9-19-85

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

004660

OFFICE OF
PESTICIDES AND TOXIC SUBSTAN

MEMORANDUM

SUBJECT: Alachlor, EPA Reg. No. 524-316. Evaluation of Newly Submitted Studies: A One year Feeding Study in Dogs, Accession # 255953; and a CHO/HGPRT Mammalian Cell Forward Mutation Study, Accessions # 255952.
Caswell No. 11

FROM: Amal Mahfouz, Ph.D.
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TO: Robert Taylor, Product Manager #25
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THRU: Laurence Chitlik, DABT
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THRU: Theodore M. Farber, Ph.D.
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Action Requested:

The registrant submitted the following 2 studies for evaluation:

1. A one year feeding study in dogs, study # 820165 (ML-82-279) by Monsanto Environmental Health Laboratory, 11/16/1984, accession # 2559530. This study was performed to determine a NOEL for Alachlor hepatotoxicity in dogs because a previously submitted 6-month feeding study in dogs (PR-80-015) failed to reflect a NOEL at the lowest dosage tested, 5mg/kg/day (see my review of 6/25/82).

2. CHO/HGPRT mammalian cell forward gene mutation assay, study # PK-83-246 by Pharmakon Reseach Int., 7/30/84, accession # 255952.

Recommendations:

1. The one year dog feeding study is an acceptable study; the NOEL is 1 mg/kg/day and the LOEL is 3 mg/kg/day based on symptoms of chronic diarrhea, and effects on organ weight data (liver,brain and pituitary) and hemolytic anemia.

Although this study is Core classified as Guideline, the data from this study should be considered together with the data from the previously submitted 6-month dog study #PR-80-015 (see my 6/25/82 review of this study) for a complete picture of the effects of alachlor on dogs at the dosage levels tested in both studies.

2. The CHO/HGPRT mammalian gene mutation assay is negative and is Core Classified as Acceptable.

The reviews of the above discussed new studies are attached to this memo. The Registration Standard for Alachlor should be amended to indicate that these two data gaps are successfully filled.

004660

EPA: 68-01-6561
TASK: 111
August 30, 1985

DATA EVALUATION RECORD

ALACHLOR

One-Year Chronic Toxicity in Dogs

STUDY IDENTIFICATION: Naylor, M. W., Ribelin, W. E., Thake, D. C., Stout, L. D. and Folks, R. M. Chronic study of Alachlor administered by gelatin capsule to dogs. (Unpublished study No. 820165 by Environmental Health Laboratory, Monsanto Company, St. Louis, MO, for Monsanto Company, St. Louis, MO; dated November 16, 1984.) Accession No. 255953.

APPROVED BY:

I. Cecil Felkner, Ph.D.
Program Manager
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Signature: I. Cecil Felkner

Date: 8-29-85

3

1. CHEMICAL: Alachlor.

2. TEST MATERIAL: Alachlor, lot No. MULT 0417B., was of 94.1 percent purity and was described as a solid material with an amber color.

3. STUDY/ACTION TYPE: One-year chronic (capsule administration) study in beagle dogs.

4. STUDY IDENTIFICATION: Naylor, M. W., Ribelin, W. E., Thake, D. C., Stout, L. D. and Folks, R. M. Chronic study of Alachlor administered by gelatin capsule to dogs. (Unpublished study No. 820165 by Environmental Health Laboratory, Monsanto Company, St. Louis, MO, for Monsanto Company, St. Louis, MO; dated November 16, 1984.) Accession No. 255953.

5. REVIEWED BY:

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7. CONCLUSIONS:

A. The LOEL for Alachlor in dogs is 3 mg/kg/day based on hemosiderosis seen in the kidney and spleen of one of six male dogs (the effect was seen in the spleen in one dog and the kidney in another) tested in the study. No hemosiderosis was associated with the liver in the mid-dose dogs. At higher levels (10 mg/kg/day) the effect was also seen in the liver (3/6) and correlated with hematologic findings showing red cell destruction and consequent red cell replenishment. The hematologic findings noted in the high-dose males were also seen as a trend (except for the increased reticulocyte count) in the mid-dose male dogs. The effect was found only in the males. Thus, the NOEL is 1.0 mg/kg/day.

