10/25/82

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

002483

MEMORANDUM JUN 25 1982

OFFICE OF
PESTICIDES AND TOXIC SUBSTANCES

TO: Robert Taylor (25)
Registration Division (TS-767)

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

SUBJECT: EPA Reg.#524-316, Alachlor. Review of Monsanto Six-Month Subchronic Feeding Study in Dogs. R.D.#387, Special Report MSL#1925; Volumes 1 and 2, 11/16/81. Accession Nos.: 246292, 246293 and 247376. CASWELL#11

Action Requested:

A review is requested for a 6-month subchronic feeding study in dogs 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.

°A NOEL could not be determined in this study at the lowest dose level tested, 5 mg/kg/day. Liver weight values (absolute, rel./bw, and rel./brw) increased at this dosage level in males and at 25 mg/kg/day and above in both sexes. The increases were often statistically significant ($p < 0.05$). Liver discoloration, fatty infiltration and billiary hyperplasia were reported at 25 mg/kg/day and above. Noted significant increases in SAP and LDH activity were also indicative of liver pathology in this study (see Clinical Chemistry section in this review). Other organ weight changes were noted at the 5 mg/kg/day dosage level, i.e. a decrease in the adrenals rel. /brw ratio and an increase in the pituitary rel. /bw and rel./brw ratios in males, and a decrease in the heart absolute and rel. /brw ratio in females; however these changes were not always consistent at the higher dosage levels (see pathology section, organ weights).

°Other relevant gross and microscopic findings were discussed in this review under the pathology section (i.e. lung congestion in females; kidney inflammation , stomach/intestine and bladder congestion in both sexes; ovaries and testes weight decrease; and heart weight decrease in females).

°Significant emaciation and a high rate of mortality were noted at 50 and 75 mg/kg/day dosage levels which indicates that these two dosages are extremely toxic to the dog.

REVIEW

Study Identification

A 6-month subchronic feeding study in dogs. A report compiled by R.W. Street on 11/16/81 and submitted on 12/1/81 by Monsanto Company, St. Louis, MO. 63166. (Accession Nos: 246292 & 246293). Data on page 80-106 were missing from this report and were submitted later on 5/4/82 (Accession #247376).

The study was performed by Pharmacopathics Research Laboratories, Inc., PR-80-015, PRL#7952(A&B). The laboratory report was dated 10/9/81 and signed by Farid E. Ahmed, Ph.D (Study Director).

The study was initiated on 1/30/80 for phase "A" animals (control, 25, 50 and 100 mg/kg/day groups) and 4/2/80 for phase "B" animals (control and 5 mg/kg/day groups). Termination of animals in both phases occurred 6 months after feeding them the Alachlor treated diet (8/11 - 14/80 for group "A" and 10/9 - 10/80 for group "B").

Material and Methods

Test Substance

Alachlor (Lasso®, Technical) was supplied by Monsanto on 11/30/79 in two five gallon drums. The shipment was from Lot#MTLT 1128X with a stated purity of 93.3% a.i. The compound was reported to crystallize at room temperature into brown crystals and to emit a pungent odor.

Study Design

The study consisted of two phases, A and B. Phase B was initiated when it was realized (by the seventh week of the study) that the low dose in phase A is toxic to the animals.

The two phases of the study (Phase "A" and "B") were nine weeks apart. Day zero for the first phase ("A") was on January 30, 1980, and for the second phase ("B") was on April 2, 1980. Each phase ran for 6 months.

