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

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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.
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TO: Robert Taylor, PM#21
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THRU: Laurence D. Chitlik, DABT
Section Head, Section V
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and
William L. Burnam, Chief
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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.

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 (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 interference 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 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.

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 to 070590). The study was performed by Bio/dynamics Inc. (BD-77-421), reviewed on 6/16/82 and classified as 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 noted in this study was triggered by epichlorohydrin. Thus, Monsanto replaced epichlorohydrin [redacted] 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 Uelmer

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 [redacted] Lot #MULT 0417B. The compound, an orange/amber solid with a low melting point, was received from Monsanto Company on 4/25/80.

Dosage Tested: 0, 0.5, 2.5 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 126 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% RH) 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.

Ophthalmic examinations were performed approximately every three months using slit-lamp biomicroscopy. The ophthalmoscopic examinations on month 13 and 24 of the study (9/18/81 and 8/20/82) were performed by Dr. Lionel F. Rubin (D.V.M.).

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

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

Laboratory Studies:

The Laboratory tests were not performed at regular intervals during the study as usually required, because the initial objective of this study was to investigate the ocular lesions which were seen in this strain of rats in a previous chronic feeding/oncogenicity study with alachlor (#BD-77-421, reviewed 6/16/82). However, a decision was later made in 1981 during the performance of the study to also examine the animals for oncogenic effects. Thus, blood chemistry tests and hematological evaluations in this study were only performed at termination.

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

"Hematology

Erythrocytes (RBC)
Leukocytes (WBC)
Platelets
Hematocrit
Hemoglobin
Mean Corpuscular Volume
Mean Corpuscular Hemoglobin
Mean Corpuscular Hemoglobin Concentration

Clinical Chemistry

Albumin
Total Protein
Blood Urea Nitrogen
Total Bilirubin
Direct Bilirubin
Glucose
Glutamate Pyruvate Transaminase
Alkaline Phosphatase
Glutamate Oxaloacetate Transaminase
Lactate Dehydrogenase
Creatinine
Cholesterol
Calcium
Phosphorus
Chloride
Sodium
Potassium"

Necropsy:

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):

"aorta
adrenals (2)
femur (with marrow)
mesenteric and submandibular lymph nodes
sciatic nerve
pituitary
stomach
thyroid with parathyroid
esophagus
ovaries (2)
testes with epididymides
seminal vesicles
rectus femoris muscle
skin
spleen
urinary bladder
uterus
kidney
liver
lung
mammary gland
salivary gland
pancreas
spinal cord
trachea
heart
brain
nose
eyes with optic nerve
duodenum
jejunum
ileum
large intestine
thymus (when present)
growths
masses or tumors
abnormal lesions"

The eyes were preserved in buffered 2% glutaraldehyde and 10% formalin. Some adrenals with tumors were preserved in 5% glutaraldehyde then 'mordanted with potassium dichromate to reveal 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	Males Mid	Males High	Females Low	Females Mid	Females 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 submitted 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 -13.5
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	-9.3

*% Difference = $\frac{\text{analytical value - target value}}{\text{target value}}$ x 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 dehydrated, 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*

Doasge 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.

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 (2/29 mid dose males and 1/23 high dose males were affected).

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.

Histopathology

Non-Neoplastic Lesions

The following microscopic lesions were noted in this study and appeared to be compound-related.

No. of Animals Affected/No. of Animals Examined

	Males				Females			
No. Organ	Control	Low	Mid	High	Control	Low	Mid	High
Brain	50	50	50	49	49	49	49	48
°Compression atrophy	6	5	0	11	23	28	30	21
Nose	45	48	45	44	42	44	47	48
°Submucosal gland hyperplasia	2	1	3	21**	2	5	5	11**
°Inflammation of nasal passages	4	4	4	9	3	7	2	11**
Heart	50	50	50	50	50	50	50	49
°Myocardial Fibrosis/Scar	1	2	0	1	0	2	4	6**
Kidney	100	100	100	100	100	100	100	100
°Mononuclear infil.	74	87	78	86	50	64	58	71**
°Tubular sclerosis	23	25	24	23	2	9	8	14**
Adrenal	50	50	50	50	49	50	50	49
°Cortical Telangiectasis	0	2	1	5**	29	21	18	27

**$p < 0.01$

Neoplastic Lesions

The incidences of tumor bearing rats in the treatment groups were similar to the control group with the exception of the low and mid dose male groups where a slightly lower incidence rate was noted as compared to the control male group, see table below:

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)

The above two summary tumor incidence tables do not reflect a strong oncogenic potential for Alachlor. However, the following table represents the incidence of individual neoplastic lesions, some of which appear to be compound related in this study:

No. of Animals Affected/No. of Animals Examined

	Males				Females			
Organ	Control	Low	Mid	High	Control	Low	Mid	High
Nose	45	48	45	45	42	44	47	48
°Epithelial adenoma	0	0	0	11**	0	0	1	9**
°Submucosal gland adenoma	0	0	1	0	0	0	0	0
Thymus	19	50	48	50	48	50	49	47
°Lymphosarcoma	0	0	1	0	0	1	2	3*
Adrenal	50	50	50	50	49	50	50	49
°Phenochromocytoma	8	7	2	6	1	1	3	5*
Thyroid	49	50	49	49	49	49	49	47
°C-cell adenoma	5	3	7	6	2	2	4	3
carcinoma	0	0	1	0	0	0	0	0
°Follicular adenoma	2	4	3	4	1	1	0	2
carcinoma	1	0	1	2	3	1	1	1
Uterus					50	50	50	48
°Benign mucosal polpys	–	–	–	–	4	4	4	5
°Malignant mucosal polpys	–	–	–	–	1	0	1	4
Mammary Gland	50	1	1	1	50	48	50	45
°Adenoma	0	1	1	0	19	23	23	20
°Carcinoma	–	–	–	–	4	3	6	0
Stomach	50	50	50	49	50	50	50	48
°Adenocarcinoma	0	0	1	0	0	0	0	0

*$p < 0.05$ **$p < 0.01$

As noted above, the most statistically significant tumor incidences in this study are the nasal turbinate tumors in both males and females of the high dose group; the thymus tumors and the adrenal tumors in the high dose females.

The stomach tumor in the mid dose male is a rare tumor and is considered biologically significant in this study because in an older study (#BD-77-421), this kind of tumor occurred at a statistically significant rate at much higher dosage levels.

Discussion:

1. In a previous study (#BD-77-421, reviewed on 6/16/82), one specific type of tumor, nasal turbinate tumors, was noted to occur only in the alachlor treated rats. In the study under review at the present time (ML-80-186) this kind of tumor appeared to be most significant in the Long-Evans strain of rats based upon daily exposure to alachlor in feed.

The incidence of the nasal turbinate tumors in this study (#ML-80-186) is much higher than in the previous study (#BD-77-421). Thus, a summary of the incidence of nasal turbinate tumors at a higher dosage level, 126 mg/kg/day (study #ML-80-224), was also forwarded for consideration in this review in order to provide a comprehensive comparison with the previous data in study #BD-77-421.

The following table reflects the incidence of nasal turbinate tumors in the above mentiond 3 studies:

Total Number of Animals Bearing Nasal Turbinate Tumors
(Nasal Respiratory Epithelium Tumors)

Study No.	Dosage mg/kg/day	No. of Animals Affected Males		No. of Animals Affected Females		Total No. of Animals Affected/ Total No. of Animals Examined	
		Adenoma	Carcinoma	Adenoma	Carcinoma	Males	Females
ML-80-186	0.0	0	0	0	0	0/50	0/50
(present study)	0.5	0	0	0	0	0/50	0/50
	2.5	0	0	1	0	0/50	1/47
	15.0	11	0	9	0	11/44[a]	9/46
ML-80-224							
(to be submitted)	126.0	42	7	11	2	49/61	13/25
BD-77-421	0.0	0	0	0	0	0/46	0/49
(previous study)	14.0	0	0	0	0	0/46	0/47
	42.0	10	1	4	1	11/41	5/45
	126.0	23	0	10	0	23/42	10/48

a: The author indicated that the number of animals examined in this group was 45, however the tissues from one additional animal were autolysed.

Note: Both studies #ML-80-186 and #ML-80-224 used [redacted] technical alachlor instead of epichlorohydrin which was used in study #BD-77-421. The registrant then suspected that epichlorohydrin, a known carcinogen was the major trigger of the oncogenicity noted in study #BD-77-421. Thus, the registrant decided to alter the protocol of the then ongoing study #ML-80-186/224 (part I and II) to include an oncogenic assessment for alachlor.

Apparently the above kind of tumor is not reversible because study #ML-80-224 included a group of animals (not listed in the above table) which was exposed to alachlor at 126 mg/kg/day for only 5 to 6 months then the animals were fed untreated diet for the remainder of the two-year study period. The incidence of nasal turbinate tumors in this group was as follows (see also appendix 1 to this review):

Study No.	Dosage mg/kg/day	No. of Animals Affected Males		No. of Animals Affected Females		Total No. of Animals Affected/ No. of Animals Examined	
		Adenoma	Carcinoma	Adenoma	Carcinoma	Males	Females
ML-80-224	126	10	0	19	1	10/17	20/46

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**/44	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 7/21 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 (molting 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 #BD-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 (molting 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.

L. K.

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 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 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:AMahfouz:4-5-84:card #9

"References:

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Appendix #1

LASSO®

Summary of Nasal Tumor Incidence

(EHL-800219, ML-80-224)

The study was originally intended to reproduce the ocular lesions induced by alachlor in an earlier study (BD-77-421). It was designed to determine the time of onset, progression of clinical signs and possible recovery following cessation of exposure. Groups of 100 male and female Long Evans rats were fed diets intended to provide 126 mg/kg/day of alachlor. At the end of the study (approximately 25 months) the animals were partitioned into three groups based upon the duration of alachlor administration and their fate during the study.