B. The study was designed and conducted in basic agreement with the EPA Pesticide Registration Guidelines of 1982. However, the dose of Alachlor was weighed "neat" into gelatin capsules and there are no data to demonstrate the variation obtained in this dosing method. The precision that can be achieved by weighing approximately 10 mg/capsule day after day is likely to be low. This technique may introduce a large variability in actual doses administered.

Core Classification: Guideline.

Item 8--see footnote 1.

9. BACKGROUND:

In the section that states the purpose of this study, a reference is made to a previously completed 6-month oral toxicity study examining the effects of Alachlor in dogs. The lowest dose tested, 5.0 mg/kg/day, produced marginal liver toxicity, such as increased liver weight and serum enzyme changes that were considered to be equivocal. This current study was conducted to establish a definitive NOEL in dogs for Alachlor.

Item 10--see footnote 1.

11. MATERIALS AND METHODS (PROTOCOLS): (The Materials and Methods section of the final report is included as Appendix A.)

A. Materials and Methods:

1. The test material used in this study was Alachlor, technical, received from Monsanto Agricultural Products Company on April 25, 1980. The material was an amber solid with a reported purity of 94.1 percent; the lot No. was MULT 0417B.

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[1]Only items appropriate to this DER have been included.

5

2. The test animals used in this study were pure-bred beagle dogs, 24 male and 24 females, obtained from Marshall Research Animals, North Rose, NY. At initiation of the study the dogs were approximately 6-1/2 months old. The dogs were randomized by body weight and assigned six per sex to a dosage group.

3. The test material was given neat (with no dilution) in gelatin capsules and administered daily at least 1 hour after feeding a ration consisting of pellatized Ralston-Purina Certified Dog Chow No. 5007. Control dogs were given empty gelatin capsules. Daily doses of Alachlor were 0, 1.0, 3.0, or 10 mg/kg body weight per day.

   The test material used in this study was analyzed by the testing laboratory at initiation and at 6 and 12 months during the study.

4. Animals were observed twice daily during the study for signs of toxicity and mortality. Body weight and food consumption were measured and clinical chemistry, hematology, and urinalysis testing were performed. All surviving animals were sacrificed after 12 months and examined for gross pathological changes; organ weights were measured for adrenal, brain, heart, kidneys, liver, pituitary, testes with epididymides, and the paired thyroids (including parathyroids). Tissue masses and lesions were preserved and examined for histological changes as were the 39 different tissues collected at necropsy (see Appendix A, page 9).

5. Statistical analysis of body weight, food consumption, and noncategorical pathological data were performed using Dunnett's (two-tailed) test; the variability was assessed with Bartlett's test. Terminal body weight and organ weight data were analyzed by ANOVA and Dunnett's test for multiple group comparisons. Organ-to-body weight ratios were analyzed by the Mann-Whitney test, and histopathologic findings were analyzed by the Fisher exact test using the Bonferoni's Inequality procedures.

B. Protocol: No separate protocol was submitted with the report.

12. REPORTED RESULTS:

A. Test Material Analysis: The analyzed purity of the test material at initiation, at 6 months, and at 12 months was reported to be 93.9, 95.2, and 93.2 percent, respectively.

B. Survival and Clinical Observations: One control female dog (NF003) died at 150 days of the study. All other animals survived to scheduled termination.

Diarrhea or loose, green mucus or dark, tary stools (melen) were seen in the high-dose animals, particularly in the males and to a lesser extent in the mid-dose animals (see Table 1). Salivation was a frequent observation in the mid- and high-dose males and especially in the mid-dose females where 140 occurrences were reported for one animal. All other clinical observations were unrelated to test material ingestion.

C. Body Weight and Food Consumption: No effects on body weight were noted in female dogs at any dosage level (Table 2); male dogs in the 10 mg/kg/day group showed reduction in body weight gain starting in month 8. Food consumption was not affected at any dosage level in either sex.

D. Ophthalmoscopic Examination: Aside from pigment spots of the tapetal fundi, which were considered incidental, congenital abnormalities, the 6-month and terminal ophthalmologic examinations revealed no effects in any of the dosage groups that were related to the test compound.