Male and female beagle dogs were randomly divided by weight into groups (in both phases of the study), and administered Alachlor daily in capsules for the entire duration of the study at the following nominal concentrations:

Group	Dosage mg/kg bw/day	Initial No. of animals/ sex/group	Number of Animals/Sex/Group			
			Hematology, Blood Chemistry and Urinalysis (prior to testing and at regular intervals during the study).	Organ Weight at Termination (No. of Survivors only) M	F	Gross Necropsy & Histology
Phase A						
Control "A"	0	6	All animals	5	6	All animals
Low-dose "A"	25	6	All animals	5	5	All animals
Mid-dose	50	6	All animals	2	3	All animals
High-dose	75*	6	All animals	0	1	All animals
Phase B						
Control "B"	0	6	All animals	6	6	All animals
Low-dose "B"	5	6	All animals	6	6	All animals

*This dosage level was 100 mg/kg bw/day for weeks 1 through 3. The dosage was then reduced to 75 mg/kg bw/day for the remainder of the study due to the noted severe toxicity at the 100 mg/kg bw/day level.

Test Animals

The animals used in the two phases (A & B) of this study were purebred beagle dogs received from Hazelton Laboratories Inc. in several shipments. The first shipment was received by PRL on 11/29/80. Fourty-eight dogs (24 M and 24 F) were taken from this shipment and used in the first phase of the study (phase A). These animals were 6 1/2 to 7 1/2 months old when initiated in the study and weighed 9.8 to 11.0 kg for males and 7.6 - 8.1 kg for females.

Three more shipments were received by PRL from 3/13-3/21/80. A total of 24 dogs (12 M and 12 F) were taken from these shipments and used in the second phase of the study (phase B). These animals were 5 1/2 to 6 1/2 months old when initiated in the study and weighed 8.1 kg for males and 6.1 to 6.6 for females. The animals were acclimatized for 62 days in phase "A", and 20, 19 and 12 days respectively for animals of phase "B" (received in 3 consecutive shipments). The dogs were examined during this period and found free of ova and parasites. The animals were then randomized into groups of 6 males and 6 females per dosage level.

The dogs were housed individually in suspended stainless steel cages in two rooms. The air in the rooms was completely changed, at the rate of 10 changes per hour with 100% fresh air. Humidity was maintained at 54% to 70% and temperature at 73 $\pm$ 3°F.

All dogs were exercised at regular weekly intervals.

Feed was offered to the dogs for approximately one hour after which feed consumption was measured. However the animals were put on different kinds of feed during the study (see section below).

Animal Feed

The diet initially selected for this study was 350 g/day of Wayne Dog Food, meal form (WDF, mf) manufactured by Wayne Feed Supply (a division of allied Mills Inc., Gaithersburg, Md.). However it was noted during the first 3 weeks of the study that the high dose animals did not consume an appropriate amount of food. Two high dose dogs were selected to be fed 100 g of 50% WDF, mf and 50% canned dog food during weeks 4 and 5. An improvement in food consumption was noted and the dogs stopped showing diarrhea. Consequently, the basic diet for all dogs was altered as follows:

	Feed Consumption	
Phase (A)	WDF, mf	Canned dog food*
Weeks		
6-8	175	175
9-15	175	175
15-27	200	200
Phase (B)		
Weeks		
1-5	175	175
6-27	200	200

From week 6-8 of Phase A in this study the canned dog food used was distributed by Compass Food Inc., Montvale, N.J.

For the remainder of the study period (Phases A & B) Giant canned food was used. This diet, distributed by Giant Food Inc. of Washington, D.C., was selected because it has a minimum guaranteed level of 10% protein, 3% crude fat and 25% crude fiber.

Test Substance Administration

Alachlor shipment (Lot#MTLT 1128X, 93.3% a.i.), was melted at 45°C by heating the entire drum in an incubator. The drum was agitated to assure uniformity of contents. The material was poured into small glass jars and both the jars and drum were stored in the dark at room temperature.

Alachlor dosages were administered in capsules to the animals because in a range finding study the animals did not consume appropriate amounts of feed containing the compound.

The capsules were prepared weekly based on the mean body weight data two weeks earlier. Usually 2 hours before the capsules were prepared, one of the Alachlor glass jars was incubated at 45°C to melt the compound and then the jar was agitated before appropriate amounts of the melted Alachlor were pipetted into the capsules.

