. 18.

The following table identifies further the incidence of individual tumors according to tissue of origin.

Individual Number of Animals with Tumors

Organs	Males				Females			
	C	Low	Mid	High	C	Low	Mid	High
Blood & Hemato-poietic system								
Spleen	50	50	50	50	50	50	50	50
Hemangioendothelioma	1	0	0	0	1	0	0	0
Lymphosarcoma	0	1	0	0	0	0	0	0
Myelosarcoma	0	0	0	0	1	2	0	0
Hemangioma	0	1	0	0	0	0	0	0
Lymph Nodes	50	50	50	50	50	50	50	50
Lymphosarcoma	0	1	1	1	1	2	1	2
Bone Marrow	50	50	50	50	50	50	50	50
Myelogenous Leukemia	1	0	0	0	0	0	0	0
Granulocytic sarcoma	0	1	1	1	0	0	0	0
Liver	50	50	50	50	50	50	50	50
Adenoma	5	1	4	7	0	0	0	1
Adenocarcinoma	0	3	1	4	0	0	1	0
Hemangioma	0	0	0	0	1	0	1	0
Lungs	50	50	50	50	50	50	50	50
Bronchiolar-alveolar adenoma	6	1	4	10	2	4	7	10
Bronchiolar-alveolar adenocarcinoma	3	5	7	2	1	1	1	1
Fibrosarcoma	0	0	0	0	0	0	0	1
Brain	50	50	50	50	50	50	50	50
Astrocytoma	0	0	0	1	0	0	0	0

	Females			
	C	Low	Mid	High
Uterus	50	50	50	50
Hemangioma	1	1	0	0
Hemangiosarcoma	0	1	0	0
Endometrial carcinoma	0	1	0	0
Leimyoma	0	2	0	0
Leiomyosarcoma	1	0	2	3
Granular cell myoblastoma	0	0	0	1
(Fibrovascular endometrial polyp)	1	3	0	3

Note: Data were collected from appendices 21-22 and table 19A.

The denominators in the above table and in the table on page 10 (non-neoplastic lesions) differ occasionally. However these differences are minimal and have no impact on the statistical evaluation of the above listed tumors.

As noted in the above table, the total number of lymphosarcoma of the blood and hematopoietic system increased slightly in treated animals of all groups, i.e. 1, 4, 2, and 3 in the combined male and female control, low-, mid- and high-dose groups respectively. One brain astrocytoma (a rare tumor) was noted in a high-dose male. Bone marrow granulocytic sarcomas were noted only in treated males. (it is important to state here that incidences of bone marrow hyperplasia almost doubled in each of the treated male groups as compared to the control group, see p. 11).

Conclusions:

This study indicates that Alachlor is oncogenic in mice.

*Lung is the major target organ for oncogenicity. Incidence of lung bronchioalveolar tumors (adenoma + carcinoma) was significant at the high-dose level (260 mg/kg bw/day) in females, $p < 0.05$, and the combined high-dose animals (males + females), $p < 0.05$. The incidence of these tumors was also highly significant, $p < 0.01$, for the high-dose females which died in extremis during the study.

This reviewer notes that the decreased significance of the lung tumors in males may be due to a high incidence of this tumor in the male control group, an incidence which is unusually high for this strain of mice (JNCI: 46 #5, 1971).

•Incidence of uterine tumors (see p. 17 for description) was only significant in the high-dose females (28% above control) which were sacrificed at the end of the study, $p < 0.05$.

•Incidence of liver tumors (adenoma + adenocarcinoma) increased in the high-dose males (12% above control). This increase was not statistically significant ($p = 0.06$ for animals sacrificed at the end of the study, and $p = 0.086$ for all high-dose males in the study).

•The incidence of animals bearing tumors increased significantly at the high-dose level in males (22% above control), $p < 0.05$. The incidence of animals bearing tumors increased in all treated female groups (8-14% above control), however these increases were not statistically significant except when the high-dose female data were combined to the high-dose male data, $p < 0.01$.

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Appendix F.

SPONTANEOUS TUMORS IN CONTROL F344 AND CHARLES RIVER-CD RATS AND CHARLES RIVER CD-1 AND B6C3HF1 MICE

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SUMMARY

The incidence of spontaneous neoplasms in outbred, inbred and F1 hybrid strains was compared using the Charles River-CD rat and mouse, the F344 rat, and B6C3HF1 mouse. These strains are commonly used in carcinogenic studies.

Each strain has a consistent pattern of tumor occurrence; testicular, pituitary and lymphoreticular neoplasms are common in F344 rats, mammary and pituitary neoplasms are common in Charles River-CD rats, liver neoplasms are uncommon in CD-1 mice, while hepatic tumors are frequent in male B6C3HF1 mice. There is considerable variation in tumor incidence in individual studies regardless of strain and there appeared to be greater variation in incidence between laboratories using the same strain than in different laboratories using unlike strains.

Therefore, the choice between these strains may be fortuitous or recommended by governmental agencies. Regardless of the strain selected, it is vital to develop sufficient historical tumor data on the strain used at the particular test laboratory

INTRODUCTION

Chronic studies in mice and rats have been used to evaluate the carcinogenic potential of drugs, food additives, and chemicals. There have been differences in opinions expressed concerning the use of inbred and outbred strains in such studies. The Canadian Food and Drug Directorate [1] has suggested that animals with heterogeneous genetic constitution (outbred strains) be used to 'determine the potential carcinogenicity of a hitherto untested compound.' When basic mechanisms in carcinogenesis are studied, an 'inbred strain that is known to respond to a particular test compound or group should be selected.' The guidelines for carci-

Abbreviation: MSDRL, Merck, Sharp and Dohme Research Laboratories.

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nogenic testing for the United Kingdom [2] recommend the use of outbred strains of rats and hamsters or an F1 hybrid mouse. Other investigators [3] have recommended the use of inbred mouse strains because of 'genetic stability and stable, reproducible background noise'.

To demonstrate the variability in spontaneous tumor incidence in commonly used strains, tumor incidence in an inbred rat strain (F344), an outbred rat strain (Charles River-CD), and F1 hybrid mouse (B6C3HF1) and an outbred strain of mouse (Charles River CD-1) were compared.

As a survey of 14 pharmaceutical companies has shown, these strains are commonly used (see below):

Strain	Number of companies
Charles River-CD rat	7
F344 rat	2
CD-1 mouse	6
B6C3HF1 mouse	5

The National Cancer Institute had used the F344 strain of rat and B6C3HF1 mouse exclusively since 1972.

METHODS AND MATERIALS

Reports of carcinogenic studies issued by the National Cancer Institute* were scanned for studies using the B6C3HF1 mouse or F344 (Fischer) rats. The tumors in control mice and rats from 22 and 23 studies, respectively, performed by Laboratory A were compiled. 20 male and 20 female controls were started on each study although the final number autopsied varied. The animals were usually 6 weeks old at initiation and were obtained principally from Charles River Breeding Laboratories or the Frederick Cancer Research Center. Data from nine control groups from similar studies performed by Laboratory B were also compiled.

Absorb Dri® hardwood chip bedding from two principal suppliers, (Wilner Wood Products Norway, Maine and Northeast Products Warrensburg, N.Y.) was used for both rats and mice in studies sponsored by the National Cancer Institute. In three of the studies, hardwood chip bedding (Sanichips®) was supplied by Shurfire Products, Beltsville, MD, or Pinewood Sawdust Co., Moonachie, NJ. Contact bedding in MSDRL studies was either Absorb Dri® or Betta Chips® hardwood bedding supplied by Lab Products, Secaucus, NJ.

Wayne Lab Blox or Wayne sterilizable lab meal (Allied Mills Inc., Chicago, IL)

*National Cancer Institute Bioassay of compounds for possible carcinogenicity Washington, DC U.S. Dept. of Health Education and Welfare, 1978–1980.

was used for both rats and mice in all NCI contract studies. Purina Lab diets supplied by Buckshire Corp., Perkasie, PA, was used in all MSDRL studies. Certified rodent diets were introduced in June, 1979. Analysis of the diets is shown below:

	Non-certified No. 5001	Certified No. 5002
Crude Protein min	23%	20.0%
Crude Fat min	4.5%	4.5%
Crude Fibre max	6.0%	5.5%
Ash max	–	7.0%
Added Minerals max	–	2.5%

Tumor data from carcinogenic studies of new human health drugs performed in the Department of Safety Assessment, MSDRL were compiled. Data from 24 groups of control CD-1 mice and 23 groups of Charles River-CD rats were tabulated. Both mice and rats were obtained from Charles River Breeding Laboratories and were 4 to 6 weeks of age when the studies were initiated. Almost all studies were of 81 weeks duration in mice and 100 to 105 weeks in rats.

RESULTS

B6C3HF1 mouse (Table I)

Overall tumor incidence in Laboratory A varied from 20% to 89% in males and 10% to 70% in females. In Laboratory B, the range of tumor incidence was 13% to 80% for males and 20% to 60% for females.

Neoplasms of the lung were much more frequent in males than in females. Lymphoreticular neoplasms were one of the most commonly observed and appeared to be more frequent in Laboratory B than Laboratory A studies. Liver neoplasms were considerably more frequent in males than in females. Tumors of the mammary glands, adrenals and thyroid were quite rare occurring in only a few studies at an incidence not exceeding 10%.

Study duration varied as shown below.

Duration (weeks)	Number of studies	
	Lab A	Lab B
90–100	10	4
101–108	12	5

TABLE I

PERCENT (%) INDICENCE OF NEOPLASMS IN CONTROL B6C3HF1 AND CD-1 MICE

Duration	Lab A B6C3HF1 90–108 weeks		Lab B B6C3HF1 91–105 weeks		MSD Studies CD-1 81–105 weeks	
	Male	Female	Male	Female	Male	Female
Number necropsied:	425	426	321	324	1232	1240
Total tumors range:	20–89	10–70	13–88	20–60	24–56	21–60
Average:	49	29	49	40	38	40
Number of groups:	22		9		24	
Lung						
Range – adenomas:	0–30	0–12	0–16	0–10	0–38	0–41
adenocarcinomas:	0–21	0–6	0–5	2	0–16	0–12
Average – adenomas:	8	1	6	3	17	14
adenocarcinomas:	5	1	2	1	5	3
Combined average:	13	2	8	4	22	17
Liver						
Range – adenomas:	0–42	0–6	0–6	0–5	0–12	0–14
adenocarcinomas:	0–37	0–5	0–35	0–10	0–8	0–6
Average – adenomas:	11	2	2	1	3	2
adenocarcinomas:	13	1	20	2	2	1
Combined average:	24	3	22	3	5	2
Lymphoreticular						
Range:	0–35	0–45	4–30	5–40	0–16	3–22
Average:	9	16	15	27	6	11

Overall tumor incidence, as well as tumors at sites of high incidence (liver, lymphoreticular) increased with study duration.

CD-1 mouse (Table I)

Lung tumors occurred at the highest incidence in both males and females. Lymphoreticular neoplasms were frequent, and at a somewhat higher incidence in females than males. Liver neoplasms were infrequent in both males and females. Overall tumor incidence was 38% in males and 40% in females.

F344 rat

The distribution of neoplasms for selected tumor sites is shown in Table II. Overall tumor incidence was quite high, 96% in males and 62% or 78% in females.

As is readily apparent, the most common tumors in males were benign testicular interstitial cell tumors and in females, pituitary tumors. Mammary tumors, principally adenomas, were frequent in females. Lymphoreticular neoplasms occurred in both males and females and at a higher incidence in Laboratory B.

Charles River-CD (Table II)

Charles River-CD rats also had a high incidence of tumors averaging 71% in males and 88% in females. Pituitary tumors, both adenomas and carcinomas, occurred commonly in both males and females. Mammary tumors were frequent in females. Liver and lymphoreticular tumors were infrequent.

DISCUSSION

Ward et al. [4] compiled spontaneous tumors in over 2500 control B6C3F1 mice of both sexes from National Cancer Institute carcinogenic studies. Laboratory variability was not analyzed. As seen below, the most common tumors were also lung, liver and lymphoreticular.

	Male (%)	Female (%)
Pulmonary	13	4
Lymphoreticular	8	17
Liver	22	4

Goodman et al. [5] also compiled tumor incidence in about 1800 control F344 rats from National Cancer Institute studies. The most common tumors observed were also interstitial cell tumors of the testis in males, mammary and pituitary tumors in females, and lymphoreticular tumors in both sexes.

	Male (%)	Female (%)
Testis	81	–
Mammary	1	18
Lymphoreticular	12	10
Pituitary	11	30

Compilation of published results in Charles River strains have shown good agreement with MSDRL results [6].

The average incidence of selected tumor types was compared in studies done at Laboratories A and B and MSDRL (Tables I and II).

