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

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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.
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10/24/84

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)

WLB
10/31/84

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, 2/12/84 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.

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 94.13% a.i.; stabilized [redacted] Lot #MULT-0417B. The compound, an orange/amber solid with a low melting point, was received from the sponsor on 4/25/80.

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 (uveal 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 epichlorohydrin 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 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 (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 report	70	31	10	20	20	49
reviewer notes	70	33	10	18	20	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, #83 and #85, appeared to belong to group I, see table #2, appendix II. Animal #83 died spontaneously and was reported as autolyzed six hours later at necropsy. Animal #85 was reported with missing 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		Group III	
	M	F	M	F	M (Interim)	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 treated 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 (%)	
	Females	Males
Group I		
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).

ALACHLOR: CHRONIC FEEDING STUDIES

SUMMARY OF OPHTHALMIC EXAMINATIONS BY L. RUBIN

INCIDENCE (% OF SURVIVORS AFFECTED) OF OCULAR ABNORMALITIES

SEX: Females

	Target Dose (mg/kg/day)/Month of Study								
Code	0[a]/13	0[a]/24	126/5	126/7	126/13	126/24	126[b]/7	126[b]/13	126[b]/24
e		3							
f		7	23	36	6		51	65	36
g			18	64	87	100	43	35	36
h			14	67	94	100	43	49	36
i			16	56	90	100	35	33	50
j			1						
k	77	100	4	11	6		10	20	64
l			1	6					
m			1						
n								4	9
q							2	2	5
r				8	55	86		4	27
s		3		6	77	100		22	59
t					3				
v		3							
z	52	29	100	36	31	7	49	49	22

[a]Includes control animals from EHL Study Nos. 800218 and 800219.

[b]Animals removed from treated diet at five to six months of the study.

ALACHLOR: CHRONIC FEEDING STUDIES

SUMMARY OF OPHTHALMIC EXAMINATIONS BY L. RUBIN

INCIDENCE (% OF SURVIVORS AFFECTED) OF OCULAR ABNORMALITIES

SEX: Males

	Target Dose (mg/kg/day)/Month of Study								
Code	0[a]/13	0[a]/24	126/5	126/7	126/13	126/24	126[b]/7	126[b]/13	126[b]/24
a			2					5	
b			1						
c			1						
d			1	1					
e			1						
f		3			90	11		30	38
g					13	93			
h					16	82			8
i		3			6	86			
k	85	100			76	18		50	100
l						4			
n		10		1	1				15
o				1					
r						25			
s					1	50			
w		3							8
z	53	29	100	70	68	28	20	20	13

[a]Includes control animals from EHL Study Nos. 800218 and 800219.

[b]Animals removed from treated diet at five to six months of the study.

ALACHLOR: CHRONIC FEEDING STUDIES

CODES AND DESCRIPTIONS USED FOR OCULAR EXAMINATIONS BY L. RUBIN

Code	Description
a	Superficial corneal scarring
b	Focal, depigmented spot (inferiorly) of retina
c	Hyaloid cataract
d	Myelinated retinal nerve fibres
e	Pigment deficiency lateral to optic disc
f	Pigment mottling in retina
g	Posterior synechiae
h	Pigment dispersion onto lens
i	Retina not clearly visible
j	Loss of iris architecture
k	Pigment hypertrophy at pupillary border
l	Hyphema
m	Fibrin in anterior chamber
n	Posterior, subcapsular cataract
o	Pigment deposition on corneal surface
q	Pigment deposition on posterior corneal surface
r	Iris dissolution
s	Complete cataract
t	Corneal edema
v	Optic nerve atrophy
w	Chronic keratitis
z	Number of survivors

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.

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
°Mylocytic 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.