Dosing of animals was performed one hour after all dogs received basic ration.

Observations

The animals were observed once daily for signs of overt toxicity and mortality. The animals were further inspected weekly for signs of local or systemic toxicity and pharmacologic effects.

Food consumption was determined daily. Body weights were measured weekly.

Ophthalmological Examinations

Ophthalmological examinations were performed prior to day zero (baseline), at months 1, 3 and 6, and prior to the terminal sacrifice. All animals in the study (phase "A" & "B") were examined at each of these intervals.

Laboratory Tests

•Clinical chemistry determinations were performed on all dogs prior to initiation of dosing and monthly thereafter until termination. These determinations included the following assays:

Total Protein (TP)
Albumin (A)
Globulin (G)
Serum Glutamic Pyruvic Transminase (SGPT)
Serum Alkaline Phosphatase (SAP)
Serum Glutamic Oxaloacetic Transaminase (SGOT)
Serum Lactic Dehydrogenase (LDH)
Blood Urea Nitrogen (BUN)
Fasting Blood Sugar (FBS)
Total Bilirubin (TB)
Direct Bilirubin (DB), if TB was elevated
Cholesterol (Chol)
Potassium (K)
Calcium (Ca)
Sodium (Na)
Chloride (Cl)
Carbon Dioxide (CO_2)

•Hematology determinations were performed on all dogs prior to initiation of dosing and monthly thereafter until termination. These determinations included the following parameters:

Erythrocyte Count (RBC)
Hemoglobin (Hgb)
Hematocrit (Hmct)
Leukocyte Count, Total (WBC, Total)
Leukocyte Count, Differential (WBC, Diff)
Platelet Count (Plat Ct)
Reticulocyte Count (Retic Ct), only in case of anemia
Erythrocyte Morphology (RBC Morph)

•Urinalysis determinations were performed on all dogs prior to initiation of dosing and at months 2, 4 and 6. The urinalysis parameters evaluated were as follows:

Color
Appearance
Specific Gravity
pH
Protein
Glucose (qualitative)
Ketones
Urobilinogen
Bilirubin
Microscopical examination of sediment for white blood cells and red blood cells.

Necropsy

All animals were subjected to a complete gross necropsy examination. The animals were sacrificed by intravenous injection of Somlethol. Moribund dogs were sacrificed within 16 hours so as to prevent autolysis of tissues.

Brain, pituitary, thyroids/parathyroid (combined), heart, liver, adrenals, kidneys and gonads were weighed at necropsy for each animal. The large organs were weighed fresh while the small ones (pituitary, thyroids/parathyroids, adrenals and gonads) were weighed after fixation in 10% buffered formalin. Organ to body weight and organ to brain weight ratios were also calculated for each animal.

Histopathology

All animals were histopathologically examined. The following tissues were processed for these examinations:

Adrenals, aorta, bladder (urinary), bone, bone marrow, brain, cecum, colon, duodenum, ears (middle), esophagus, eye and optic nerve, gall bladder, heart, ileum, jejunum, kidneys, larynx, liver, lungs and bronchi, lymph nodes, mammary glands (if palpable), muscle (skeletal), peripheral nerve (sciatic), nose and nasal sinuses, ovaries, pancreas, parathyroids, penis, pharynx, pituitary, prostate, salivary glands (submaxillary), seminal vesicles, skin, spinal cord, spleen, stomach, testes, tongue, thymus, trachea, thyroids, uterus and vagina.

Statistical Analysis

Statistical analyses of data was performed using the independent two-sided Student's t-test at the 95% confidence level.