There frequently was a greater variation in incidence between laboratories using the same strain than between different laboratories using unlike strains. For

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TABLE II

PERCENT (%) INCIDENCE OF NEOPLASMS IN CONTROL F344 AND CHARLES RIVER-CD RATS

	Lab A F344		Lab B F344		MSD Studies Charles River-CD	
Duration	102-107 weeks		104-106 weeks		98-128 weeks	
	Male	Female	Male	Female	Male	Female
Number of necropsied:	459	459	448	450	1211	1204
Total tumors range:	85-100	35-95	90-100	72-90	35-90	57-100
Average:	96	62	96	78	71	88
Number of groups:	23		9		23	
Liver						
Range - adenomas:	0-5	0-5	0-10	0-6	0-6	0-2
adenocarcinomas:	0-10	0	0-4	0-4	0-16	0-12
Average - adenomas:	1	1	3	1	1	1
adenocarcinomas:	2	0	1	1	5	2
Combined average:	3	1	4	2	6	2
Mammary gland						
Range - adenomas:	0-5	0-6	0-2	14-38	0-10	27-72
adenocarcinomas:	0-5	0-5	0-4	0-4	0-4	6-40
Average - adenomas:	1	13	1	24	3	49
adenocarcinomas:	1	1	1	2	1	20
Combined average:	1	14	1	26	4	69
Pituitary						
Range - adenomas:	0-65	5-90	2-14	28-48	16-62	32-90
adenocarcinomas:	0	0-10	0-2	0-2	0-10	0-16
Average - adenomas:	14	34	7	39	36	65
adenocarcinomas	0	1	1	1	2	5
Combined average:	14	35	8	39	38	70
Testis						
Range - benign:	0-100	-	78-92	-	> 0-20	-
malignant:	0-90	-	0-2	-		-
Average - benign:	80	-	86	-	> 7	
malignant:	8	-	0.2	-		-
Combined average:	88	-	86.2	-		-
Lymphoreticular						
Range:	0-30	0-20	14-46	6-32	0-12	0-6
Average:	11	9	26	16	4	3
Adrenal medulla						
Range:	0-15	0-10	6-26	0-8	0-20	0-7
Average:	8	2	17	3	9	2

Female Charles River CD-1 Mice
Bio/dynamics, Inc.

77-2064

LIVER

CONTROL DATA

STUDY I.D.	A		B		C		D		E		F		G	
	*	**	*	**	*	**	*	**	*	**	*	**	*	**
Tissue/Finding														
Liver - Neoplastic														
# Examined	34	48	57	58	44	47	56	59	60	59	60	60	60	60
(M)-hepatocellular carcinoma/adenocarcinoma	4	0	1	0	0	0	0	0	0	1	1	0	2	1
(B)-hepatocellular adenoma	2	3	4	5	1	0	2	2	1	1	1	1	0	0

* Control Group A
** Control Group B

Table 5. Q_1^* Potency Estimates for Alachlor Based on Rat Tumor Data

Nasal Turbinates					Turbinates, Stomach or Male Thyroid (Follicular Ad/Ca)			
Combining Sexes					Combining Sexes			
Males	Females	Program InPut	Geometric Mean	Study Data	Males	Females	Program Input	Geometric Mean
4×10^{-2}	1×10^{-2}	2×10^{-2}	2×10^{-2}	First Study	3×10^{-3}	2×10^{-2}	2×10^{-2}	2×10^{-2}
9×10^{-2}	7×10^{-2}	8×10^{-2}	8×10^{-2}	Second Study	1×10^{-1}	1×10^{-1}	1×10^{-1}	1×10^{-1}
5×10^{-2}	1×10^{-1}	6×10^{-2}	8×10^{-2}	Second Study without High Dose	1×10^{-1}	1×10^{-1}	9×10^{-2}	1×10^{-1}
6×10^{-2}	3×10^{-2}	4×10^{-2}	4×10^{-2}	Combined Studies	6×10^{-2}	4×10^{-2}	5×10^{-2}	5×10^{-2}
5×10^{-2}	2×10^{-2}	3×10^{-2}	4×10^{-2}	Combined without second study High-Dose	5×10^{-2}	4×10^{-2}	4×10^{-2}	5×10^{-2}

example, lymphoreticular neoplasms in female B6C3HF1 mice were almost twice as frequent in Laboratory B as in Laboratory A studies (27% vs. 16%). In MSDRL studies of CD-1 mice, the overall incidence of lymphoreticular neoplasms was 11%. The same pattern held for lymphoreticular tumors in male B6C3HF1 and CD-1 mice (9%, 15% and 6%) in Laboratory A, B, and Merck studies, respectively.

Overall tumor incidence was higher in female B6C3HF1 mice in Laboratory B studies than in Laboratory A studies (40% vs. 29%). Overall tumor incidence in male CD-1 MSDRL studies was 40%.

Adrenal medullary tumors on the average were twice as frequent in male Laboratory B F344 rats as in Laboratory A F344 rats (17% vs. 8%) compared to 9% in Merck CRCD rats. Lymphoreticular neoplasms were more frequent in both males and females in Laboratory B than Laboratory A studies (26% vs. 11% and 16% vs. 9%).

Tarone et al. [7] have recently reported on the variability in spontaneous tumor rates in two strains, F344 rats and B6C3HF1 mice. Data from 72 control F344 rat groups from six laboratories and 54 control B6C3HF1 mice from five laboratories were analyzed. The data were obtained from the NCI Carcinogenesis Bioassay Program. This group also found significant intralaboratory variation for certain tumor types for both the rat and mouse. Significant interlaboratory variability occurred in 2 of 6 laboratories for the F344 rat and 1 of 5 laboratories for the B6C3HF1 mouse.

The data presented in this report show that the outbred strains of Charles River-CD rat and Charles River CD1 mouse, as well as the F1 hybrid mouse (B6C3HF1), are commonly used in carcinogenic studies. Each strain has a relative pattern of tumor occurrence; testicular, pituitary and lymphoreticular neoplasms are common in the F344 rat, mammary and pituitary neoplasms are common in the Charles River-CD rat, and liver neoplasms are relatively uncommon in the CD-1 mouse. There is considerable variation in tumor incidence in individual studies regardless of strain and there frequently was greater variation in incidence between laboratories using the same strain that different laboratories using unlike strains. In recent years understanding of the relationship of spontaneous tumors to certain environmental factors including the type of bedding used, the type of cage, the presence of aflatoxin in the diet, etc. has improved [8]. The variation in spontaneous tumor incidence observed may be related to other environmental factors not clearly identified including wild viruses, stress, etc. [8, 9].

Whichever strain is selected, it is vital to develop sufficient historical tumor data on the strain used at the particular test laboratory. Gart et al. [10] and Ward et al. [4] have commented on the value of historic controls. Historic control information may call attention to tumor incidences that are unusually low or high, e.g., as a result of inadvertant environmental contamination or randomization error. Historic data may also 'indicate the degree of expected variability of spontaneous tumor types from study to study and allow more critical evaluation of the incidences in test animals.'

Tarone et al. [7] have recently pointed out that 'the most appropriate and important comparison of a treated group is with its matched control...when the comparison...leads to equivocal results, however, the historical control rats can sometimes provide data needed to make a clear interpretation of the results.'

REFERENCES

1 Draft of Preclinical Toxicologic Guidelines, Bureau of Drugs Health Protection Branch - Health and Welfare, Canada, October, 1979.

2 Notes for Guidelines on Carcinogenicity Testing on Medicinal Products, MAIL 24 (Medicines Act Information Letter), June, 1979.

3 M.B. Shimkin, in L. Goldberg (Ed.), Carcinogenic Testing of Chemicals, CRC Press, Cleveland, OH, 1974, p. 15.

4 J.M. Ward, D.G. Goodman, R.A. Squire, K.C. Chu and M.S. Linhart, Neoplastic and nonneoplastic lesions in aging C57BL/6N X C3H/HeN)F_1 (B6C3F1) Mice, J. Natl. Cancer Inst., 63 (1979) 849–854.

5 D.G. Goodman, J.M. Ward, R.A. Squire, K.C. Chu and M.S. Linhart, Neoplastic and nonneoplastic lesions in aging F344 rats, Toxicol. Appl. Pharmacol., 48 (1979) 237–248.

6 S.P. Sher, Tumors in control hamsters, rats, and mice: literature tabulation, Crit. Rev. Toxicol., (1981) in press.

7 R.E. Tarone, K.C. Chu and J.M. Ward, Variability in the rates of some common naturally occurring tumors in Fischer 344 rats and (C57BL. 6N × C3H/HeN)F_1 (B6C3F1) Mice, J. Nat. Cancer Inst., 66 (1981) 1175–1181.

8 F.J.C. Roe, Spontaneous tumors in rats and mice, Food Cosmet. Toxicol., 3 (1965) 707–720.

9 V. Riley, Psychoendocrine influences on immunocompetence and neoplasia, Science, 212 (1981) 1100–1109.

10 J.J. Gart, K.C. Chu and R.E. Tarone, Statistical issues in interpretation of chronic bioassay tests for carcinogenicity, J. Natl. Cancer Inst., 62 (1979) 957–974.

Appendix G.

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Aging Changes in CD®-1 HaM/ICR Mice Reared Under Standard Laboratory Conditions [1,2]

L. Hoenburger,[3] A. B. Russfield,[3,4] J. H. Weisburger,[3,4] S. Lim,[3,5] S. P. Chak,[3,5] and E. K. Weisburger [3,6]

SUMMARY—Three hundred CD®-1 HaM/ICR mice were observed for 2 years, and useful necropsies were done on 99 males and 102 females. Mortality was 50% at 16 months in the males and 18 months in the females. Among mice that came to autopsy, total tumor incidence was 54% for males and 75% for females, with most neoplasms occurring after 18 months. Adenomas or adenocarcinomas of the lung were the most frequent, followed by lymphoreticular tumors, vascular tumors, hepatomas, subcutaneous fibrosarcomas, and adenocarcinomas of the mammary glands. Some degree of amyloidosis was seen in half the mice of both sexes, beginning at 8 months in males and 12 months in females. Variability in tumor incidence among small groups of mice emphasized the need for adequate samples.—J Natl Cancer Inst 55: 37–45, 1975.

THE SO-CALLED SWISS MOUSE was descended from the 7 mice imported from Lausanne, Switzerland, by Clara Lynch in 1926; since then, several million offspring of these animals have been used in various experiments (*1*). Despite the intensive study to which various sublines of this stock have been subjected, information as to the natural history of these mice is fragmentary and scattered throughout the literature on virology, toxicology, and carcinogenesis. A paper on the incidence of spontaneous tumors in 1,000 consecutively necropsied mice from a breeding colony of CD®-1 HaM/ICR (Swiss-derived) mice describes only 38 animals over 20 months old (*2*). Spontaneous tumors were found in specific pathogen-free Swiss (Webster) mice used as controls for a carcinogenesis study terminated at 18 months (*3*), and in HaM/ICR mice used for lifetime carcinogenesis studies (*4*, *5*).[10]

This paper describes the natural history of CD®-1 HaM/ICR mice used as controls for a 2-year feeding study of potentially carcinogenic compounds. During this experience, several characteristics of the strain were noted which are relevant to the planning of future experiments.

MATERIALS AND METHODS

Six groups of 25 male and 25 female CD®-1 HaM/ICR mice (300 mice) were obtained when they were 4–8 weeks of age from the Charles River Breeding Laboratory in batches of 50 (25 males and 25 females) at the following times: October 1969, January 1970 (2 groups), March 1970 (2 groups), and June 1970. The study was begun 2–4 weeks later. The animals were randomized by weight and placed (5 per cage) in plastic cages measuring 7×11½×5 inches, with metal tops which included a food hopper. The San-I-Cel bedding was changed once a week when the cages were machine washed at 180° F. The mice were given Cambridge City tap water and powdered Purina meal ad libitum. For the first year, the diet was offered in a tunnel feeder of our own design which is practically spillproof and permits the measurement of food consumption. These animals served as controls in a study on the carcinogenicity of several compounds and were kept in the same animal rooms as the experimental mice. The temperature of the rooms was kept at 74±4° F with occasional wider differences in midsummer and winter. There were 8.2 to 8.5 air changes per hour.

The animals were inspected in the morning and again in the late afternoon every day, including weekends and holidays. During the first year, they were weighed individually at each weekly cage change, but during the second year they were weighed monthly. All animals that died after the first 6 months of the study were necropsied unless there was advanced autolysis or cannibalism. The tissues were fixed in neutral buffered formaldehyde or in Tellyesniczky's solution followed by 70% alcohol after 24-hour fixation. All urinary bladders were inflated with fixation fluid, later sectioned into two hemispheres, and examined under a ×10 dissecting microscope before being embedded. Hematoxylin and eosin-stained sections of these organs were made: liver, stomach, intestines, spleen, kidneys, adrenal glands, urinary bladder, ovaries or testes, uterus, lungs, and heart, plus all lesions observed grossly, regardless of site. Toward the end of the study, moribund animals were killed and processed when possible. However, some mice that appeared well in the late afternoon were in a state of advanced autolysis the next morning and often largely cannibalized. Animals still alive at the end of 2 years were killed; a few mice survived into the 25th month.

RESULTS

Food Consumption and Body Weights

The average food consumption remained fairly constant for the first 30 weeks of the experiment and amounted to 5.0 g per day for the males and 5.2 g for the females.

Weight curves for males and females are shown in text-figures 1 and 2. In both sexes there was good agreement of average weight among the 6 groups into which the mice were divided. Males were heavier, attaining an average weight of about 45 g, as compared with about 35 g for the females. An occasional male was exceptionally large, weighing well over 50 g.