E. Hematology: High-dose (10 mg/kg/day) males had slightly reduced red blood cell (RBC) counts, hemoglobin values, and hematocrit values while mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), and reticulocyte counts were elevated compared to controls (Table 3a) at all evaluation intervals (approximately 3, 15, 24, 32, and 52 weeks).

The hematologic values for females (Table 3b) were comparable to those of the controls for all values and intervals except at 32 weeks when the MCV and MCH were significantly elevated ($p \leq 0.05$ and $p \leq 0.01$, respectively) in the high-dose animals, and the mid-dose (3 mg/kg/day) females showed a significant ($p \leq 0.05$) increase in reticulocytes at 24 weeks.

F. Blood Chemistry and Urinalysis: Serum alkaline phosphatase levels were higher at all intervals in all dosed male groups compared to the control groups (Table 4); they were significantly different from the controls ($p \leq 0.05$) for the 1 mg/kg/day dosage level at all intervals except at the 24-week measurement. However, the mean pretest value of all groups combined was higher than the controls. The authors reported a "slight" increase in total bilirubin (Table 5) values for the high-dose males for all measurement intervals compared with controls. Bromsulphalein (BSP) values showed a trend (this was not significant because of the high variations) toward retention in the mid- and high-dose groups at 24 weeks (Table 6) and showed significant ($p < 0.05$) retention in the high-dose males at 52 weeks; no effect was seen in any male group at 15 weeks or in any of the female dogs at any time during the study. Clinical chemistry values for the other parameters measured in the dosed groups were comparable to control values.

Urinalysis results revealed no effects that could be attributable to the test material in either sex at any sampling period.

7

TABLE 1. Clinical Observations in Dogs Administered Alachlor for 1 Year

Observation	Number of Animals with Observation							
	Males Dose Level (mg/kg)				Females Dose Level (mg/kg)			
	0	1	3	10	0	1	3	10
Emesis	3(7)[a]	3(8)	1(3)	5(6)	3(5)	2(4)	2(2)	2(5)
Diarrhea	1(1)	2(3)	4(6)	5(13)	1(1)	4(6)	1(1)	4(6)
Mucus stool	-	-	2(2)	4(4)	-	1(1)	1(2)	3(4)
Black stool	-	-	-	3(4)	-	1(1)	-	-

[a]The numbers in parentheses are the number of occurrences.

TABLE 2. Selected Mean Body Weight Data for Dogs Fed Alachlor for 1 Year

Oral dose level (mg/kg/day)	Mean (± S.D.) Body Weight (kg) at Day					
	0	32	103	172	270	369
Males						
0	7.9±0.59	8.8±1.17	10.1±1.51	10.5±1.56	11.1±1.64	11.1±1.74
1	7.7±0.58	8.8±0.85	10.1±0.75	10.6±0.67	11.2±0.77	11.2±0.88
3	7.7±0.53	9.0±0.56	10.0±0.53	10.3±0.73	11.3±1.09	11.2±1.13
10	7.8±0.55	8.9±0.78	9.9±0.94	10.4±0.82	10.7±0.68	10.4±0.67
Females						
0	6.5±0.31	7.2±0.40	7.9±0.55	8.3±0.53[a]	8.5±0.54[a]	8.5± .51[a]
1	6.6±0.44	7.3±0.63	8.2±0.76	8.5±0.78	9.0±0.90	9.0± .67
3	6.6±0.34	7.4±0.68	8.2±0.87	8.8±1.07	9.0±1.15	9.6±1.39
10	6.5±0.43	7.4±0.50	8.2±0.54	8.9±0.79	8.8±0.67	8.8± .83

[a]Mean of five animals; all other values are means of six animals.