Results

Mortality and Observations

All high-dose animals (6 M, 6 F), except one female, were dead by the seventh week of the study; over half of the mid-dose animals (4/6 males and 3/6 females) died by month 5-6 of the study; and two low-dose "A" animals (1/6 males and 1/6 female) died by month 5 of the study period. Also one male control "A" died accidentally by suffocation due to a blank capsule that lodged in the animal's throat. No death was reported for control "A" females or low-dose "A" females or for control "B" and low dose "B" animals (males and females). See the table below:

Dosage Group	Dosage mg/kg bw/day	Mortalities No. Dead/No Initiated Males	Females
Control "A"	0	1	0
Low "A"	25	1	1
Mid	50	4	3
High	75*	6	5
Control "B"	0	0	0
Low "B"	5	0	0

*Initially 100 mg/kg/day for the first 3 weeks of study.

All deaths in treated animals were attributable to Alachlor toxicity.

Progressive emaciation was noted in all treated animals that died during the study.

Diarrhea and vomiting were reported in the high-dose animals before changing the diet from 100% Wayne Dog Food, meal form to 50% canned food.

Alopecia was reported in 2/6 male low-dose "B" animals, however, it was not considered compound related because it did not occur at higher dosage levels.

NOTE: Individual clinical observations data for animals in this study were not submitted.

Body Weight and Food Consumption

Progressive emaciation and severe reduction in food intake were noted in animals that died during the study.

Survivors (males and females) of the 25, 50 and 75 mg/kg/day dosage groups exhibited lower mean body weight values at week 27 than at week 0 of the study (with the exception of the mid-dose female group which gained[1] an average of 0.7 kg during the study) as compared to an increase of 0.1 kg (males) and 1.5 kg (females) in the mean body weight of the control "A" animals. Animals in the 5 mg/kg/day dosage group and their concurrent control "B" group exhibited an increase in the mean body weight values at the end of the study (week 27). However the body weight increase was slightly lower in the 5 mg/kg/day group than the respective control group.

Food consumption data also reflected a dose-dependent decrease at all dose levels tested with the exception of data for the 5 mg/kg/day group which was similar to the controls. These differences from the control group, though not always statistically significant, are considered compound related. The following table reflects the mean body weight change for survivors from week 0 to week 27 and the mean food consumption data at week 27:

Group	Dosages mg/kg/day	Mean BW Change (kg) Males	Females	Mean Food Consumption (g) Males	Females
Control "A"	0.0	+ 0.1	+ 1.5	397	359
Control "B"	0.0	+ 1.9	+ 2.3	398	369
Low-dose "B"	5.0	+ 1.7	+ 1.9	400	353
Low-dose "A"	25.0	- 0.3	- 0.2	360	305
Mid-dose	50.0	- 0.2	[(-.4), + 0.7[1]]	266*	325
High dose	75.0	-	- 0.8	-	246*

*p < 0.05
- All animals died before termination.

[1]The increase noted in the mean body weight of the mid-dose female group is misleading if we consider the fact that only 3 females survived (#1660, #1662, and #1666) to termination. The average weight of these animals was 8.7 kg at day zero and 8.3 kg at termination. This fact indicates that the mid-dose female group did lose an average of 0.4 kg during the study period.

NOTE: The data in the above table are limited by the fact that all animals of the high-dose group, except one female, died before the end of the study; and that only 2/6 males and 3/6 females of the mid-dose group survived.

Ophthalmology

No significant difference was reported between the control and treatment groups.

Clinical Chemistry

The most noted changes in the clinical chemistry parameters of the treated animal groups as compared to the controls were observed at the low-dose "A", (25 mg/kg bw/day) and above. These changes included the following:

- Reduction in total protein and globulin in both sexes.

- Increase in SAP and occasionally SGPT activity in both sexes.

- Increase in LDH activity (especially in treated males and to lesser extent in treated females).

- Inconsistant changes in SGOT values in both sexes which indicate a potential injury to the muscle cells (i.e., heart).

- Occasional increase in cholesterol in some treated males.

- Occasional decrease in calcium in some treated females.

- Occasional increase in total bilirubin in few treated males that later died in extremis.