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[2] Supported by Public Health Service contract NIH-NCI-E-68-1311 from the National Cancer Institute.

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[9] We gratefully acknowledge the cooperation of Dr. Gilbert Friedell, Chief of Pathology, St. Vincent Hospital, for pathologic observations.

[10] Recently, S. P. Sher (Toxicol Appl Pharmacol 30:357–359, 1974) tabulated tumors in Charles River control (CD-1, ICR/Ha) mice from six studies involving 1,076 males and 1,404 females. Tumor incidence varied from 7.6 to 64%. The survival times also varied or were not apparent from the table.

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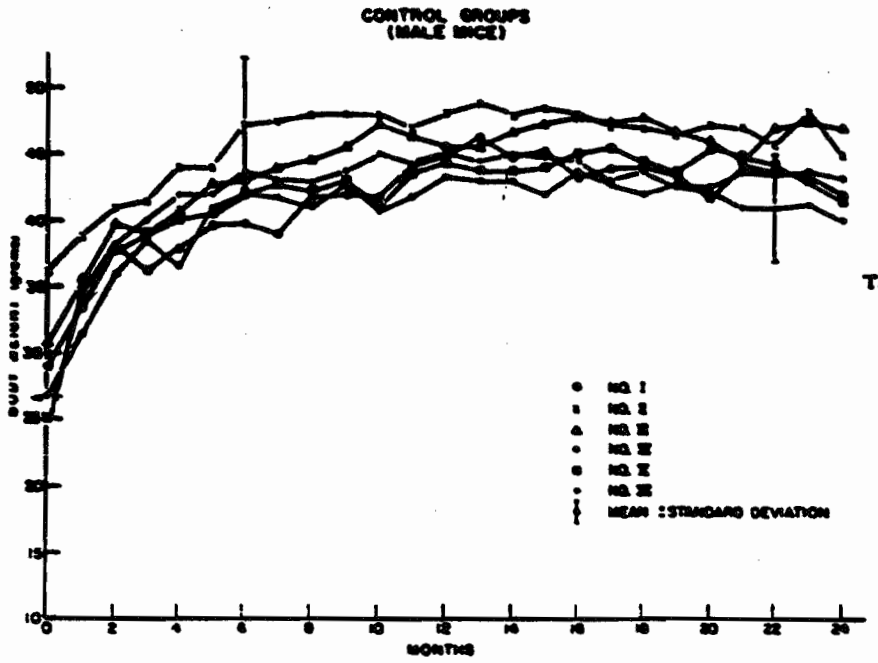
Survival

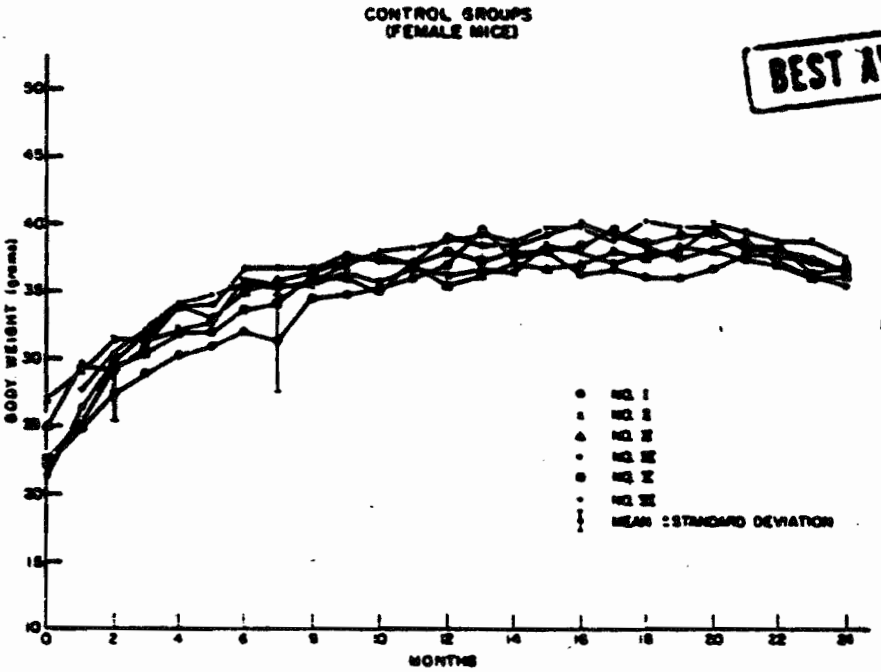
Survival curves for both sexes are shown in text-figures 3 and 4. During the first 6 months of the experiment, equal numbers of males and females died (11% of the initial group of 150). Of the mice dying after 6 months, 29% of the males and 21% of the females were lost through autolysis and/or cannibalism. Survival was somewhat better in females than in males, 50% mortality occurring at approximately 16 months in the males and 18 months in the females. At 24 months only 3% of the males and 18% of the females were still alive.

Tumors

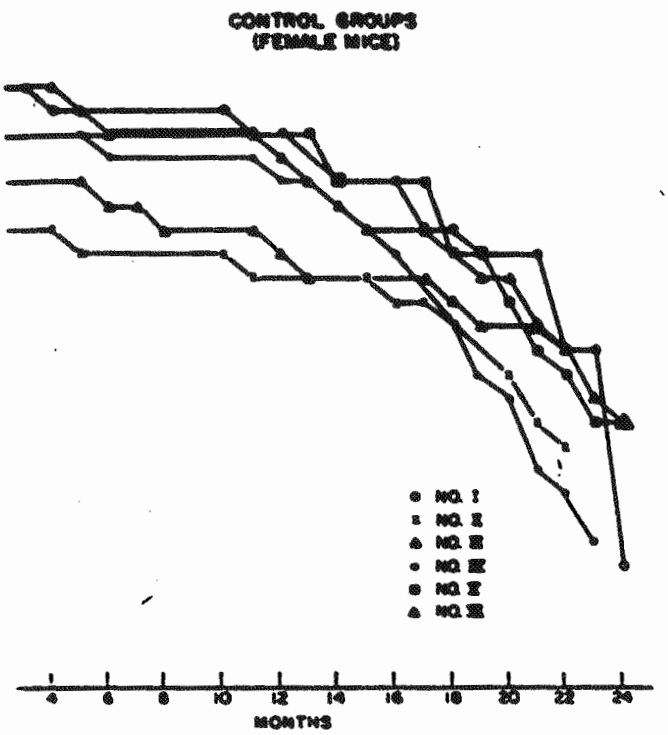
The 72 tumors occurring during the life-spans of the 99 effective males and the 99 tumors found in the 10[illegible] effective females are listed in table 1. Most common were adenomas or adenocarcinomas of the lung (6) and malignant lymphomas (7), both being slightly more fr[illegible]



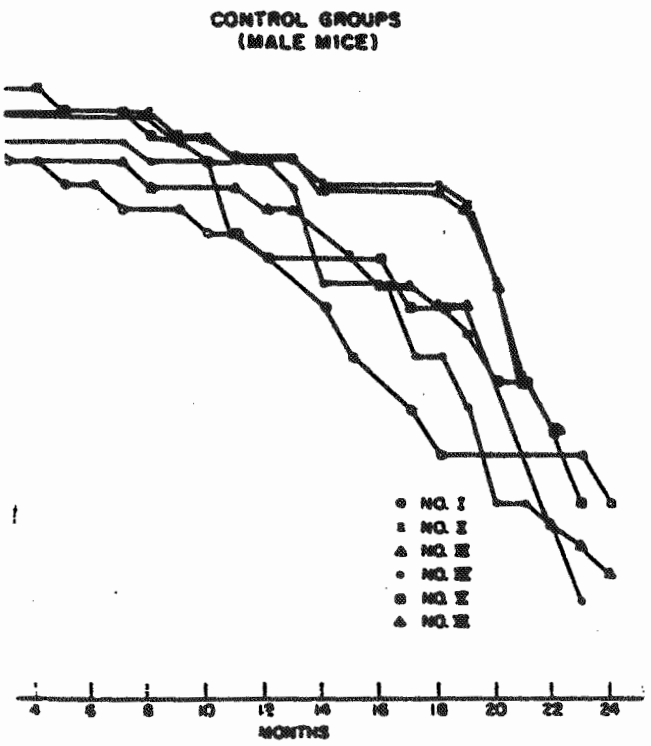
TEXT-FIGURE 1.—Body weight changes observed in 6 groups of untreated male controls.



TEXT-FIGURE 2.—Body weight changes observed in 6 groups of untreated female controls.



TEXT-FIGURE 3.—Survival curves: survival of animals of 6 untreated female control groups were plotted against time after beginning of study.



TEXT-FIGURE 4.—Survival curves: surviving animals of 6 untreated male control groups were plotted against time after beginning of study.

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ales. There were moderate numbers of he- subcutaneous fibrosarcomas (in males), f the breast (females), and vascular tumors tes (both sexes). Other tumors occurred

Most tumors occurred after the mice were 18 months old; only 16 tumors were found in males that age or less and 19 tumors in females. The principal tumors found before 18 months were adenomas of the lung, lymphosarcomas, and carcinomas of the breast (table 1).

TABLE 1.—*Tumors in CD®-1 Hall/ICR mice in relation to age*

Site or organ (tumor)	99 Males				102 Females			
	Total tumors		Tumors at 18 mo		Total tumors		Tumors at 18 mo	
	Number	Average age (mo)	Number	Ages (mo)	Number	Average age (mo)	Number	Ages (mo)
Lung								
Adenoma	19	21.8	2	9, 18	24	22.6	4	16, 17, 18, 18
Adenocarcinoma	5	22.8	—	—	8	22.4	1	16
Total	24	—	2	—	32	—	5	—
General*								
Lymphosarcoma	9	17.9	5	11, 14, 15, 16, 17	15	19.8	5	6, 13, 14, 17, 18
Reticulum cell sarcoma	6	20.7	2	14, 16	14	21.6	2	6, 14
Leukemia	2	20	1	18	3	18.7	1	13
Total	17	—	8	—	32	—	8	—
Liver								
Hepatoma	7	22.6	1	17	1	24	—	—
Hemangioma	2	24.5	—	—	1	13	1	13
Subcutaneous								
Fibroma	1	25	—	—	—	—	—	—
Fibrosarcoma	6	21	2	14, 16	1	16	1	16
Hemangioma	1	21	—	—	—	—	—	—
Breast								
Adenocarcinoma	—	—	—	—	6	17.8	3	12, 13, 15
Carcinosarcoma	—	—	—	—	1	22	—	—
Stomach								
Adenocarcinoma	3	24.7	—	—	4	24	—	—
Adenoacanthoma	1	22	—	—	—	—	—	—
Squamous papilloma	1	20	—	—	—	—	—	—
Adrenal gland								
Cortical adenoma	1	25	—	—	1	24	—	—
Pheochromocytoma	2	16	1	9	—	—	—	—
Uterus								
Adenocarcinoma	—	—	—	—	2	24.5	—	—
Adenoacanthoma	—	—	—	—	1	25	—	—
Leiomyoma	—	—	—	—	3	23.7	—	—
Leiomyosarcoma	—	—	—	—	1	25	—	—
Lymphangioma	—	—	—	—	1	22	—	—
Hemangioma	—	—	—	—	2	23	—	—
Hemangiosarcoma	—	—	—	—	1	25	—	—
Ovary								
Papillary cystadenoma	—	—	—	—	1	22	—	—
Luteoma	—	—	—	—	1	25	—	—
Hemangioma	—	—	—	—	3	22	—	—
Kidney								
Tubular adenoma	1	25	—	—	—	—	—	—
Bladder								
Transitional cell carcinoma	1	22	—	—	—	—	—	—
Spleen								
Hemangioma	2	20	1	18	2	23.5	—	—
Ear								
Sebaceous adenoma	1	8	1	8	—	—	—	—
Testis								
Interstitial cell adenoma	1	23	—	—	—	—	—	—
Pituitary								
Adenoma	—	—	—	—	1	25	—	—
Gastrointestinal tract								
Adenocarcinoma of undetermined origin	—	—	—	—	1	21	—	—
Totals	72	—	16	—	99	—	19	—

* The classification of lymphomas was made according to Dunn (7). No blood smears were made and the diagnosis of myeloid leukemia was based on histopathology of spleen, liver, and bone marrow. There was a preponderance of type-B reticulum cell sarcomas among females (75%); males had equal numbers of A and B type.

No unusual types of tumors were found, but 3 require comment. The 1 transitional cell carcinoma of the bladder occurred in a 22-month-old male, which also had a bladder calculus over 1 cm in diameter (fig. 1). Bladder calculi have been implicated in the pathogenesis of these lesions (*8*).

Two males (24 and 25 months old) and 1 female (25 months old) showed polyploid hyperplasia of the mucosa of the gastric fundus, with penetration of atypical glands through the muscularis mucosa (fig. 2). In 3 more males (average age, 24.7 months) and 4 females (average age, 24 months), atypical glands extended through the serosal layer of the stomach. The latter 7 lesions were classified as adenocarcinomas by the criteria of Rowlatt (*9*).