TABLE 3a. Selected Hematologic Values in Male Dogs Fed Alachlor for 1 Year

Parameter/ Dose Level (mg/kg/day)	Pretest[a]	Mean Value (± S.D.) at Week (approximate) 3	15	24	32	52
RBC (10^6/mm^3)	6.2±0.58					
0		6.32±0.47	7.23±0.62	7.27±0.68	7.70±0.64	7.75±0.67
1		6.23±0.52	7.13±0.53	7.45±0.54	7.84±0.41	7.87±0.44
3		6.25±0.43	6.90±0.43	6.95±0.50	7.35±0.35	7.55±0.42
10		5.74±0.61	6.29±0.67*	6.27±0.99	6.65±0.90*	6.81±0.94
Hemoglobin (g/dL)	14.4±1.2					
0		14.6±0.9	16.1±1.5	16.5±1.7	17.1±1.6	17.0±1.8
1		14.4±1.3	16.0±1.4	17.1±1.6	17.8±1.4	17.4±1.4
3		14.8±0.7	15.6±0.6	15.9±0.7	16.7±0.5	17.0±1.0
10		13.6±1.5	14.5±1.0	14.7±1.9	15.5±1.8	15.6±2.0
Hematocrit (%)	42.8±3.7					
0		43.2±3.0	48.6±4.8	49.3±5.4	54.0±5.3	54.5±5.8
1		42.8±4.4	48.5±4.8	51.2±5.1	55.9±4.2	55.7±3.9
3		44.0±2.1	47.4±2.2	48.1±2.2	52.9±1.8	54.5±2.3
10		40.6±4.8	43.5±3.5	43.7±5.8	48.9±5.6	50.2±6.2
MCV (μ^3)	69.0±1.7					
0		68.3±1.2	67.1±1.2	67.7±1.2	70.0±1.3	70.1±1.7
1		68.5±2.0	67.9±2.0	68.5±2.1	71.2±2.0	70.7±1.7
3		70.4±1.9	68.7±2.0	69.3±2.2	71.9±2.2	72.1±2.1
10		70.6±2.2	69.4±2.4	69.9±2.8	73.7±2.4*	73.9±2.2*
MCH (pg)	23.2±0.7					
0		23.1±0.5	22.2±0.4	22.7±0.3	22.2±0.4	21.9±0.5
1		23.2±0.3	22.4±0.6	22.9±0.6	22.6±0.7	22.1±0.7
3		23.7±0.8	22.7±1.0	23.0±1.0	22.8±0.9	22.5±1.0
10		23.7±0.8	23.1±1.1	23.7±1.0	23.4±0.7*	23.0±0.6*
Reticulocytes (%)	0.45±1.17					
0		0.78±0.44	0.47±0.24	0.47±0.16	0.58±0.40	0.50±0.28
1		0.62±0.28	0.30±0.20	0.30±0.22	0.62±0.21	0.65±0.25
3		0.50±0.25	0.28±0.10	0.42±0.31	0.50±0.18	0.37±0.21
10		1.82±1.80	1.08±1.45	1.23±1.65	2.50±2.89	2.57±3.39*

[a] Mean for 26 animals; animals were assigned to dosage groups after the pretest value was obtained.

* Significantly different from control value at $p \leq 0.05$.