The above noted changes were frequently statistically significant but not biologically significant, with the exception of SAP values which increased significantly both biologically and often statistically ($p < 0.05$) at 25 mg/kg/day and above in both sexes.

°No remarkable changes were noted in males or females of the low-dose "B" group, 5 mg/kg bw/day, as compared to the concurrent control "B" with the exception of the statistically significant decrease ($p < 0.05$) in the female group of the total protein, albumin, globulin, and carbon dioxide at month 4 interval, and the of the SGOT value at month 5 interval. In males a significant increase ($p < 0.05$) of the SGOT value was also noted at month 5 interval.

°The Laboratory Animals Data Bank, (LSRO Interim Report, March 1980) indicates that the normal range for SAP is 5-75 IU and for LDH is 10-100 IU. The effect of Alachlor administration on SAP and LDH activities may be underestimated in this study due to the fact that PRL's normal range for these two parameters are much higher (SAP: 15-318 IU and LDH: 20-390 IU) than the normal range reported in the LADB reference.

When compared to LADB's normal range, SAP and LDH values appeared to be high in all determinations in phase "A" treated animal groups and in some determinations in the control "A" groups. In phase "B" these values were high in both the control and treatment groups for the baseline determinations and the the first 3 months of the study. Consequently the effect of Alachlor on these phase "B" animals could not be clearly assessed.

Also PRL's normal range for total protein (TP) is much higher (3.8-9.9 g/dl) than the normal range (4.5-7.5 g/dl) in the same reference (LADB); however this difference does not affect the analysis of TP data in this study because all dogs had a total protein value commensurate with the normal range in both references (PRL and LADB).

Hematology

Slight changes were noted in the hematology parameters of the treated animals as compared to the respective control groups. These changes were occasionally statistically significant but within PRL's normal range. These changes are as follows:

- RBC consistently decreased in all treated groups as compared to the respective control groups. This decrease was statistically significant ($p < 0.05$) in female groups at 25 mg/kg/day for months 3 and 5, at 50 mg/kg/day for months 2, 3 and 5, and at 75 mg/kg/day for months 2 and 3 (~~then~~ it should be noted that only one female survived for months 4, 5 and 6 determinations and also reflected lower RBC values). In males the decrease was significant ($p < 0.05$) at 50 mg/kg/day for months 2, 3 and 6; all the 75 mg/kg/day male group died after a month of exposure to Alachlor.

- WBC generally increased in all treated animal groups. The increase was statistically significant ($p < 0.05$) in female groups at 5 mg/kg/day for months 1, 5 and 6, and at 75 mg/kg/day for month 3. In males, the increase was significant ($p < 0.05$) at 25 and 50 mg/kg/day dosages for month 5.

- Hemoglobin values generally decreased in treated animal groups as compared to the respective control groups. The decrease was statistically significant ($p < 0.05$) only in the 50 mg/kg/day female group at month 2 and 3.

- Hematocrit values also tended to decrease in treated groups. The decrease was statistically significant ($p < 0.05$) in the 25 mg/kg/day female group for month 5 and in the 5 mg/kg/day male group for month 5.

Platelets counts tended to increase in treated female groups but they were inconsistent in treated male groups. In females, the increase was statistically significant ($p < 0.05$) at 5 mg/kg/day for month 1; at 25 mg/kg/day for months 2 and 4; at 50 mg/kg/day for month 5; and at 75 mg/kg/day for months 1 and 3 (it should be noted that the baseline value for this dosage group was significantly higher than the control group). In males, the only significant ($p < 0.05$) change was a decrease in the platelet count at 75 mg/kg/day for month 1 (it should be noted that all these high-dose males died by month 2 of the study period).

- Reversal of P:L ratio (polymorphonuclear counts lower than lymphocyte counts) was noted occasionally in the one low-dose "A" male and the 2/4 mid-dose males that died in extremis during the study. This reversal in the P:L ratio could not be noted in the treated females that died in extremis.