In the older females, the endometrium frequently showed severe cystic hyperplasia and occasionally adenomyosis. In the 2 females (24 and 25 months old) in which histologically dysplastic glands extended through the serosa, the lesion was classified as adenocarcinoma (fig. 3). One adenoacanthoma of the uterus was also found.

Tumors occurred in 54% of the total necropsied males and in 75% of the females (table 2). Multiple

...idence of tumors during life-span in groups of untreated CD®-1 HaM/ICR mice*

Group	Final No.		Percent with tumors		Average No. of tumors/mouse		Percent with multiple tumors		Percent lymphoma-leukemia		Percent lung tumors		Percent hepatomas		Percent vascular tumors		Percent other tumors	
	M	F	M	F	M	F	M	F	M	F	M	F	M	F	M	F	M	F
I	18	20	67	85	1.2	1.2	33	30	33	30	28	30	17	0	17	0	39	35
II	14	15	57	73	0.6	0.8	7	7	14	7	29	60	7	0	0	0	14	13
III	16	13	56	69	0.7	1.0	13	23	13	15	13	23	6	0	13	15	25	46
IV	14	15	50	67	0.7	0.9	14	20	14	13	28	33	0	7	14	13	14	20
V	19	17	21	65	0.2	0.8	0	17	5	35	5	24	5	0	0	6	5	17
VI	18	22	72	82	0.9	1.1	16	23	22	50	44	23	6	0	6	18	16	18
Total Average	99	102	54	73	0.7	1.0	14	21	17	31	24	31	7	1	5	9	19	25

* Initial number of mice in all groups was 25.

... were found in 14 and 21% of the males and females respectively. Except for two males, 17 and 18 months old and two females, 15 and 16 months old, all instances of multiple tumors occurred in mice over 20 months of age. Some neoplasms occurred as part of a multiple tumor syndrome more often than might be expected by chance. Thus 5 of the 7 fibrosarcomas, 5 of the 7 adenocarcinomas of the stomach, 6 of the 8 hepatomas, 3 of the 3 tumors of the endometrium, and 3 of the 4 tumors of the myometrium were associated with other neoplasms. In contrast, only 19 of the 56 lung tumors and 13 of the 49 lymphomas or leukemias were found with other tumors. However, the number of mice with multiple tumors was too small to permit final conclusions. An unusual association between lung tumors and malignant lymphomas was reported in other laboratories (*10*). In our material this association occurred in 7 mice, about the number expected by chance.

When tumor incidence was calculated for the 6 individual groups of mice, considerable variation between the small groups was noted (table 2). Thus the percentage of tumor-bearing males varied from 21 to 72%, multiple tumors in males from 0 to 33%, lung tumors from 7 to 44%, and hepatomas from 0 to 17%.

Amyloidosis

The most common abnormal finding in these animals, aside from neoplasms, was amyloid. The identity of this substance was confirmed by Congo red staining and birefringence with polarized light (*11*). Amyloid was found in 56 males (the youngest, 8 months old) and 54 females (the youngest, 12 months old). Most instances of amyloid occurred after 18 months in 44 of 56 males and 43 of 54 females. The sites are listed in table 3.

Renal amyloidosis was sometimes associated with papillary necrosis, mild hydronephrosis, and distention of the bladder (*12*). In 11 males and 11 females, severe amyloidosis was the only pathologic finding and appeared to have been the cause of death. Abnormalities associated with amyloid are shown in table 4.

Cardiovascular Disease

Anatomically demonstrable cardiovascular disease, comparatively rare in these animals, is summarized in table 5.

Adrenal Glands

In addition to the frequent deposition of amyloid and rare benign tumors, the adrenal glands often exhibited fibrous downgrowths of capsular cells into the cortex. This lesion may be the forerunner of hormone-secreting adrenal neoplasms in some strains of mice and has been designated A-cell hyperplasia (*13*). It occurred in 18% of the males and 56% of the females.

TABLE 3.—*Distribution of amyloid in CD®-1 HaM/ICR mice*

Site	56 Males		54 Females	
	Number	Percent	Number	Percent
Kidney	44	78	46	85
Small intestine	29	51	23	42
Liver	22	39	11	20
Testis	22	39	—	—
Ovary	—	—	18	33
Adrenal	19	34	22	41
Heart	17	30	7	13
Spleen	14	25	7	13
Stomach	12	21	9	17
Lung	4	7	1	2
Peritoneal fat	—	—	5	9
Uterus	—	—	2	4
Lymph node	1	2	—	—

Reproductive Organs

In males, active spermatogenesis was the rule, even in the oldest mice. Only 1 mouse (20 months old) showed total tubular atrophy. Two mice had focal tubular atrophy, 3 had Leydig cell hyperplasia, and 3 had blocked ducts with small spermatoceles; 11 had calcification of scattered tubules. In 2 mice, cells of the rete testis were hyperplastic; in 1 mouse, the seminal vesicles were extremely dilated.

TABLE 4.—*Abnormalities associated with amyloidosis in CD®-1 HaM/ICR mice*

Abnormalities	56 Males		54 Females	
	Number	Percent	Number	Percent
Neoplasms	28	50	38	70
Chronic pneumonitis	6	11	4	7
Intestinal worms	6	11	1	2
Healed pericarditis	1	2	—	—
Acute and chronic cystitis	1	2	—	—
Retroperitoneal abscess	1	2	—	—
Acute and chronic prostatitis	1	2	—	—
Focal necrosis, liver	1	2	—	—
No pathologic diagnosis	11	20	11	20

TABLE 5.—*Cardiovascular disease in CD®-1 HaM/ICR mice*

Site and lesion	99 Males		102 Females	
	Number	Age (mo)	Number	Age (mo)
Myocardium				
Fibrosis	4	21*, 21, 21, 24	2	16*, 24
Necrosis and mural thrombi	1	22	—	—
Coronary artery				
Calcification	1	24	—	—
Periarteritis				
Kidney	2	18, 21	—	
Intestine	1	21	—	—
Testis	1	24	—	—
Coronary artery	—	—	2	24, 24
Uterus	—	—	1	24

* Associated with pheochromocytoma.
* Associated with large necrotic carcinoma of the breast.

In contrast, most of the changes in older females suggested reproductive senescence. Follicular development was seen in only 16 mice, actual ova in 8. Only 9 mice (less than 1 year old) had corpora lutea present. In 48 mice, the bulk of the ovary was replaced by a large, simple, serous cyst. The endometrium in mice with cystic ovaries was characteristically stimulated, and a diagnosis of cystic endometrial hyperplasia was made in 39. Three had severe hydrouterus. In only 2 mice was the endometrium atrophic—in both, the ovaries were totally replaced by leukemic or lymphomatous infiltrate.

DISCUSSION

The body weights of our CD®-1 HaM/ICR mice are in the same range as those CD®-1 HaM/ICR mice bred in Schroeder's laboratory (*4*) and are somewhat higher than those of an outbred Swiss stock maintained at the Eppley Institute (*14*). Neither laboratory observed the marked sex differential seen by us.

Our survival figures are also in the same range as Schroeder's for CD®-1 HaM/ICR mice, i.e., 50% mortality in males at approximately 14 and 17 months of age in 2 male control groups and at 17 and 21 months in females (*4*). The normal life-span of this stock is too short to make it suitable for use in a 24-month feeding study. In the present study, as in Schroeder's, a little over 20% of mice were lost through autolysis, whereas a 10% loss was reported from Yale University in laboratory-bred CD®-1 HaM/ICR mice (*2*). Housing conditions, such as the weekly cage changing, could not explain the early deaths of these animals, because, in another experiment, at the time (76 wk) when half the females were dead, we recorded 90% survivors in 200 (BALB/c × A/J)F_1 (CAF_1) female mice kept under similar conditions; this has been our experience with several other studies involving CAF_1 and other mice. Fighting among males had no effect, since sex difference was not significant in mortality rates.

There seems little doubt that the tumor incidence in the present Swiss sublines is lower than that of the initial animals, in which Lynch found 44% lung tumors, 19% mammary tumors, and 11% leukemias in mice surviving over 4 months, and 70–80% lung tumors in animals living to 18 months (*1*).

Our figures for the total tumor incidence in CD®-1 HaM/ICR mice (54% in males, 75% in females) are somewhat higher than those reported by some other workers [16.3–26.8%, both sexes (*5*); 15.8%, both sexes (*2*)]. However, most of the tumors we found in the gastrointestinal tract, adrenal glands, and reproductive organs were not grossly apparent. These tissues were not always sectioned routinely by some of the other laboratories (*5*). The tumor spectrum and incidence found in our mice are similar to those reported for outbred Swiss mice maintained elsewhere (*10*, *14–16*), although one Swiss subline (Wisconsin) has a much higher incidence of leukemia (*17*). It is possible that the presence of carcinogenic aniline derivatives in the same room may have influenced the tumor spectrum of the controls. (This is unlikely, since the tumors induced in the experimental mice of our study by such compounds occurred with much higher doses than the controls could possibly have received through airborne contamination, and the nature of the induced tumors was different from the spontaneous types, consisting mostly of hepatomas and hemangiosarcomas, and occurred much earlier than spontaneous neoplasms.)

Two findings in our study have practical importance for future work in carcinogenesis. First, the many spontaneous tumors found in our mice made the detection of weak carcinogens difficult in a 2-year experiment. However, since most spontaneous tumors occurred after 18 months, this problem might have been obviated by a shorter experimental time. Second, the variability of tumor incidence within the initial 25-mouse control groups suggests the need for larger samples.

Our incidence of approximately 50% amyloidosis in CD®-1 HaM/ICR mice was higher than the 30% reported by Schroeder (*4*) and the 8.6% reported by Anderson (*18*). The lack of a sex difference and the occurrence of severe amyloidosis in many mice without other disease favor a genetic origin for the amyloid (*19*) rather than an environmental basis for the disease (*20*). It is known, however, that Swiss mice are easily susceptible to the induction of experimental secondary amyloidosis (*17*). Amyloidosis obviously must be considered when one uses these mice in long-term work, since it probably shortened the average life-span of our animals.

In a previous report on these mice (*2*), neither amyloidosis nor the presence of spontaneous cardiovascular disease was mentioned. However, in a recent publication on various strains of mice, Akamatsu and Barton (*21*) reported that this disease varied from 0 to 19% in control males and from 0 to 3% in females. In some strains the incidence was appreciably higher in 3-methylcholanthrene-treated animals than in controls, which indicated an association between treatment and the disease. Since amyloidosis and the presence of tumors can be life-threatening, the occurrence of this disease should be reported, since it influences the life expectancy of the animals.

The incidence of spontaneous cardiovascular disease in our mice is much lower than the 44% reported in old males of a specific pathogen-free outbred albino stock maintained at the Imperial Chemical Industries laboratories in England (*22*). This type of degeneration presented no problems in our experiments. The hyperplastic endometrium and myometrial changes seen in our old females suggest that this mouse stock might be useful in the investigation of spontaneous reproductive pathology.

It cannot be emphasized too strongly that this report reflects only the present situation, and that similarly labeled mice from the same source are capable of differen

behavior in a different environment at a different time. Mühlbock and van Ebbenhorst-Tengbergen's work (*23*) on the instability of characteristics in inbred strains of mice has led them to the conclusion that "Genetic changes in inbred strains cannot be prevented. Environmental changes may also occur over the years. The practical consequences of the instability of characteristics in inbred strains is that control experiments must always be done with the same inbred generation at the same time."