TABLE 3b. Selected Hematologic Values in Female Dogs Fed Alachlor for 1 Year

Parameter/ Dose Level (mg/kg/day)	Pretest[a]	Mean Value (± S.D.) at Week (approximate)				
		3	15	24	32	52
RBC ($10^6/mm^3$)	6.61±0.39					
0		6.72±0.47	7.30±0.76	7.13±0.23	7.92±0.48	7.41±0.53
1		6.70±0.41	7.18±0.50	7.04±0.50	7.65±0.45	7.48±0.46
3		6.55±0.26	7.15±0.44	7.03±0.29	7.74±0.43	7.18±0.33
10		6.45±0.30	7.15±0.45	7.03±0.18	7.64±0.21	7.51±0.44
Hemoglobin (g/dl)	15.4±1.0					
0		15.6±1.1	16.4±1.5	16.3±0.60	17.7±1.1	17.0±1.4
1		15.7±1.1	16.6±0.80	16.5±1.0	17.4±0.70	17.1±0.80
3		15.3±0.5	16.3±0.80	16.4±0.50	17.8±1.2	16.6±0.80
10		15.2±0.8	16.5±0.70	16.6±0.70	17.8±0.60	17.4±0.80
Hematocrit (%)	46.0±2.9					
0		46.6±2.9	49.7±4.7	49.0±2.0	56.2±3.0	53.3±5.1
1		46.9±3.1	50.0±2.6	49.2±2.9	54.9±2.0	54.5±3.7
3		45.7±1.5	49.2±2.9	49.0±1.5	55.9±3.3	52.2±2.6
10		45.5±2.2	49.8±2.5	49.6±1.8	56.5±1.7	54.8±2.7
MCV (μ^3)	69.4±1.6					
0		69.3±2.1	68.1±1.2	68.7±1.7	70.9±1.4	71.9±2.5
1		69.8±1.9	69.7±2.1	69.9±1.7	71.7±1.8	72.8±1.9
3		69.8±1.3	68.7±0.90	69.7±1.0	72.2±1.0	72.7±2.0
10		70.5±1.1	69.7±1.2	70.5±1.3	73.8±1.0**	72.9±1.4
MCH (pg)	23.3±0.6					
0		23.3±1.1	22.5±0.30	22.9±0.30	22.5±0.50	22.9±0.50
1		23.5±0.6	23.2±0.70	23.4±0.60	22.7±0.50	22.9±0.60
3		23.4±0.2	22.8±0.60	23.3±0.40	23.0±0.40	23.1±0.50
10		23.6±0.4	23.2±0.60	23.6±0.60	23.3±0.50*	23.1±0.60
Reticulocytes (%)	0.46±0.36					
0		0.40±0.26	0.27±0.16	0.34±0.21	0.22±0.11	0.22±0.15
1		0.55±0.25	0.38±0.29	0.58±0.21	0.60±0.15	0.32±0.31
3		0.58±0.37	0.33±0.22	0.72±0.34*	0.40±0.29	0.53±0.33
10		0.38±0.16	0.27±0.05	0.50±0.40	0.30±0.20	0.38±0.28

[a] Mean for 26 animals; animals were assigned to dosage groups after the pretest value was obtained.

*Significantly different from control value at $p \leq 0.05$.

**Significantly different from control value at $p \leq 0.01$.

10

TABLE 4. Mean (±S.D.) Serum Alkaline Phosphatase Activity (IU/L) in Male Dogs Fed Alachlor for 1 Year

Dose Level (mg/kg/day)	Approximate Week on Test				
	Pretest[a]	3	15	24	32	52
	381±153					
0		281±30.9	208±49.2	148±27.9	120±25.7	106±27.5
1		407±106*	320±92.2*	219±66.6	186±66.1*	166±61.8*
3		371±80.7	283±51.5	220±51.0	181±22.7	155±33.4
10		335±51.9	296±54.4	209±48.4	147±43.2	136±26.9

[a] Mean for 26 animals; animals were assigned to dosage groups after the pretest value was obtained.

* Significantly different from control value at ($p \leq 0.05$).

TABLE 5. Mean (±S.D.) Total Bilirubin (mg/dL) in Blood of Male Dogs Fed Alachlor for 1 Year

Dose Level (mg/kg/day)	Approximate Week on Test				
	Pretest[a]	3	15	24	32	52
	0.10±0.02					
0		0.07±0.05	0.07±0.05	0.10±0.00	0.10±0.00	0.10±0.00
1		0.07±0.05	0.10±0.00	0.08±0.04	0.10±0.00	0.12±0.04
3		0.10±0.00	0.10±0.00	0.10±0.00	0.10±0.00	0.12±0.04
10		0.13±0.10	0.15±0.08*	0.17±0.10	0.22±0.18	0.28±0.25

[a] Mean of 26 animals; animals were assigned to dosage groups after the pretest value was obtained.

* Significantly different from control ($p < 0.05$); two-tailed Dunnett's test.

11

TABLE 6. Mean (±S.D.) Bromsulphalein (BSP) Retention (%) in Male Dogs Fed Alachlor for 1 Year

Dose Level (mg/kg/day)	Approximate Week of Test				
	Pretest[a]	3	15	24	32	52
	4.4±0.9					
0		ND[b]	4.2±1.4	3.5±3.0[c]	ND	4.8±0.9
1		ND	3.9±1.0	3.8±2.1	ND	5.0±1.1
3		ND	4.6±1.0	6.2±4.4[d]	ND	4.8±0.9
10		ND	3.6±0.9	8.0±9.4[e]	ND	7.0±1.5*

[a] Mean of 26 animals; animals were assigned to dosage groups after the pretest value was obtained.