Urinalysis

A decrease in the urine specific gravity was noted in both sexes at 25 mg/kg bw/day on month 6 and at 50 mg/kg/day on months 4 and 6. This noted decrease appeared to be dose-related. No treatment-related changes were noted at the 5 mg/kg bw/day dosage, however, appearance of red blood cells was noted in urine in both sexes at this dosage level and in the control "B" animals.

Pathology

I. Gross Necropsy

General emaciation was noted in all animals that died in extremis in this study. Analysis of data indicated the following findings:

Liver:

Dose dependent discoloration (nutmeg to yellow) of the liver was noted at 25 mg/kg/day and above in males (1/6, 4/6 and 6/6 animals at 25, 50 and 75 mg/kg/day respectively), and at 50 mg/kg/day and above in females (2/6 and 3/6 animals at 50 and 75 mg/kg/day respectively). Changes in liver texture (thick and firm) was also noted in some males at 50 mg/kg/day and above. All animals identified with dicolored liver in the male groups died in extremis during the study; and 0/1, 2/3 and 3/5 animals of the total number of females that died in extremis during the study respectively at the 25, 50 and 75 mg/kg/day dosage levels had discolored livers.

Kidneys:

Inflammation of the kidneys (with capsule adhering to both kidneys) was noted in 1/6 mid-dose males; 2/6 low-dose "A" females and 1/6 high-dose females. Congested kidneys were noted in 4/6 high-dose males, and in 1/6 mid-dose females. A yellow cortex was noted in a cross section in 1/6 high-dose females. In summary, gross findings in the kidneys were noted at the 25 mg/kg/day dosage level and above in females; and at 50 mg/kg/day and above in males. No remarkable changes were noted in the control groups (A or B) or the 5 mg/kg/day dosage group.

Lungs:

Lungs were not grossly affected in males but at least one animal at each dosage level was affected in the treated female groups, i.e. at 5 mg/kg/day, 1/6 females had elevated tan lesions (0.5-0.7 cm) in several lung lobes; at 25 mg/kg/day, 1/6 females had a congested lung at the right lobe; at 50 mg/kg/day, 2/6 mid-dose females had atelectic lungs; and at 75 mg/kg/day 1/6 females had a focal terminal congestion in both lung lobes.

Bladder:

The bladder was dilated and sometimes contained red stained material or fluid in 3/6 high-dose males; and 1/6 mid and 1/6 high-dose females.

Stomach/Intestines:

These organs were congested and sometimes contained red stained material in 1/6 mid-dose males (stomach) 3/6 high-dose males (3 animals with congested intestine, one of these animals had also red fluid in both intestine and stomach); in 1/6 mid-dose females (focal congestion in intestine) and 2/6 high-dose females (focal congestion in intestine, one of the two affected animals had also a congested stomach).

Note: The bladder and stomach/intestines were only affected in the 50 and 75 mg/kg/day animal groups; the liver and kidneys were affected at the 25 mg/kg/day dosage level and above; and the lungs were affected at 5 mg/kg/day and above but in females only. None of these gross findings were noted in the control "A" or "B" animals.

II. Organ Weights

The major changes noted in organ weights at termination are reflected in the table below:

Dosage mg/kg/day		Liver		Kidney		Gonads	
		Female	Male	Female	Male	Female	Male
5	Absolute	100	118*	96	102	107	98
	Rel. to bw	112	119*	103	103	118	101
	Rel. to brain	92	109	92	95	106	91
25	Absolute	102	122	96	70	68*	67
	Rel. to bw	126*	126*	122	80	89	68*
	Rel. to brain	106*	126*	98	82	64	70
50	Absolute	128	137*	138*	90	71	44*
	Rel. to bw	141*	148*	157*	93	82	50*
	Rel. to brain	139	162*	135*	135	82	55
75	Absolute[1]	147	-	138	-	79	-
	Rel. to bw	189	-	178	-	96	-
	Rel. to brain	110	-	126	-	82	-

*p < 0.05

1: All high-dose animals (M&F) died with the exception of one female survivor.