REFERENCES

(*1*) Lynch CJ: The so-called Swiss mouse. Lab Anim Care 19:214–220, 1969

(*2*) Percy DH, Jonas AM: Incidence of spontaneous tumors in CD-1 HaM/ICR mice. J Natl Cancer Inst 46:1045–1065, 1971

(*3*) Prejean JD, Peckham JC, Casey AE, et al: Spontaneous tumors in Sprague-Dawley rats and Swiss mice. Cancer Res 33:2768–2773, 1973

(*4*) Schroeder HA, Mitchener M: Scandium, chromium (VI), gallium, yttrium, rhodium, palladium, indium in mice. Effects on growth and life span. J Nutr 101:1431–1437, 1971

(*5*) Kanisawa M, Schroeder HA: Life term studies on the effects of arsenic, germanium, tin and vanadium on spontaneous tumors in mice. Cancer Res 27:1192–1195, 1967

(*6*) Stewart HL: Pulmonary tumors in mice. *In* Physiopathology of Cancer (Homburger F, ed.), 2d ed. New York, Paul B. Hoeber, 1959, pp 18–57

(*7*) Dunn TB: Normal and pathologic anatomy of the reticular tissue in laboratory mice, with a classification and discussion of neoplasms. J Natl Cancer Inst 14:1281–1433, 1954

(*8*) Wang WF, Curtis MR, Segaloff A: Strain differences in response to estrone and the induction of mammary gland, adrenal, and bladder cancer in rats. Cancer Res 13:147–152, 1953

(*9*) Rowlatt UF: Neoplasms of the alimentary canal of rats and mice. *In* Pathology of Laboratory Rats and Mice (Cochin E, Roe FJ, eds). Oxford, Blackwell Scientific Publications, 1967, pp 57–84

(*10*) Rustia M, Shubik P: Induction of lung tumors and malignant lymphomas in mice by metronidazole. J Natl Cancer Inst 48:721–729, 1972

(*11*) Cohen AS, Shirahama T: Animal model. Spontaneous and induced amyloidosis. Am J Pathol 68:441–444, 1972

(*12*) Babcock VI, Southam CM: Obstructive uropathy in laboratory mice. Proc Soc Exp Biol Med 120:580–581, 1965

(*13*) Dunn TB: Normal and pathologic anatomy of the adrenal gland of the mouse, including neoplasms. J Natl Cancer Inst 44:1323–1389, 1970

(*14*) Toth B: Lung tumor induction and inhibition of breast adenocarcinomas by hydrazine sulfate in mice. J Natl Cancer Inst 42:469–475, 1969

(*15*) Chieco-Bianchi L, De Benedictus G, Tassenti G, et al: Influence of age on susceptibility to liver carcinogenesis and skin initiating action by urethane in Swiss mice. Br J Cancer 17:672–680, 1963

(*16*) Nelson JB: Respiratory infections of rats and mice, with emphasis on indigenous mycoplasma. *In* Pathology of Laboratory Rats and Mice (Cochin E, Roe FJ, eds.). Oxford, Blackwell Scientific Publications, 1967, pp 259–294

(*17*) Cohen SM, Ertürk E, Bryan GT: Carcinogenicity of formic acid 2-[4-(5-nitro-2-furyl)-2-thiazolyl]-hydrazide in Swiss mice. Cancer Res 30:906–912, 1970

(*18*) Anderson RF: Disseminated amyloidosis in germ-free mice. Spontaneous prevalence, relationship to ionizing radiation and pathogenetic implications. Am J Pathol 68:43–50, 1971

(*19*) Cornelius EA: Amyloidosis and renal papillary necrosis in male hybrid mice. Am J Pathol 59:317–325, 1970

(*20*) Page DL, Glenner GG: Social interaction and wounding in the genesis of "spontaneous" murine amyloidosis. Am J Pathol 67:555–570, 1972

(*21*) Aramatsu Y, Barton BP: Neoplasms and amyloidosis in strains of mice treated with 3-methylcholanthrene. J Natl Cancer Inst 52:377–385, 1974

(*22*) Tucker MJ, Baker SB de C: Diseases of specific pathogen-free mice. *In* Pathology of Laboratory Rats and Mice (Cochin E, Roe FJ, eds.). Oxford, Blackwell Scientific Publications, 1967, pp 787–824

(*23*) Mühlbock O, van Ebbenhorst-Tengbergen WP: Instability of characteristics in inbred strains of mice. *In* Defining the Laboratory Animal. IV Symposium, International Committee on Laboratory Animals (Schneider HA, chairman). Washington D.C., National Academy of Sciences, 1971, pp 230–249

Appendix H.

007699

Bio/dynamics Inc.

Division of Biology and Safety Evaluation

CONTROL DATA FOR CD-1

COBS (ICR DERIVED) MICE

February 27, 1986

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Page_____ is not included in this copy.

Pages 263 through 267 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.

____ Description of quality control procedures.

____ Identity of the source of product ingredients.

____ Sales or other commercial/financial information.

____ A draft product label.

____ The product confidential statement of formula.

____ Information about a pending registration action.

✓ FIFRA registration data.

____ The document is a duplicate of page(s) _____.

____ The document is not responsive to the request.

The information not included is generally considered confidential by product registrants. If you have any questions, please contact the individual who prepared the response to your request.

Table 5. Q_1^* Potency Estimates for Alachlor Based on Rat Tumor Data

Nasal Turbinates					Turbinates, Stomach or Male Thyroid (Follicular Ad/Ca)			
Combining Sexes					Combining Sexes			
Males	Females	Program InPut	Geometric Mean	Study Data	Males	Females	Program Input	Geometric Mean
4×10^{-2}	1×10^{-2}	2×10^{-2}	2×10^{-2}	First Study	3×10^{-3}	2×10^{-2}	2×10^{-2}	2×10^{-2}
9×10^{-2}	7×10^{-2}	8×10^{-2}	8×10^{-2}	Second Study	1×10^{-1}	1×10^{-1}	1×10^{-1}	1×10^{-1}
5×10^{-2}	1×10^{-1}	6×10^{-2}	8×10^{-2}	Second Study without High Dose	1×10^{-1}	1×10^{-1}	9×10^{-2}	1×10^{-1}
6×10^{-2}	3×10^{-2}	4×10^{-2}	4×10^{-2}	Combined Studies	6×10^{-2}	4×10^{-2}	5×10^{-2}	5×10^{-2}
5×10^{-2}	2×10^{-2}	3×10^{-2}	4×10^{-2}	Combined without second study High-Dose	5×10^{-2}	4×10^{-2}	4×10^{-2}	5×10^{-2}

Appendix G.

6/29/86 007699

REVIEWER

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

JUN 23 '86

005205

OFFICE OF
PESTICIDES AND TOXIC SUBSTANCES

MEMORANDUM

Subject: Ames/Salmonella Mutagenicity Assay on [redacted] Metabolites
Submitted by Monsanto 11/25/85. Accession No: 260510.
Caswell # 11

From: Judith W. Hauswirth, Ph.D.
Mission Support Staff
Toxicology Branch/HED

To: Robert Taylor, PM #25
Registration Division

and

Vicki C. Walters
PM Team #25
Registration Division

Through: Reto Engler, Ph. D., Chief
Mission Support Staff
Toxicology Branch/HED

and

Theodore M. Farber, Ph. D., Chief
Toxicology Branch/HED

Action Requested:

Monsanto is requesting review of the final reports of Ames/Salmonella mutagenicity assays with seven synthesized alachlor metabolites and with bile and urine from alachlor treated Long-Evans rats.

Conclusions:

1. Five synthesized metabolites of alachlor, N-(methoxymethyl)-2-(methylsulfonyl)-2', 6'-diethylacetanilide, N-methoxymethyl)-2-(methylthio)-2', 6'-diethylacetanilide, N-(methoxymethyl)-2-(methylsulfinyl)-2', 6'-diethylacetanilide, 2-(methylsulfinyl)-2', 6'-diethylacetanilide and 2-(methylsulfonyl)-2', 6'-diethylacetanilide, were tested in the Ames/Salmonella mutagenicity assay. None of the five tested were mutagenic toward Salmonella typhimurium strains TA 98 and TA 100.

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2. Alachlor metabolite, N-2-ethyl-6-(1-hydroxyethyl)-phenyl-2-(methylsulfonyl)

acetamide, was mutagenic toward TA 100 both in the presence and absence of metabolic activation. Alachlor metabolite, N-2-ethyl-6-(1-acetoxyethyl)-phenyl-2-(methylsulfonyl)acetamide, was weakly mutagenic toward TA 100 in the presence of metabolic activation. Neither metabolite was mutagenic toward TA 98.

3. Urine from alachlor-treated rats produced a weak mutagenic response in TA 98 without metabolic activation but with beta-glucuronidase and in TA 1537 in the presence of both metabolic activation and beta-glucuronidase. In TA 100, this reviewer questions whether a positive response with urine from alachlor-treated rats wasn't masked due to histidine and other nutrients present in control (corn oil) urine. This is especially evident when the assay is run in the presence of both S-9 and beta-glucuronidase. [The authors claim histidine and other nutrients are responsible for the mutagenic response in both urine from alachlor and corn oil-treated rats].

Should the registrant want to pursue this further in light of the discussion above, this reviewer suggests that urine be concentrated and/or put through a column such as XAD-2 to remove histidine (Yamasaki and Ames. PNAS 74: 3555-3559, 1977) prior to being tested. Such testing could clarify the weak mutagenic responses seen in this study.

4. Bile from alachlor-treated rats did not induce a mutagenic response toward Salmonella strains TA 98, TA100, TA 1535 and TA 1537.

Classification: The studies discussed under Conclusions #1 and 2 above are incomplete in that only Salmonella strains TA 98 and 100 were used. At a minimum, in addition to TA 98 and TA 100, strains TA 1535 and TA 1537 should be routinely used in the Ames Salmonella assay. Otherwise these assays were adequately performed. The assays run with urine from alachlor-treated rats were inconclusive (see Conclusion #3 above). The assays using bile from alachlor-treated rats are acceptable.

3/27/84

007699

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

OFFICE OF
PESTICIDES AND TOXIC SUBSTANCES

MEMORANDUM

To: Michael McDavit, PM #61
Special Review Branch
Registration Division (TS-767C)

From: Judith W. Hauswirth, Ph.D. Judith W. Hauswirth 3/27/84
Mission Support Staff
Toxicology Branch
Hazard Evaluation Division (TS-769C)

Thru: Theodore M. Farber, Ph.D., Chief Theodore M. Farber 3/31/80
Toxicology Branch
Hazard Evaluation Division (TS-769C)

and

Reto Engler, Ph.D. Chief
Mission Support Staff
Toxicology Branch
Hazard Evaluation Division (TS-769C)

Subject: Monsanto's Rebuttal to the Alachlor PD-1

Action Requested:

Review of Monsanto's rebuttal to the alachlor PD-1. This memorandum addresses the following sections of Monsanto's rebuttal:

1. Section II. Hazard and Risk Evaluation
2. Section X. Appendix A. Rebuttal of the Presumption of Human Cancer Risk. (Part 1 through Part 4 and Part 6, including exhibits a-g.

Appendix D (Toxicity of Alternatives to Alachlor - Analysis of Available Information) will be addressed in a forthcoming memorandum from Toxicology Branch.

Monsanto Comment:

The [EPA] conclusion that alachlor is oncogenic in mice is not supportable.

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EPA Response:

The major basis for Monsanto's argument is the high spontaneous rate of lung tumors in CD-1 mice. They rely primarily on two literature references to support

1. F. Homburger, et al. Aging Changes in CD-1 (ICR/HaM) Mice Reared Under Standard Laboratory Conditions. J. Natl. Cancer Inst. 55(1):37-43, 1975;

2. S. P. Sher, et al. Spontaneous Tumors in Control F344 and Charles River-CD Rats and Charles River CD-1 and B6C3HF1 Mice. Toxicology Letters 11:103-110, 1982.

The Homburger data on lung tumors are given for 102 female CD-1 mice at different ages, at an average of 22.6 months for adenomas and 22.4 months for adenocarcinomas and at 18 months. From the data given, the difference between lung tumor incidence at 22 months and 18 months is quite striking. At an age of 22 months, 24 lung adenomas were reported as compared to only 4 adenomas at 18 months and for adenocarcinomas eight versus one, respectively. It is not clear from the report if data is reported for 102 mice at both time periods.

The Sher paper gives tumor incidence data for 1240 female CD-1 mice. According to the table in the paper the length of the studies was from 81 to 105 weeks. There were 24 groups of mice. The percentage incidence of adenomas ranged from 0-41% and of adenocarcinomas 0-12%. The average incidence was 14% for adenomas and 3% for adenocarcinomas.

In considering Monsanto's argument, Toxicology Branch also had available historical control data on this strain of mouse from the conducting contract laboratory (Bio/dynamics). Data on the incidence of alveologenic carcinomas and adenomas in female CD-1 mice were available on six studies with each study having two control groups. The average length of these studies was 24 months. the incidence of carcinomas was 70/690 or 10.1% and of adenomas 60/690 or 8.6%; the combined incidence was 130/690 or 18.7%. A comparison of this data with that obtained in the Monsanto alachlor oncogenicity study in CD-1 mice is found in the following table.

Lung Tumors (females only)

	adenomas	carcinomas	combined
Alachlor Study Dose Group			
control	2/50 (4%)	1/50 (2%)	3/50 (6%)
low	4/50 (8%)	1/50 (2%)	5/50 (10%)
mid	7/50 (14%)	1/50 (2%)	8/50 (16%)
high	10/50 (20%)	1/50 (2%)	11/50 (22%)
Bio/dynamics Control Data	60/690 (8.6%)	70/690 (10.1%)	130/690 (18.7%)
Range	0 - 8.4%	0 - 18%	2 - 23%

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The Toxicology Branch agrees that the control rate for lung tumors in the alachlor study appears low when compared to the contemporary historical control data from the contract laboratory; however, historical control data was supplied on studies which were conducted for an average of 6 months longer than the alachlor study. One of Monsanto's own references (Sher et al., see above) indicates that there is

Furthermore, in the Monsanto study there was an increased incidence of lung ($p<0.01$) tumors in alachlor treated female mice that died or were killed in extremis during the study.

	Lung Tumors in Female Mice that Died in extremis	Length of Time on Study (mos.)
Control	0/30	-
Low	1/17	17
Mid	3/27	17, 17, 18
High	7/35	13, 14, 15, 17, 17, 17, 18

The NTP (Ad Hoc Report, August 17, 1984) states that "primary reliance should be placed on the comparison of treated animals with concurrent, randomized controls for evaluation of commonly occurring tumors". They further state that "for example, the low rate in control animals could be due to a shift in the genetic makeup of the source animals or a change in diet that applied just as well to treated as to control animals. It is precisely because of such uncertainties that one uses a randomized design and places greatest weight on the comparison with concurrent randomized control".

