8

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

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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).

•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 (812 to 813 days) for males and from 4/12/78 through 4/21 to 24/80 (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#XHI-167 (92.6% a.i.), stabilized with 0.5% epichlorohydrin, was used from 4/12/78 to 3/6/79; and Lot#MHK-6 (92.19% a.i.), stabilized [redacted] was used from 3/7/79 to termination.

INERT INGREDIENT INFORMATION IS NOT INCLUDED

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	42	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 7 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 wer available ad libitum.

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 115 (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.

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 oxaloacetic 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

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]

arenals (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

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

lungs with mainstem bronchi and trachea
lynph 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 Hardenian 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 $p < 0.05$.

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	Weeks 2 to 4 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	17	27
42	18	32
126	19	20

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.

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 ophthalmoscopic 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 diminition 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]		Week 106		Week 115	
		No.[b]	%	No.	%	No.	%
0	Males	0/44	0	0/37	0	0/28	0
	Females	0/45	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; Ophthalmoscopic exam conducted Week 86, 106 and 115.

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

[c]Both rats #334 & 337, affected unilaterally with the mildest form of uveal syndrome died prior to Week 106 examination.

10

At week 88 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.

Necropsy

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*	109.6	-	-	-	-	-
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	109.9*	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: $\frac{\text{W for treatment group} \times 100}{\text{W for control group.}}$

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	2
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	30
Myofiber hypertrophy atria	1	0	6	1	1	0	5	5
Lung:	48	50	50	50	50	49	49	49
°Parabronchial Lymphoid hyperplasia	25	32	34	30	35	33	30	27
°Alveoli filled with foamy macrophages	3	5	8	4	0	7	7	7
Mesenteric Lymph Nodes:	48	50	49	49	47	49	45	48
°Plasma cell hyperplasia	-	0	-		-		-	8

	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	7	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	8	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	-	18	-	24
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	18/43
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	21
°Dimpling of liver surface	0	0	1	0	0	1	0	4

(-): Unremarkable difference from control.

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

*: $p < 0.05$

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

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	23***
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	0	0	2	0	0	2	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

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

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: Epichlorohidrin (ECH), used as a stabilizer in the technical Alachlor's Lot#XHI-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, 922-923 , December, 1980). However ECH's stomach tumors are not malignant in nature as described above.

•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 tumorsin 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.

20

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, 922-923), 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 070585, 070591 and 070592 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

WSD for LDC

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TS-769:th:TOX/HED:AMAhfouz:6-14-82:card5

21