Note: Organ weight calculation = $\frac{\text{W for treatment group} \times 100}{\text{W for control group}}$

The mean absolute organ weights (g) for the animal groups at-termination are as follows:

Phase "B"	Liver		Kidney		Gonads	
	F	M	F	M	F	M
0.0 mg/kg/day	263	288	48	57	1.13	13.60
5.0 mg/kg/day	263	340*	46	58	1.21	13.36
Phase "A"						
00 mg/kg/day	258	288	48	77	0.94	18.00
25 mg/kg/day	264	350	46	60	0.64*	12.29
50 mg/kg/day	330	395*	65*	70	0.67	8.43*
75 mg/kg/day	380	-	66	-	0.74	-

*p < 0.05

1: See note on page 10 in regard of the number of animals that survived to termination.

As noted in the above tables, liver weight (absolute and relative) for both males and females increased in all treatment groups (with the exception of the 5 mg/kg/day female group which had an absolute liver weight and organ/brain weight ratio similar to the control group). This effect on the liver appears to be dose-related and statistically significant ($p < 0.05$) in males at 5 mg/kg/day dose level and above and in females at 25 mg/kg/day and above (the noted 12% increase in the liver rel./bw ratio in the 5 mg/kg/day female group was not statistically significant).

A statistically significant increase ($p < 0.05$) in the kidney weight (absolute, rel./bw, rel./brw) was noted in the 50 mg/kg/day female group, this increase was also noted in the only surviving high-dose female. In males, the kidney data were inconsistent.

A biologically significant dose-related decrease in the weight of gonads was noted in both sexes (absolute, rel./bw, rel./brw) at 25 mg/kg/day dose level and above. However, the decrease was only statistically significant ($p < 0.05$) for the absolute ovaries weight at 25 mg/kg/day and for the testes rel./bw at the 25 and 50 mg/kg/day dosage levels.

Other organ weight changes, not reported in the above table, were noted, i.e, 1) a decrease in the heart absolute weight in all treated female groups (78, 65, 68, 77 and 65 g at 0, 5, 25, 50 and 75 mg/kg/day dose level respectively); 2) an increase in the pituitary rel./bw ratio in the 5 mg/kg/day male group, this increase was statistically significant ($p < 0.05$) but the effect was not noted at the higher dose levels; and 3) inconsistent changes (statistically significant $p < 0.05$) in adrenal weights in males, these changes included a decrease in the rel./brw ratios at 5 mg/kg/day, an increase in the absolute, rel./bw and rel./br ratios at 25 mg/kg/day and in the rel./brw ratio at 50 mg/kg/day dose levels. The increase in the adrenal weights usually indicates that the animals are under stress, however the decrease noted at the 5 mg/kg/day in this organ rel./br weight ratio cannot be explained. See table below:

Dosage (mg/kg/day)

Phase"B"	Mean[1] Body Weight	Heart Female	Adrenals Males	Adrenals Females	Pituitary Males
0.0	absolute (%)	78 (100)	1.38 (100)	1.27 (100)	0.05 (100)
	rel . bw (%)	8.9 (100)	0.45 (100)	0.145 (100)	0.003 (100)
	rel . to brain (%)	1.121 (100)	0.018 (100)	0.018 (100)	0.001 (100)
5.0	absolute (%)	65 (83)	1.30 (94)	1.25 (98)	0.06 (120)
	rel . bw (%)	8.05 (91)	0.135 (93)	0.155 (107)	0.007* (233)
	rel . to brain (%)	0.880 (79)	0.015* (83)	0.018 (100)	0.003 (300)
Phase "A"[2]					
00	absolute (%)	78 (100)	1.04 (100)	1.37 (100)	0.06 (100)
	rel . to brain (%)	1.024 (100)	0.013 (100)	0.018 (100)	0.001 (100)
25	absolute (%)	68 (87)	1.46* (140)	1.14 (83)	0.05 (83)
	rel . to brain (%)	0.932 (91)	0.019* (146)	0.016 (89)	0.001 (100)
50	absolute (%)	77 (99)	1.25 (120)	1.52 (111)	0.05 (83)
	rel . to brain (%)	1.016 (99)	0.018* (139)	0.02 (111)	0.001 (100)
75	absolute (%)	65 (83)	- -	1.43 (104)	- -
	rel . to brain (%)	0.813 (79)	- -	0.018 (100)	- -