Toxicology Branch cannot accept Monsanto's contention that alachlor is not oncogenic to female CD-1 mice for the reasons cited in the above discussion. The fact remains that (1) there was a statistically significant increase in the incidence of lung tumors in alachlor treated female mice ($p<0.05$) along with an increase in the number of lung tumors in female mice killed in extremis ($p<0.01$) and (2) the incidence of lung tumors in the high dose female mice was slightly above the average historical control rate from the conducting laboratory and was barely within the historical control range, despite the differences in the lengths of the studies (alachlor study, 18 months versus an average of 24 months for the contemporary studies).

Monsanto Comment:

There is substantial evidence that alachlor is not genotoxic or mutagenic.

EPA response:

The results of mutagenicity testing conducted on alachlor and found to be acceptable by the Toxicology Branch are summarized in the following table.

Test	Result	Comments
Ames assay	negative	a positive response was seen at 5000 ug/plate in TA 1535 but the response was not repeated for consecutive doses.
Gene mutation in CHO	-	

In vivo bone marrow chromosome	negative	no structural or numerical chromosomal aberrations
In vivo - in vitro hepatocyte DNA repair assay	positive	positive at highest dose tested (1.0g/kg), "weakly genotoxic"
DNA damage in B. subtilis M45 and H17	negative	did not cause DNA damage (20-20,000 ug/plate)

The following assays were requested in the Registration Standard and are still outstanding :

1. In vitro cytogenetic damage: both chromosomal aberration and SCE (in CHO cells or human lymphocytes or other rodent/human cell lines/strains test; and
2. Dominant lethal test in rats or mice.

The package of mutagenicity assays requested by the agency is not yet complete; however, the available data indictes that alachlor, itself, is not mutagenic but is "weakly genotoxic" in an in vivo - in vitro hepatocyte DNA repair assay.

A metabolite of alachlor, N-[2-ethyl-6-(1-hydroxyethyl)-phenyl]-N-(methoxymethyl)-2-(methylsulfonyl)acetamide, found in rat urine, was tested in the Ames salmonella assay and found to be positive both with and without metabolic activation.

Monsanto Comment:

Numerous studies demonstrate that alachlor does not produce mutagenic responses, thereby providing supportive evidence that alachlor is not a human carcinogen.

EPA Response:

The Toxicology Branch cannot concur with this statement and cites the Report of the NTP Ad Hoc Panel on Chemical Carcinogenesis Testng and Evaluation, August 17, 1984 in support of their opinion:

> .....because of the limitations of the present battery (for example, relative inability to detect non-initiating/non-mutagenic carcinogens, the fact that it is not known which endpoint(s) are critical in carcinogenesis, etc.), a lack of positive results, by itself, could not justify a conclusion that a positive animal test was a "false positive." Therefore, for purposes of interpreting animal data, negative short term test data do not by themselves provide a basis for discounting positive results from animal bioassays. As IARC has already done for purposes of classifying a chemical regarding carcinogenicity where animal data were limited, short term test positives can be regarded as support for a conclusion of potential human carcinogenicity based on experimental data. However, a positive bioassay cannot be invalidated or discounted by negative short term data alone.

Monsanto Comment:

The results of the rat test do not suggest human oncogenic effects because the rat is not an appropriate model for man in the case of alachlor.

Summay of Monsanto's argument to support their conclusion:

1. The rat is not an appropriate metabolic model for man in the case of alachlor. The monkey would be a better model.

2. The MTD was exceeded at the dosage level of alachlor that induced stomach and thyroid tumors, leaving only nasal turbinate tumors induced at lower dosage levels of alachlor.

3. The other tumor type possibly induced by alachlor at the highest dose tested was neuroepithelioma in brain. Evidence now suggests that these tumors were actually extensions of nasal turbinate adenocarcinomas.

4. Alachlor induces tumors only in one strain of rats. The induction of nasal turbinate tumors in Long-Evans rats is species specific and, therefore, has no relevance to man.
5. A study of the mortality records of workers who manufacture alachlor indicates that there was no association between cancer deaths and the manufacture of alachlor.

EPA Response:

On point #1:

The following differences in the metabolism of alachlor in the Sprague-Dawley rat and the monkey can be noted from studies submitted by Monsanto:

1. number of identifiable urinary metabolites: five in the monkey versus 14 in rat;
2. ratio of urinary to fecal recovered radioactivity: rat 1:1, monkey 9-10:1. Monsanto claims that this difference is due to the different molecular weight thresholds for biliary excretion in rats and monkeys (325±50 versus 475±50, respectively);
3. only two metabolites were common to both rat and monkey urine (two mercapturate conjugates);
4. sulfate conjugaton and side chain hydroxylation were not metabolic pathways identified for alachlor in the monkey (urine only); and
5. the half-life of elimination in the rat is 8.2 to 10.6 hours for the first phase and 5 to 16 days for the second phase, while the half-life in the monkey was calculated to be approximately 5 hours (considering both the first and second phases of elimination).

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In blood binding studies with alachlor, submitted by Monsanto in their rebuttal [illegible] #MSL-1498 and #4105 reviewed in the Review section, pp. 10-15, of this [illegible] the following was reported:

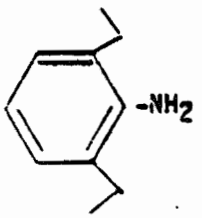
- a greater amount of ^{14}C-alachlor equivalents (alachlor + metabolites) are [illegible] rat hemoglobin as compared to mouse, monkey and human; and
- [illegible] preferentially forms a glutathione conjugate in the red blood [illegible]

While the Toxicology Branch would certainly agree that there are differences in the metabolism of alachlor by rat and monkey, the data that they have submitted to date do not convincingly support the rest of their argument.

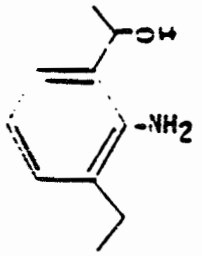
Although the threshold molecular weight for biliary excretion in human has not been adequately determined, it is estimated to be approximately 500 which is closer to the monkey (475) than the rat (325) (J. R. Gillette, in Drug Metabolism for Microbe to Man. D. V. Parke and R. L. Smith, eds. Taylor and Francis Ltd. London, 1977. P. 149). It should be noted, however, in man that 15% of a given dose of indomethacin (molecular weight = 358), 15% of a given dose of diazepam (molecular weight = 285) and 23-40% of a given dose of practolol (molecular weight = 264) were excreted in the bile (Douglas E. Rollins in Pharmacokinetic Basis for Drug Treatment. edited by L. A. Benet, et al. Raven Press, N.Y. 1984, pp. 77-78). Molecular weight plays a role in biliary excretion but other factors also influence it.

Although the Rhesus monkey is a good metabolic model for man for a series of arylacetic acids, amphetamine and isoniazid for example, the rat is a better model for man for oxisurn and 2-acetamidofluorene and as good as the Rhesus monkey for hydratropic acid and diphenylacetic acid (R. L. Smith and J. Caldwell in Drug Metabolism from Microbe to Man. D. V. Parke and R. smith, eds. Taylor and Francis Ltd. London, 1977 p. 149). Furthermore, the rates of elimination of drugs are generally lower in man than in experimental animals including the monkey (see D. S. Davies in Drug Metabolism from Microbes to Man. D. V. Parke and R. L. Smith, eds. Taylor and Francis Ltd. London, 1977. pp. 357-368).

In addition, alachlor metabolism in monkeys and man may be different: the identification of metabolites in the urine of monkeys indicated that only metabolites which contained the DEA moiety were present, while in the human biomonitoring studies, metabolites which contained the HEEA moiety were also present in urine at a level that required attention (i.e., DEA:HEEA was generally 4:1 but in one individual it was 1:2). Hence, the monkey may not be the best model for man and all available data from other animal species should be considered for extrapolation to man. The DEA and HEEA moieties refer to the following structures:



DEA



HEEA

The blood binding studies submitted by Monsanto in their rebuttal are certainly interesting but do not give any insight into the mechanism of cancer induction by alachlor in the Long-Evans rat. By their own admission, and the Toxicology Branch concurs, the toxicological significance of these studies is not known.

Monsanto also cites the results of four other studies to support their argument that the monkey is a better model for man than the rat. These include:

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1. Alachlor Pharmacokinetics and Metabolism in the [illegible]

2. *In Vitro* Metabolism of alachlor by Liver and Kidney Homogenates

3. Alachlor Metabolism in CD-1 Mice

4. Animal Whole-Body Autoradiography Studies.

Only summaries of these studies were submitted in the rebuttal and, therefore, it is impossible at this time to adequately evaluate Monsanto's conclusions that were based on the data generated. Monsanto claims that full reports of these studies will be submitted by May 1986. Each of these studies is briefly discussed on page of this memorandum.

On point #2:

The Toxicology Branch would agree that the MTD was exceeded at the highest dose (126 mg/kg/day) of alachlor in the Long-Evans rat; however, nasal turbinate tumors were induced at lower levels of alachlor and one mixed carcinoma-sarcoma stomach tumor was induced in a male rat of the 42 mg/kg/day dosage level. The one stomach tumor at this lower level is considered alachlor related because of its unusual etiology.

Monsanto's argument that nasal turbinate tumors induced by alachlor is species specific since no neoplasic lesions were seen in a one year beagle dog study (even though nasal and stomach tumors can be induced in the dog) cannot be accepted by the Toxicology Branch. To make such a conclusion from a dog study of such short duration is not scientifically sound.

The argument that nasal turbinate tumors were not induced in the CD-1 mouse also cannot be accepted since it is quite common for a chemical carcinogen to induce tumors at different sites in different rodent species. Data from the entire NTP carcinogenesis testing program supports this conclusion.

On Point #3:

Monsanto submitted an addendum to the special chronic feeding study (MSL-3492) in Long-Evans rats that contains a reevaluation of the brain tumors (neuroepitheliomas) seen in this study. The only level of alachlor administered in the feed was equivalent to 126 mg/kg/day. Three brain tumors were reported, two in female rats and one in males. Reevaluation, according to the report, indicated that these tumors were extensions of nasal adenocarcinomas and not brain tumors. However, several discrepencies between the original report of this study and the addendum need to be resolved prior to accepting the conclusion reached in the addendum, that alachlor does not induce neuroepitheliomas.

On Point #4:

The Toxicology Branch cannot agree that alachlor causes tumors in only one strain of rats. Alachlor has only been tested for oncogenicity in one strain of rats and we maintain that alachlor is also oncogenic in female CD-1 mice (see the discussion of the mouse study on pp. 1-3 of this memorandum).

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On Point #5:

to alachlor and cancer deaths is not sufficient to conclude that there is "no association between deaths from cancer and the manufacture of alachlor" or that alachlor is not a human carcinogen. Death records on alachlor workers, which were used by Monsanto to reach their conclusion, are not always reliable for determining the underlying cause of death. Frequently only the immediate cause of death is given. Only a well-designed epidemiology study on alachlor workers could generate the information necessary to make such a decision.

Conclusion:

After considering Monsanto's rebuttal to the PD-1, Toxicology Branch still concludes that alachlor is probable human carcinogen.

Appendix A Part 6 Exhibit a.

This report contains a summary of a study entitled "Alachlor Pharmacokinetics and Metabolism in the Long-Evans Rat". The full study report has not been submitted. Toxicology cannot comment on the adequacy of this study without a full report. Since chronic toxicity/oncogenicity studies were conducted in the Long-Evans rat, submission of this report could be important in the interpretation of the results of these long term studies.

Core Classification: Invalid - summary report without adequate identification of the test material. This classification may be changed upon submission and review of the final report.

Appendix A Part 6 Exhibit b.

This report contains a summary of a study entitled "In Vitro Metabolism of Alachlor by Liver and Kidney Homogenates". The full study report has not been submitted. From the results of the study the following was concluded by Monsanto:

> The results from in vitro incubations with liver and kidney preparations from rats, mice, and monkeys showed that alachlor can be metabolized by GST and cyt P-450 enzymes in all three species. In general, monkeys showed less enzymatic activity toward alachlor than rats and mice. The glutathione conjugate was further metabolized by enzymes which revealed distinctive differences between rats and monkeys. Prior alachlor administration is expected to have inductive effects on the activities of key liver enzymes responsible for its degradation.

Without the full report Toxicology cannot assess the adequacy of this study or comment on Monsanto's conclusions.

Core Classification: Invalid - summary report without adequate identification of the test material. This classification may be changed upon submission and review of the final report.

Appendix A Part 6 Exhibit c.

This report contains a summary of a study entitled "Alachlor Metabolism in CD-1 Mice". The full study report has not been submitted. Toxicology cannot comment on the adequacy of this study without a full report. Since an oncogenicity study was conducted the CD-1 mouse, submission of this report could be important in the interpretation of the results of this long term study in relation to Monsanto's rebuttal position.

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Monsanto concluded that the data "from this mouse study revealed rapid and extensive metabolism of alachlor and excretion primarily in the feces. The higher proportion of fecal metabolites and the lack of significant blood binding differed markedly from results with the Sprague-Dawley rat. The half-life for elimination of alachlor following oral dosing was calculated to be approximately one hour

the test material. This classification may be changed upon submission and review of the final report.