Lesions Possibly Related to Treatment
(Percentage by Group)

A) Tumors

	I Animals Exposed Lifetime		II Animals Exposed and Killed Less than 8 Months		III Animals Exposed 5-6 Months then Basal Diet	
	Male	Female	Male	Female	Male	Female
Brain, Neuroepithelioma	1	3	0	0	0	2
Liver, Hepatoma	4	3	0	0	0	0
Neoplastic Nodule	0	3	0	0	0	2
Hepatocellular Carcinoma	3	6	0	0	0	2
Nose, Adenoma, Papillary Adenoma	69	44	*	*	59	41
Adenocarcinoma	11	8	*	*	0	2
Stomach, Mixed Carcinosarcoma	3	19	0	0	0	0
Anaplastic Sarcoma	1	3	0	0	0	0
Adenocarcinoma	0	10	0	0	0	0
Leiomyosarcoma	0	10	0	0	0	0
Undifferentiated Sarcoma	0	16	0	0	0	2
Undifferentiated Carcinoma	0	3	0	0	0	0
Total	4	61	0	0	0	2
Thyroid, Follicular Adenoma	12	13	0	0	5	4
Follicular Adenocarcinoma	14	0	0	0	5	4

B) Non-Tumor Lesions

Bone Marrow, Myelocytic Hyperplasia	21	42	0	0	21	20
Eye, most frequent lesion of uveo-retinopathy (proteinous fluid or excess pigment)	62	97	20	45	10	41
Liver, Focus of cellular alteration	36	3	10	0	30	16
Nose, Submucosal gland Hyperplasia	10	0	*	*	29	28
Inflammation nasal passages	16	24	*	*	35	20
Thyroid, Follicular Cyst/Adenomatous Cysts	54	26	10	0	20	8
Urinary Bladder, Epithelial Hyperplasia	13	13	0	6	5	0

*Noses were not saved nor microscopy performed on these animals.

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)

A. K.

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 #...-80-22...				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	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	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 hyperperplasia	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-80-186								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	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-year; 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 61% 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 [redacted] 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] (unknown potential as initiator or promotor)	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.

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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.

SUMMARY

Title: An Evaluation of the Potential of Alachlor to Induce Unscheduled DNA Synthesis in the In Vivo-In Vitro Hepatocyte DNA Repair Assay.

Conducted by: SRI International, Menlo Park, CA 84025

Project No.: SRI LSC 83-201, 6642

Study No.: SR-83-293

Date of Report: April 5, 1984

Date Monsanto Staff Toxicology Review Completed: April 13, 1984

Study Director: J. C. Mirsalis

The accompanying report contains the results of the referenced study with alachlor. Quality assurance reviews were conducted by Stanford Research Institute (SRI) International. A review of the data and an evaluation of the conclusions presented in this report are summarized below.

Purpose

The purpose of this assay was to assess the potential of the test material to produce DNA damage in mammalian cells using an in vitro technique following in vivo administration of the test material. Hepatocyte primary cultures (HPC) were used as the cell model for the test.

Methods

Alachlor (Lot No. MDLT 1114D; purity 95.2%) was administered by stomach tube to six groups of three male Fischer-344 rats at dosage levels of 50, 200 and 1000 mg/kg body weight, at two and 12 hours prior to sacrifice. The high-dose level of 1000 mg/kg was selected because it is the approximate LD50 for alachlor in rats. Two groups of two male rats received 3 ml/kg corn oil vehicle as the negative control and 50 mg/kg 2-acetylaminofluorene as the positive control, respectively, 12 hours prior to sacrifice. At the appropriate time of sacrifice, hepatocytes were isolated from the livers.

For the DNA repair test, hepatocyte cultures were incubated with ^{3}H-thymidine for four hours, followed by incubation with unlabeled thymidine for 14 to 16 hours. After slide preparation, the slides were dipped in Kodak NTB-2 emulsion and exposed at 20°C for 12-14 days. Cells were stained with 1% methyl-green Pyronin Y.

Nuclear and cytoplasmic tritium-exposed silver grains on the radiographic films were counted using an automatic grain counter. The net nuclear grain counts were determined by subtracting the highest count of two nuclear-sized areas of the cytoplasm from the nuclear count. A minimum of 50 cells/slide, 3 slides/animal, 3 animals/dose group/time point were scored for a total of 450 cells/dose/time point. A test result was considered positive if the net grain counts and percent of cells in repair were markedly elevated above those in the vehicle control group. A cell was considered "in repair" if the net grain count was five or greater.