[1]absolute in grams, rel ./brw in g/kg and rel ./brain g/g.

[2]rel ./bw not reported in this table due to the weight loss noted in the phase "A" treated animals.

*$p < 0.05$

- animals died before termination.

III. Histopathology

Liver fatty degeneration (mild to severe) and mild to moderate billiary hyperplasia were noted in both sexes at 25 mg/kg/day and above i.e. 1/6, 6/6 and 6/6 males and 2/6, 6/6 and 6/6 females were affected at 25, 50 and 75 mg/kg/day dose levels respectively. The degree of these microscopic lesions was dose related. No effect was noted in the control (A or B) or the 5 mg/kg/day dose groups.

Other microscopic findings included a chronic granuloma in the lungs of one female of the 5 mg/kg/day group; a focal myocardial hemorrhage in the heart of one female of the 25 mg/kg/day in group and in the control "A" male that died accidentally during the study; focal tubular cysts in the kidney of one female of the 25 mg/kg/day group; an ultramobronchial parathyroids cyst in one male of the 5 mg/kg/day group; and a Rathke's pouch cyst of the pituitary was noted in one male in each of the control "B", 25 and 50 mg/kg/day groups. No remarkable microscopic findings were noted in the control (A or B) groups with the exception of two control "B" females which had mammary cystic hyperplasia, one of these females had also a simple cyst in the uterus.

Conclusions:

A NOEL has not been demonstrated in this study at the lowest dosage level tested 5 mg/kg/day. A dose related increase in liver weight (absolute, rel./bw, and rel./brw) was noted at this level and above in males and at 25 mg/kg/day and above in females. Discoloration of the liver, fatty degenerations and billiary hyperplasia were also noted in both sexes at 25 mg/kg/day and above. The increase in SAP and LDH activity demonstrates further the pathological pattern of Alachlor hepatotoxicity in this study (see Clinical Chemistry section in this review). Other organ weight changes were noted at the 5 mg/kg/day dosage level, i.e. a decrease in the adrenals rel ./brw ratio and an increase in the pituitary rel ./bw and rel ./brw ratios in males, and a decrease in the heart absolute and rel ./brw ratio in females; however these changes were not always consistent at the higher dosage levels (see Pathology section, organ weights).

Other pathological findings in this study that appear to be potentially compound related are described under the pathology section in this review (i.e. lung, kidney, stomach/intestine, bladder, gonads and heart).

Dose-related emaciation and mortality were noted in both sexes at 25 mg/kg/day dosage level and above; and a slight reduction in body weight gain was also noted at 5 mg/kg/day dosage level as compared to the control group (1.9 g and 1.7 g in males and 2.3 g and 1.9 g in females in the control and 5 mg/kg/day groups respectively). It is important to note that mortality was high at the 50 and 75 mg/kg/day dosage groups, i.e. all but one high dose animal (a female) died during the first 2 to 3 months of the study and 4/6 males and 3/6 females of the mid-dose group died in extremis during the study.

Note: Food consumption was severely reduced especially in the 2 highest dose groups and the diet was changed several times during the study until a 'Giant' canned food ration was acceptable to the animals.

Study Classification: Core-Minimum

FDC 6/24/82

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