Appendix A Part 6 Exhibit d.

This report contains a summary of a study entitled "Animal Whole-Body Autoradiography Studies". The full study report has not been submitted. Toxicology cannot comment on the adequacy of this study without a full report. From the results of the study Monsanto claims that the most radioactivity was found in the nasal turbinates of rats, less in those of mice and none in the nasal turbinates of monkeys.

Core Classification: Invalid - summary report without adequate identification of the test material. This classification may be changed upon submission and review of the final report.

Appendix A Part 6 Exhibit e.

Study Title: A Mechanistic Study of the Interaction of Alachlor with Blood. Part I. Distribution of Alachlor in Blood Components after Oral and Dermal Dosing in the Rat.

Conducted By: Monsanto Company
Environmental Health Laboratory

Report No.: MSL-4498

Job/Project No.: 830179/MSL-84-020

Study Date: January 29, 1985

Authors: W. P. Ridley and J. Warren

Purpose of Study: According to the report, the purpose was as follows:

1. To provide a time course profile of the distribution of alachlor or its metabolites between plasma and cellular components of the blood following oral and dermal administration. Other organs and tissues will not be examined;
2. To determine the nature of the macromolecules in the blood associated with alachlor and/or its metabolites;
3. To fractionate the macromolecules in the blood associated with alachlor residues and determine the active regions of interaction; and
4. To characterize, and to the extent possible, identify and quantify significant metabolites associated with macromolecular components in the blood.

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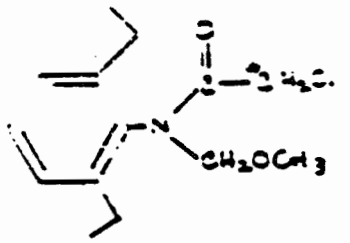
Test Material: ^{14}C and ^{13}C labelled alachlor. Alachlor was uniformally labelled in the phenyl ring and was enriched with ^{13}C at the C-2 carbon at the level of 90%. ^{14}C-Alachlor was at least 98% pure, chemically and radiochemically. ^{13}C-Alachlor was at least 98% pure chemically and unlabelled alachlor was greater than 98% pure.

Structure:



* Position of ^{13}C

Materials and Methods: See Appendix A.

Animals: Male, Long-Evans rats (7-10 weeks old)

Groups of Animals:

	No. of Rats	Rt. of Admin.	Sp. Act. (dpm/umole x 10^{-7})	Dose of Alachlor (mg/kg)
Grp. 1*	3	single, oral**	1.2419	7.33
Grp. 2*	3	single, oral	0.04173	755
Grp. 3	5	single, oral	1.2090	7.44
Grp. 4	5	single, oral	0.04159	780
Grp. 5	5	single, dermal	1.2552	8.04[1]
Grp. 6	5	single, dermal	0.04122	852[2]
Grp. 7	6	10 daily, oral	0.02172	219

*preliminary study groups only to develop techniques
**by gavage
[1]equivalent to 0.32 mg per cm^2 skin
[2]equivalent to 32.6 mg per cm^2 skin

Collection of Blood and Urine:

	Collection Time After Dosing (hr.)		
	Urine	Feces*	Blood
Grp. 1,2	6,12,24,48	6,12,24,48	1,3,6,24,48
Grp. 3-6	24,48,72	24,48,72	1,6,24,48,72
Grp. 7	24 hr intervals	24 hr intervals	24 hr intervals

*Although fecal samples were taken, they were not analyzed for radioactivity in this study.

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Vehicle: oral - corn oil
dermal - Lasso EC formulation without alachlor

Results: The following table summarizes the data obtained at terminal sacrifice.

Alachlor Equivalents

Grp. #	3	4	5	6	7
Route of Admin.	oral	oral	dermal	dermal	oral
Dose (mg/kg)	7.44	780	3.04	852	10 x 219
Whole Blood (ppm)[1]	1.39	178	0.93	59.3	231
Blood plasma(ppm)[1]	0.135	19.8	0.236	14.3	14.6
Blood cells (ppm)[1]	5.61	681	2.76	203	554
% Dose in Urine	37.1	34.2	6.18	5.31	33.3
Est. % dose in[2] Blood	1.20	1.44	0.68	0.42	0.71

[1]To correct for different dosage levels given, ppm values were divided by the actual individual animal dose (mg/kg b.wt.)
[2]assuming 6.7% of the final body weight is blood.

Overall the amount of alachlor equivalents (alachlor plus metabolites) found was less for whole blood, blood plasma, blood cells and urine obtained from dermally treated rats as compared to orally treated rats. The one exception is the level of alachlor equivalents found in blood plasma for Groups 3 and 5 (0.135, oral low dose versus 0.236, dermal low dose).

The values obtained for the uptake and elimination of alachlor equivalents over time was plotted for each group. These figures, as presented by Monsanto, can be found in Appendix B.

Conclusions: The results of this study indicate that alachlor is absorbed more rapidly into the circulatory system orally than it is dermally.

Core Classification: Supplementary. This study was not required for the registration/continued registration of alachlor as outlined in "Guidance for the Registration of Pesticide Products Containing Alachlor as the Active Ingredient" E.P.A., O.P.P., November 20, 1984 and, since human dermal absorption, i.e. biomonitoring, data on alachlor is available, is of questionable value in supporting Monsanto's rebuttal to the PD-1.

Appendix A Part 6 Exhibit f.

Study Title: A Mechanistic Study of Interaction of Alachlor with Blood. Part II. Characterization of Alachlor Residues Associated with Blood after Oral Administration and In Vitro Interspecies of Alachlor Interaction with Blood.

Conducted By: Monsanto Company

Report No.: MSL-1105

Job/Project No.: 7824

Study Date: October, 1984

Author: K. S. Anderson

Purpose of Study: According to the report the purpose of the study was as follows:

1. To provide a time course profile of alachlor distribution between blood plasma and cellular components after oral and dermal administration of alachlor;
2. To determine the nature of the macromolecules associated with alachlor and/or its metabolites;
3. To fractionate the macromolecule associated with alachlor and determine the active regions of interaction;
4. To characterize, and to the extent possible, identify, and quantify, significant metabolites associated with macromolecular components;
5. To conduct in vitro incubations of alachlor and rat blood. The distribution between plasma and cellular components will be determined and, if feasible, alachlor residues associated with macromolecular components will be characterized and compared with results from in vivo studies; and
6. To conduct an interspecies comparison of blood binding with alachlor in vitro.

Materials and Methods: The same rats (Grps. 1-7) used in Part I of this study were also used for this part of the study. The Materials and Methods Section for this study is similar to that of Part I (see Appendix A). Additional methods used in this part can be found in Appendix C.

Results:

1. The values obtained for the uptake and elimination of alachlor equivalents (alachlor plus its metabolites) over time was plotted for each group. These figures, as presented by Monsanto, can be found in Appendix B (they were also included in Part I of this study). The highest level of alachlor equivalents was found in red blood cells followed by blood plasma and finally whole blood. Approximately equivalent doses of alachlor were absorbed more rapidly orally than dermally into the circulatory system.

2. The majority of the radioactivity in red blood cells was found to be associated with the soluble hemoglobin fraction (see Appendix D, Figure 9). Hemoglobin was fractionated into its alpha- and beta-subunits. The majority of the radioactivity was found associated with the beta-subunit. The report states that this result "is not surprising since it has previously been reported that a reactive cysteine residue is located in the 125 position of the beta-subunit of rat hemoglobin" (Hughes, et al. Biochem. J. 199: 61, 1981) and the sponsor suspects an alachlor-cysteine linkage as depicted in Figure 9 (Appendix D).

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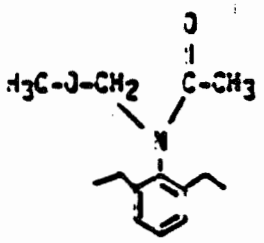
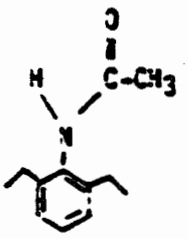
3. Trypsin digestion and treatment with Raney nickel (a desulfurization catalyst) was used to release bound radioactivity from the protein of hemoglobin of rats from Group 7. Three metabolites were positively identified [illegible]

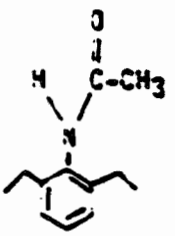
below.



II

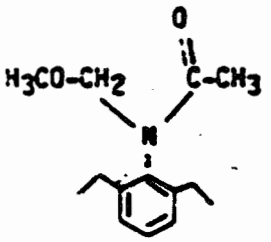


III



IV

A fourth metabolite was also identified whose structure was thought to be:



V

A ring hydroxylated metabolite of alachlor was not identified in a Sprague-Dawley metabolism study nor in three different Rhesus monkey metabolism studies (three routes of administration, I.M., I.V. and dermal).

The predominate metabolite isolated was IV. The ratio of II:III:IV:V was 1:1:3:1.

4. When ^{14}C-alachlor was incubated with whole blood, the isolated hemoglobin contained 58.61% of the radioactivity. The only metabolite isolated after trypsin digestion and Raney nickel reduction was metabolite II as shown above.

When the incubation was carried out in the presence of iodoacetamide, a reagent which reacts with sulfhydryl groups, very little binding of ^{14}C-alachlor to hemoglobin took place. The report states that this is "further evidence for the involvement of a cysteine residue in alachlor-hemoglobin interaction".

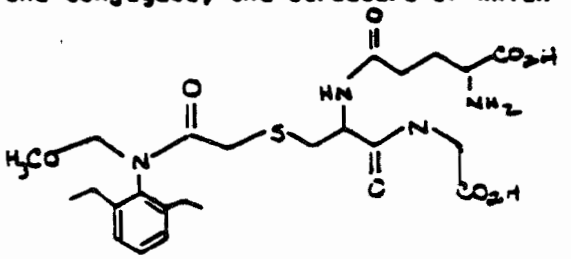
Comparable results were obtained using red blood cells instead of whole blood in the incubation. There was little difference found when using either Sprague-Dawley or Long-Evans rats.

5. The differences in binding of ^{14}C-alachlor equivalents to blood components was determined for humans, monkeys, rats and mice. These results are found in the following table.

Alachlor Equivalents

Blood Fraction	Rat		Mouse		Monkey		Human	
	Incubation Time (hrs.)							
	0.5	24	0.5	24	0.5	24	0.5	24
	% of ^{14}C activity							
Plasma	21.29	19.45	66.80	58.32	56.60	45.50	62.79	60.94
Hexane extract of plasma	0.1	0.14	1.49	0.88	8.70	1.80	5.92	0.61
Saline washes	10.75	19.79	26.60	23.36	30.50	28.40	28.01	20.34
Sol. hemoglobin fraction	58.61	55.28	4.67	15.93	3.40	19.80	2.69	15.80
Cellular pellet	8.95	5.34	0.44	1.51	1.00	4.60	0.59	2.31

The greatest amount of radioactivity was, by far, associated with rat soluble hemoglobin fraction. Mouse, monkey and human soluble hemoglobin fraction contained comparable levels of radioactivity which increased with time (0.5 hour values versus 24 hour values). When the soluble hemoglobin fraction was further fractionated with trichloroacetic (TCA) acid treatment, 35% of the radioactivity remained in the supernatant and 65% in the protein precipitate. The radioactivity in the soluble fraction by comparison of HPLC retention times was identified as an alachlor-glutathione conjugate, the structure of which is shown below.



VI

In the rat, essentially no radioactivity was found in the TCA supernatant.

Conclusions:

1. Several metabolites of alachlor bind to the beta-subunit of rat hemoglobin, possibly through a reaction with cysteine on the subunit (structures II-V as shown above). 285

2. A greater amount of ^{14}C-alachlor equivalents is bound to rat hemoglobin as compared to mouse, monkey and human.

3. Alachlor forms a glutathione conjugate in the red blood cells of mouse, monkey and human (metabolite VI as shown above).

4. The toxicological significance of these results is not known by Monsanto's own admission and, therefore, the results do not support their contention that the rat is not a good model for man in the case of alachlor toxicity/oncogenicity.

Core Classification: Supplementary. This was a research project designed to answer questions concerning the binding of alachlor to blood components. It was not designed to fulfill any requirements for registration/continued registration of alachlor.

Appendix A Part 6 exhibit g.

Study Title: The Metabolites of Alachlor in Monkey Urine Obtained from Dermal Penetration Studies.

Report No.: MSL-3386

Job/Project No.: 7824

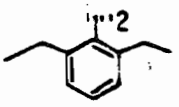
Study Date: February, 1984

This study has been previously submitted (1/5/82) and reviewed in a Toxicology memorandum dated 10/18/85. The reviewer's conclusions were as follows:

"In this study three major metabolites were identified in monkey's urine: two mercapturic acid conjugates and one conjugate of thioacetic acid. The three metabolites were clearly identified as DEA derivatives, and they apparently were detected at a similar ratio in both urine samples collected from the intramuscular test and the dermal test. The average total percentages of these metabolites (three monkeys in each test) are 69.87 ± 4.30% and 61.70 ± 1.55% in the intramuscular and dermal test, respectively.