Results

The average net grain counts and the average number of cells in repair for each alachlor dosage level and control and for each time point are shown in the following table.

Treatment	Dose	Time (hr)	Average Net Grain Count (mean±S.E.)	Average % in Repair (mean±S.E.)
Corn Oil	3.0ml/kg	12	-6.0±1.5	0±0
2-Acetylamino fluorene	50 mg/kg	12	18.7±4.6	82±11
Alachlor	50 mg/kg	2	-6.6±0.4	1±0
		12	-6.5±1.0	3±2
Alachlor	200mg/kg	2	-2.4±4.6	19±18
		12	-5.4±0.8	5±2
Alachlor	1000mg/kg	2	-3.1±3.2	11±11
		12	2.1±2.4	35±12

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.

SUMMARY

Title: In Vivo Bone Marrow Chromosome Study in Rats with Alachlor.

Conducted by: Hazleton Laboratories, America Inc.
Vienna, VA 22180

Project No.: 241-154

Study No.: HL-83-165

Date of Report: March 1, 1984 (page correction April 16, 1984)

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The accompanying report contains the results of the referenced study with alachlor. Quality Assurance reviews were conducted by Hazleton Laboratories America Inc. A review of the data and an evaluation of the conclusions presented in this report are summarized below.

Methods

Alachlor (Lot No. MDLT-08-02B; purity 95.4%) was administered by stomach tube to three groups of 24 male and 24 female Sprague-Dawley (Crl:COBS CD®(SD)BR) rats at dosage levels of 100, 330 and 1000 mg/kg body weight. The dosage levels were determined from the results of preliminary dose range-finding studies (appendices A and B of accompanying report). A fourth group of 24 male and 24 female rats received 5 ml/kg of vehicle only (corn oil) and served as the negative control group. An additional group of six male and six female rats received 40 mg/kg cyclophosphamide and served as the positive control.

Approximately 4, 10, 22 and 46 hours after the administration of alachlor or corn oil, six males and six females of each group received a single, intraperitoneal (IP) injection of 2.0 mg/kg colchicine to inhibit mitosis and arrest the cells in metaphase. Approximately two hours after the injection of colchicine, the animals were sacrificed, the bone marrow cells were aspirated from both femurs of each animal and the cells were processed, fixed, stained and mounted on slides. The positive controls received the IP injection of colchicine at the 22 hour time point only and were sacrificed at 24 hours.

When possible, 300 cells per sex per group were analyzed at each time point for cytogenetic aberrations. Cytogenetic aberrations were classified and characterized as follows:

1) chromatid breaks - including fragments and deletions
2) chromosome breaks - including acentric fragments, deletions and minutes
3) chromatid and chromosome gaps
4) exchanges - rings, dicentrics, translocations, quadriradials and triradials
5) cells with more than 10 aberrations
6) pulverized cells.

Results

In the dosage range-finding study, dosages of alachlor up to 1300 mg/kg produced no change in the mitotic index (number of cells in metaphase/number of cells examined) but caused mild clinical signs such as depression, urine stained fur and chromodacryorrhea and chromorhinorrhea (colored discharge about the eyes and nose). Based on the clinical signs of toxicity the high dosage level was selected to be 1000 mg/kg/day. This was considered to be sufficient as it approximates the oral LD50 value for alachlor in rats.

In the definitive test, no fatalities were observed but similar clinical signs of toxicity as were seen in the range-finding study were noted. In addition, the male animals which received 1000 mg/kg alachlor exhibited statistically significant weight loss at 22 and 46 hours.

When compared with the negative control group, alachlor caused no statistically significant increases in the number of chromosome or chromatid aberrations at any dosage level at any time point. Because no mitotic delay was observed, cells from the 48 hour sacrifice period were not examined. There was no difference in the modal number (average number of chromosomes in the examined metaphases) between alachlor-treated and control animals. Cyclophosphamide, however, caused statistically significant increases in both the percent aberrant cells and the average number of aberrations per cell, thus demonstrating the validity of the assay.