Group I -- Rats were maintained on alachlor treated diets until spontaneous death or terminal sacrifice at the end of the study. Animals which died spontaneously prior to month 9 are not included. This group contained 31 females and 70 males.

Group II -- Rats were maintained on alachlor treated diets until spontaneous death or sacrifice prior to month 9. This group contained 20 females and 10 males. No nasal tissues were examined for these animals.

Group III -- Rats were maintained on alachlor treated diets for five months or six months (depending on ocular status) and were then maintained on basal diet until death or terminal sacrifice. This group contained 49 females and 20 males.

Nasal Turbinate Tumors

Number with Tumor per Number Examined

	Group I		Group III	
	Male	Female	Male	Female
Adenoma	42/61 (69%)	11/25 (44%)	10/17 (59%)	19/46 (41%)
Adenocarcinoma	7/61 (11%)	2/25 (8%)	0/17 (0%)	1/46 (2%)

It is apparent from these data that continuous exposure for less than six months is sufficient to eventually induce nasal tumors in Long Evans rats at a dosage level of 126 mg/kg/day. Full lifetime exposure at a dosage of 15 mg/kg/day induces a lower incidence of nasal tumors, while lifetime dosages of 0.5 to 2.5 mg/kg/day are essentially tumor free.

Monsanto 27

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November 10, 1983

Missing from Monsanto's copy

Director
Registration Division (TS767C)
Office of Pesticide Programs
U. S. Environmental Protection Agency
1921 Jefferson Davis Highway
Crystal Mall #2, Room 716D
Arlington, VA 22202

Attention: Mr. Robert J. Taylor
Product Manager (25)

Dear Sir:

Last week EPA inquired about the status of an ongoing chronic feeding/oncogenicity study in rats with alachlor. On a number of occasions we have indicated to the Agency that such a study was being conducted.

The purpose of this letter is to provide a detailed factual account of the status of this study and to respond to the questions that have been raised.

1. The study was initiated on August 20, 1980. As initially designed, the study consisted of two parts or two separate studies. The objective of study ML-80-224 was to assess possible onset and recovery from eye lesions which had been observed previously and reported in the Bio/dynamics chronic rat study, BDN-77-421. The second study, ML-80-186 was designed to determine a NOEL for these ocular effects.

   Study ML-80-224 consisted of 100 male and 100 female rats which were fed a dietary level of 126 mg/kg/day. In addition, there was an untreated control group of six animals of each sex. The protocol called for periodic eye examinations and distribution of the animals into subgroups with and without treated diet during the course of the study to assess onset and recovery from the ocular disease.

   Study ML-80-186 consisted of 50 animals/sex/group which were fed 0, 0.5, 2.5, or 15 mg/kg/day alachlor treated diet for about 25 months. These levels constituted a titration of the low-dose level (14 mg/kg/day) of the Bio/dynamics study, BD-77-421.

2. On July 8, 1981, the protocol for study ML-80-224 was amended to provide a better overall assessment of oncogenicity. All treated animals which were scheduled to be taken off treated diets were kept on treated diet. Other changes were made to upgrade the study to a standard oncogenicity study.

3. The final sacrifice for ML-80-186 was completed on October 4, 1982. The final sacrifice for ML-80-224 was completed on October 5, 1982.

4. Histopathology for ML-80-186 was completed on September 26, 1983. Histopathology for ML-80-224 was completed in early October, 1983.

5. The laboratory is currently in the process of evaluating the data and writing the final report narrative for study ML-80-186. Study ML-80-224 is not as far along. No additional analysis or data development is taking place at this time. Other than the scheduled opthalmoscopic examinations conducted by Dr. Lionel Rubin, no consultants, no special slide reading, and no protracted deliberations have been involved.

6. The conduct, analysis of the data, and preparation of the final report of these studies has required more time than the normal chronic rat study for several reasons. First, as indicated above, these studies are not typical chronic rodent studies. Combined, they consist of over 600 animals in four treatment groups and controls, whereas, the typical chronic/oncogenic rat study consists of about 400 animals. The special features of the onset recovery of study ML-80-224 will make analysis and interpretation of the data more complicated.

In summary, these studies are complicated by study design and numbers of animals involved. We are endeavoring to complete these studies and submit them to the Agency as soon as possible. Our target date for submission of these reports is early 1984.

This letter, in the opinion of Monsanto Company, includes confidential or proprietary information. We request, therefore, that the Agency handle it accordingly.

If you should have any questions, please contact our Washington office or me.

Very truly yours,

Robert W. Street

/ms

cc: L. L. Gingerich