It is not clear if these percentages refer to the level of radioactivity in the analyzed urine samples or refer to the total amount of the dose recovered in urine (71.4% and 15.6% of the administered dosages in the intramuscular study and the dermal study, respectively). In the absence of this information, it is not possible to verify these findings.

Although this reviewer agrees with the registrant that the major metabolites in the monkey's urine in both the intramuscular test and the dermal test are conjugates of metabolites which contain the DEA moiety, several issues presented in the discusston section of this review compromise the quantitative data obtained from this study. This study remains classed as Core Suoplementary (pilot study)".



DEA moiety of Alachlor

Page_____ is not included in this copy.

Pages 387 through 394 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.

____ Description of quality control procedures.

____ Identity of the source of product ingredients.

____ Sales or other commercial/financial information.

____ A draft product label.

____ The product confidential statement of formula.

____ Information about a pending registration action.

__✓__ FIFRA registration data.

____ The document is a duplicate of page(s) _____.

____ The document is not responsive to the request.

The information not included is generally considered confidential by product registrants. If you have any questions, please contact the individual who prepared the response to your request.

Newspaper Clipping

3/16/89

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NOTE TO: Addressees

RE: Additional Alar/Food Safety Information

Attached are the latest pieces of information on the Alar/food safety issue. They are 1) a joint EPA, FDA, USDA press release, and 2) an "Update on Alar on Apples", from the Food and Nutrition Service, USDA. Copies of these documents are being sent to ASTHO, NASDA, AAPCO, SFIREG, State Extension Pesticide Coordinators, and EPA Regional Division Directors and Branch Chiefs.

Steve

Steve Johnson

Attachments

Addressees:

Doug Campt
OPP Division Directors
Bill Jordan
Reto Engler
Judith Hauswirth
Edward Zagler
Walt Waldrop
Patricia Roberts
Richard Levy
Robert Tomerlin

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United States
Environmental Protection
Agency

Office of
Public Affairs (A-107)
Washington DC 20460

EPA

Environmental News

FOR RELEASE: THURSDAY, MARCH 16, 1989

The following statement is being issued jointly by the three federal agencies noted below.

Dr. Frank E. Young
Commissioner
Food and Drug Administration

Dr. John Moore
Acting Deputy Administrator
Environmental Protection Agency

John Bode
Assistant Secretary for Food and Consumer Services
U.S. Department of Agriculture

"In the last few weeks there has been a growing public controversy over the potential harmful effects of a chemical called Alar, which is used by apple growers to retain the crispness of their fruit as it goes to market. It is used primarily in the growing of Delicious, Staymen, and McIntosh apples.

"The federal government believes that it is safe for Americans to eat apples, and the responsible federal agencies are working together to reassure the public of this fact.

"Recently, the Natural Resources Defense Council (NRDC) has claimed that children face a massive public health problem from pesticide residues in food. Data used by NRDC, which claims cancer risks from Alar are 100 times higher than the Environmental Protection Agency (EPA) estimates, were rejected in 1985 by an independent scientific advisory board created by Congress. Alar has been used for decades in apple growing, and it has been the subject of many studies on possible harmful side effects.

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(more)

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"A recent progress report on preliminary results from an ongoing study shows that a breakdown product of Alar caused certain kinds of tumors in mice. Based on this report, EPA has begun the process to phase-out Alar in apple growing if the final data, which will be independently reviewed, demonstrate a need for cancellation. Cancellation could then occur by July 1990. EPA believes the potential risk from Alar is not of sufficient certainty and magnitude to require immediate suspension of the use of this chemical. EPA and others have pointed to lack of scientific validity in the suggestion by the NRDC that the risk is much greater than has been stated by EPA. The Food and Drug Administration (FDA) of the Department of Health and Human Services, the agency responsible for monitoring pesticide residues in food, has found either no residues or residues that are far below EPA's tolerance. Both FDA and EPA believe that Alar use over this interim period is safe and does not pose a health risk to the American public. Available data show overwhelmingly that apples carry very small amounts of Alar. In addition its use has decreased dramatically over the past several years; estimates are that 95 percent of the apple crop was not treated in 1988.

"It should also be noted that risk estimates for Alar and other pesticides based on animal testing are rough and are not precise predictions of human disease. Because of conservative assumptions used by EPA, actual risks may be lower or even zero.

"The FDA, EPA and the U.S. Department of Agriculture believe there is not an imminent hazard posed to children in the consumption of apples at this time, despite claims to the contrary.

"Therefore, the federal government encourages school systems and others responsible for the diets of children to continue to serve apples and other nutritious fruit to American children.

"This is an issue that will continue to be monitored closely by the responsible federal agencies that have acted in the past to cancel pesticide uses which pose a cancer risk."

For more information contact Al Heier at 202-382-4374.

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# # #

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United States Department of Agriculture

Food and Nutrition Service

3101 Park Center Drive
Alexandria, VA 22302

Final

Reply to Attn. of:

MAR 15 1989

Subject: Update on Alar on Apples

To: Regional Administrators
All Regions

This is to provide you with the latest information available to us on the issues raised by the Natural Resources Defense Council (NRDC) and the recent media attention regarding the treatment of apples with the chemical daminozide (trade name Alar). This supplements information already provided your Program Directors and Public Information Staff.

Apples are a nutritious food; there is no reason to stop eating apples or apple products. While the Government cannot assure that any food is absolutely free of risk, on the basis of current information we believe apples to be safe. We do not advocate the removal of apples or apple products from school lunch menus.

We have consulted with appropriate officials in the Food and Drug Administration (FDA) and the Environmental Protection Agency (EPA). As you know, EPA is responsible for determining whether chemicals like Alar can be legally used and in what amounts.

EPA has indicated that the NRDC assertion "seriously misleads the public." EPA also indicated "NRDC's estimates of risk posed by pesticide residues in food are far out of line with existing data."

Please pass this information on to your State agencies immediately so that they can be as responsive as possible to questions and concerns raised by local school districts and others. In addition, State agencies should be directed to provide the information to their local school districts.

G. Scott Dunn

G. SCOTT DUNN
Acting Administrator

In a first public notice of a delisting petition filed in June by A.L. Laboratories, EPA said bacitracin is one of 40 it is proposing for deletion from the list (See Nov. 12, 1986, Page 3).

Setting a comment deadline of Jan. 7, EPA said: "Evaluation of readily available literature indicates that although bacitracin may induce two types of adverse health effects, the likelihood of any such effects resulting from an exposure to a release of bacitracin into the environment is extremely remote."

■

REVIEW BOARD URGES AGRICULTURE CANADA TO REINSTATE ALACHLOR

Restoration of alachlor's registration has been recommended to Canada's Agriculture Minister, John Wise, by the Alachlor Review Board (See Oct. 14, Page 2).

The recommendation added that the board "recognizes that there may be some potential risk associated with the use of both alachlor and metolachlor. Accordingly, the board also recommends the following:

"1. As potential substitutes for alachlor and metolachlor are evaluated for registration purposes, they should be carefully compared with alachlor and metolchlor. The registrations of alachlor and metolachlor should then be reviewed to ascertain if they remain acceptable in light of the potential availability of a substitute which may not be an animal carcinogen.

"2. In any event, the entire toxicological data base for chloracetanilide herbicides should be reviewed within five years to determine whether continued registration of alachlor and metolachlor is acceptable in light of any new toxicological information on this class of compounds that may be available at that time.

"3. The registration of alachlor and metolachlor should be reviewed annually within the above five-year period, if any relevant new information becomes available.

"4. Application rates should be examined with the aim of lowering the recommended application rates while maintaining adequate weed control, particularly on highly permeable soils and near surface water.

"5. The development of improved methods for formulating and packaging alachlor and metolachlor products, in order to minimize applicator exposure, should be encouraged.

"6. Applicator safety programs should be conducted each spring to provide farmers with instruction in the proper use of chloracetanilide products, including container disposal and the use of protective clothing."

The board's recommendations were based on the following eight conclusions:

"1. Alachlor is an animal carcinogen and should be considered to be a potential human carcinogen for regulatory purposes.

"2. The primary substitute for alachlor, metolachlor, is also an animal carcinogen and should be considered to be a potential human carcinogen for regulatory purposes.

"3. There is no valid scientific basis for concluding that either of the two chemicals, alachlor or metolachlor, is a more potent carcinogen, in rats or in humans, than the other.

"4. Alachlor and metolachlor are the only two chloracetanilide herbicides registered for use on corn and soybean crops in Canada. The continued availability of at least one member of this class of herbicides is essential if corn and soybean production in Canada is to remain economically viable and internationally competitive.

"5. If either alachlor or metolachlor is removed from the market, relatively minor aggregate economic impacts will result from the near monopoly situation. Adverse economic effects to particular farmers, in particular circumstances, however, may be significant.

"6. Reasonable worst case estimates of applicator exposure are 1,000 to 10,000 times lower (three to four orders of magnitude) than the lowest dose at which a tumour was observed in one long-term rat feeding trial. In this situation, the board considers that this is a reasonable margin of safety.

"7. Reasonable worst case estimates of potential public exposure through drinking water are lower than applicator exposure estimates. In this situation, the board considers that this is a reasonable margin of safety.

"8. Estimates of applicator and public exposure to metolachlor are similar and at least as high as estimates of exposure to alachlor, using similar assumptions."

The five-member board was chaired by B. Barry Shapiro, a former Senior Judge of the District Court of Ontario.

The report noted that Health and Welfare Canada identified alachlor risks and that officials in Agriculture Canada had the responsibility of "advising the Minister as to whether these identified risks were acceptable. This involved the dual function of identifying the benefits of having alachlor remain on the market and balancing the benefits and the risks to determine acceptability. Agriculture Canada officials also had the responsibility of communicating both the identified benefits and the risks ... and the results of the balancing exercise to the Minister for his ultimate decision." It continued:

> "As far as could be determined by the board, these functions of Agriculture Canada were not carried out, or at least were not documented, with the same degree of comprehensive effort that had been exhibited by Health and Welfare. The evaluation of the benefits associated with alachlor use was based on the assumption that metolachlor was a safer alternative and did not, in the board's view, adequately convey to the Minister the extent of the benefits associated with having alachlor on the market.
>
> "Perhaps more importantly, Agriculture Canada officials appear to have accepted from the outset that the matter would be determined by the recommendation from Health and Welfare Canada. There is no evidence that anyone at Agriculture Canada ever objected to Health and Welfare officials reaching the conclusion that the risk was unacceptable. In several documents Health and Welfare officials drew conclusions about agronomic issues that should have been the preserve of Agriculture Canada.... It

appears that Agriculture Canada adopted too passive a posture in the decision-making process."

The report concluded that data were insufficient to determine whether alachlor or metolachlor was a more potent carcinogen in rats or humans and that if exposure patterns of the two pesticides are similar, "the risk to humans should be considered similar."

It further concluded that both public and applicator exposure to metolachlor is at least as high if not higher than alachlor.

According to the report, "If alachlor and metolachlor are both removed from the Canadian market, the likely outcome would be serious adverse impacts on domestic corn and soybean production. These sectors generated over $684 million in farm receipts in 1986."

In its findings, the board stated, "At the present time there is no scientifically established way to extrapolate the relative carcinogenic potential of two or more compounds to humans from animal data. Thus, while it is known that both alachlor and metolachlor induce cancer in rats, it is not possible to infer their relative potencies in humans. The board considers that there is presently no valid scientific basis for concluding that either alachlor or metolachlor is safer than the other, from a human perspective."

The board further found, "Cancelling its registration has eliminated exposure to alachlor. However, it has resulted in a substantial increase in the use of metolachlor, the primary substitute. Since there is no basis for assuming that metolachlor is safer, and because exposure patterns are at least comparable, it cannot be concluded that the cancellation of alachlor has improved public safety."

■

FOOD EXECUTIVE URGES INDUSTRY TO HELP MEDIA UNDERSTAND ISSUES

Declaring that "issues relating to pesticides are increasingly driven by the media rather than sound science," a high-ranking Quaker Oats Company executive called on the food industry to help the media understand and report on scientific issues "better than they have in the past, particularly with reporting of risk assessment calculations."

Luther C. McKinney, Senior Vice-President, Law and Corporate Affairs, for the Chicago-based corporation, was the opening speaker Nov. 17 at a conference on pesticide regulation sponsored by the Forum on Pesticide Residues, which is supported by 27 trade associations. The two-day conference was held in Washington, D.C. (See Oct. 21, Page 25).

"It is not in the public interest to create unwarranted cancer fears," McKinney said, "with reports on assessments that are so conservative as to have only a remote chance of representing a true risk."

He added, "The controversy caused last May by the National Academy of Sciences' (NAS) report on pesticide regulation points out the need to recognize the public tendency to misinterpret worse-case risk estimates. I agree with Dr. Sanford Miller's (former FDA official) comment after the NAS report was released. He said, as you'll recall, that it is 'scientifically immoral' to report risk assessments without taking steps to insure they're correctly understood," (See July 29, Page 10, and Oct. 21, Page 8).

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