[b] ND = No data present in the final report.

[c] Individual values were 7, 5, 3, 0, 6, 0.

[d] Individual values were 6, 7, 14, 2, 6, and 2.

[e] Individual values were 0.5, 15, 23, 3.5, 0.5, 0.5.

* Significantly different from control ($p < 0.05$); two-tailed Dunnett's test.

13

G. Gross Pathology: The female control dog that died showed a 2-cm white focus in the right anterior lobe of the lung; a few insignificant gross lesions were found at necropsy but none that could account for the dog's death.

The remaining animals were examined at final sacrifice. Lung foci were observed in one mid-dose male and in one low- and two mid-dose females dogs; high-dose females had no foci. No other gross lesions were reported for any of the dosed animals.

H. Organ Weight and Organ-To-Body Weight Ratios: For the organs weighed, significant differences from controls were observed for the brain, liver, and pituitary. Data for these three organs are provided in Table 7.

Mean absolute brain weights were significantly lower ($p \leq 0.05$) only in mid-dose females. Liver weights (absolute and relative to body weight) were significantly ($p \leq 0.05$) higher in high-dose males; females also showed dose related and higher but not statistically significant values in the high-dose group. Absolute pituitary weights were significantly lower ($p \leq 0.05$) in mid-dose males.

I. Microscopic Pathology: A compound-related incidence of hemosiderosis (Table 8) occurred in the kidney, liver, and spleen of males at the high dose. Hemosiderosis occurred in kidney and spleen but not in the liver of the mid-dose males. No hemosiderosis was seen in any organ in the females regardless of dose.

The main noncompound-related lesions consisted of localized interstitial pneumonia in seven dogs in the control, low-, and mid-dose groups and thymic atrophy in all groups.

13. STUDY AUTHORS' CONCLUSIONS/QUALITY ASSURANCE MEASURES:

A. The study authors concluded that Alachlor at doses of 3 and 10 mg/kg/day in dogs produced evidence of apparent hemolytic anemia in males but not in females. Hemosiderosis was apparent in liver, kidney, and spleen at doses of 3 and 10 mg/kg/day in males only. Elevated alkaline phosphatase, increased bromsulphalein retention, and increased absolute and relative liver weights were less meaningful. The study authors concluded that 1.0 mg/kg/day was the NOEL.

B. Approximately 17 quality assurance inspections were conducted during the course of the study. The Quality Assurance Manager indicated in the report that the report accurately reflects the raw data developed during the study.

13

TABLE 7. Mean Organ Weight Data for Dogs Given Alachlor for 1 Year

Dose Level (mg/kg)	Mean Terminal Body Weight (kg)	Mean Organ Weights (Absolute) & Organ-To-Body Weights (Relative[a])							
		Brain		Liver			Pituitary		
		Absolute (g)	Relative to Body Weight	Absolute (g)	Relative to Body Weight	Relative to Brain Wt.	Absolute (g)	Relative to Body Weight	Relative to Brain Wt.
Males									
0	11.117	80.770	0.744	279.319	2.548	3.46	0.071	0.001	0.09
1	11.183	81.284	0.729	278.310	2.490	3.42	0.062	0.001	0.08
3	11.150	81.388	0.737	292.150	2.625	3.59	0.059*	0.001	0.07
10	10.400	79.705	0.770	320.677*	3.083*	4.02*	0.063	0.001	0.08
Females									
0	8.540	79.387	0.931	228.683	2.687	2.88	0.066	0.001	0.08
1	9.017	75.428	0.840	242.414	2.705	3.21	0.058	0.001	0.08
3	9.550	71.346*	0.757	257.233	2.736	3.61*	0.060	0.001	0.08
10	8.000	74.886	0.857	266.154	3.051	3.55*	0.056	0.001	0.08

[a]Organ to body weight = 100 x $\frac{\text{absolute organ weight (g)}}{\text{terminal body weight (g)}}$

*Significantly different from control value ($p \leq 0.05$) using Dunnett's test as calculated by these reviewers.

TABLE 8. Incidence[a] of Hemosiderosis in Dogs Fed Alachlor for 1 Year

Dose group (mg/kg/day)	Males			Females		
	Kidney	Liver	Spleen	Kidney	Liver	Spleen
Control	0/6	0/6	0/6	0/6	0/6	0/6
1.0	0/6	0/6	0/6	0/6	0/6	0/6
3.0	1/6[b]	0/6	1/6[c]	0/6	0/6	0/6
10.0	3/6[d]	3/6[d]	2/6[e]	0/6	0/6	0/6

[a]Number affected/Number examined.

[b]Dog No. 1.

[c]Dog No. 6.

[d]Dog Nos. 3, 4, 6.

[e]Dog Nos. 3 and 4.

14. REVIEWERS' DISCUSSION AND INTERPRETATION OF STUDY RESULTS:

A. Lower RBC count, hematocrit, and hemoglobin, elevated MCV, MCH, and reticulocyte counts, and pathology showing hemosiderosis (effect seen as low as 3 mg/kg/day) in liver, kidney, and spleen are consistent with a syndrome of red blood cell destruction. There was also an overall trend for these hematologic effects in the mid-dose males, but the effect on reticulocytes was not seen. However, complicating the interpretation is the gastrointestinal involvement reported in the clinical findings, particularly the dark, tary stools, which generally indicates hemorrhage in the upper colon; however, this was not corroborated by confirming pathologic findings. Additional factors complicating the interpretation are the elevated alkaline phosphatase values and increased serum bilirubin, which could be due to cholestasis. ~ Alkaline phosphatase levels were significantly ($p < 0.05$) elevated at the lowest dose in the male dogs at all intervals except at 24 weeks. However all values at all intervals were higher, though not significant, than the control values for that interval. We assess that the control values are low, hence the high variation and small numbers of animals used account for this trend.

The absence of bilirubin in the urine, the absence of increased direct bilirubin in the serum, and the fact that gamma glutamyl transpeptidase is not elevated leads to the conclusion that the mechanism of action of Alachlor toxicity is an effect on the red cells causing destruction, with the pigment being excreted as bile salts and the iron being spared by storage as hemosiderin. The apparent hemorrhage noted could be responsible for the hematologic findings but not for the hemosiderosis. The hematologic effect seemed sex related; it was noted almost exclusively in the males, although there were some slight positive trends in the high-dose females at 32 weeks (elevated MCV and MCH). There was no hemosiderin storage in the fema

The decreased brain weight in the mid-dose females is not considered biologically meaningful in the absence of pathologic findings. The same conclusion can be made for the mid-dose finding in the males with regard to the pituitary. The increased liver weight, liver-to-body weight ratio, and liver-to-brain weight ratio in the high-dose males cannot be dismissed in light of the hemosiderosis in this organ and associated hematological findings. The significant liver-to-brain weight ratios for the two highest dosed female groups are not meaningful as they represent the effect on the brain weight as much as that on the liver.

16

Although the study authors concluded that bromsulphalein (BSP) retention was increased in the high-dose males, this effect was not presented in the authors' results or discussion sections; it was mentioned by Dirks in the "Results" and "Conclusions" in the pages that were not numbered at the beginning of the report. However, nothing can be said with regard to the bromsulphalein retention without having more detail on the method used. The high variation in bromsulphalein data could invalidate or compromise the terminal value that is significantly ($p \leq 0.05$) different from the controls. At 24 weeks the males showed one value elevated (14) in the mid-dose group and two values elevated (15 and 23) in the high-dose group compared to a mean of 3.5 for the controls. At that same interval there were three dogs in the high-dose group with values of 0.5. One must conclude that the BSP values are too variable in terms of the number of animals used to make any conclusion, particularly when there are no corresponding histopathologic parameters in the study that would account for the elevated retention time; hemosiderosis would not cause BSP retention.

15. COMPLETION OF ONE-LINER FORM FOR STUDY:

Appendix B, One-Lines Table.

16. CBI APPENDIX:

Appendix A, Methods and Materials, CBI pp. 4-10.

17

APPENDIX A

Materials and Methods

D ALACHLOR

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____ Identity of product inert ingredients.

____ Identity of product impurities.

____ Description of the product manufacturing process.

____ Description of quality control procedures.

____ Identity of the source of product ingredients.

____ Sales or other commercial/financial information.

____ A draft product label.

____ The product confidential statement of formula.

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APPENDIX B

One-Liner Table

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EPA: 68-01-6561
TASK: 111
July 18, 1985

DATA EVALUATION RECORD

ALACHLOR

Mutagenicity: CHO/HGPRT Forward Gene Mutation in Mammalian Cells

STUDY IDENTIFICATION: Godek, E. and Barfknecht, T. CHO/HGPRT mammalian cell forward gene mutation assay with Alachlor Technical. (Unpublished study No. PK-83-249 prepared by Pharmakon Research Int., Inc., Waverly, PA for Monsanto Company, St. Louis, MO; dated July 30, 1984.) Accession No. 255952.

APPROVED BY:

I. Cecil Felkner, Ph.D.
Program Manager
Dynamac Corporation

Signature: I. Cecil Felkner

Date: 7-18-85

27

1. CHEMICAL: Alachlor technical, Lasso.

2. TEST MATERIAL: Alachlor technical, Lot No. MDLT 08-02B, a cream tan low-melting solid with a purity of 95.4%.

3. STUDY/ACTION TYPE: Mutagenicity: CHO/HGPRT Forward Gene mutation in mammalian cells.

4. STUDY IDENTIFICATION: Godek, E. and Barfknecht, T. CHO/HGPRT mammalian cell forward gene mutation assay with Alachlor Technical. (Unpublished study No. PK-83-249 prepared by Pharmakon Research Int., Inc., Waverly, PA for Monsanto Company, St. Louis, MO; dated July 30, 1984.) Accession No. 255952.

5. REVIEWED BY:

Brenda Worthy, M.T.
Principal Reviewer
Dynamac Corporation

Signature: Brenda Worthy
Date: July 18, 1985

I. Cecil Felkner, Ph.D.
Independent Reviewer
Dynamac Corporation

Signature: I. Cecil Felkner
Date: 7-18-85

6. APPROVED BY:

William L. McLellan, Ph.D.
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Signature: William L. McLellan
Date: 7-18-85

Amal Mahfouz, Ph.D.
EPA Reviewer

Signature: Amal Mahfouz
Date: 7/25/85

Laurence Chitlik, D.A.B.T.
EPA Section Head

Signature: Laurence Chitlik
Date: 9/17/85

28

7. CONCLUSIONS:

A. Under the conditions of the assay Alachlor technical, Lot No. MDLT 08-02B, was not mutagenic in the CHO/HGPRT Mammalian Cell Forward Gene Mutation Assay at dose levels from 15 to 150 µg/ml in the nonactivated assay or at dose levels from 15 to 200 µg/ml in the S9 activated assay.

B. The study is acceptable.

8. MATERIALS AND METHODS (PROTOCOLS):

A. Materials and Methods:

(1) The test material, Alachlor technical, Lot No. MDLT 08-02B, was described as a substance that can be a cream tan low-melting solid or liquid. This variation in physical form was reported to be due to the low-melting point and temperature variation within the laboratory. Purity of the test material was 95.4%. Ethyl alcohol (USP 200 proof) was used to solubilize the test material.

(2) The Chinese hamster (CHO) cell line (CHO-K1-BH4) was obtained from Dr. Abraham W. Hsie, Oak Ridge, TE.

(3) The S9 fraction used for metabolic activation was prepared from the livers of rats induced with Aroclor 1254 (sex and age not specified). The components for the S9 mix were as follows:

	S9 mix (per 1 ml)			
Components	1%	2%	5%	10%
$MgCl_2.6H_2O$	8 µmol	8 µmol	8 µmol	8 µmol
$CaCl_2.2H_2O$	8 µmol	8 µmol	8 µmol	8 µmol
KCL	33 µmol	33 µmol	33 µmol	33 µmol
Glucose-6-phosphate	5 µmol	5 µmol	5 µmol	5 µmol
NADP	4 µmol	4 µmol	4 µmol	4 µmol
Phosphate Buffer	50 µmol	50 µmol	50 µmol	50 µmol
S9 fraction	0.05 ml	0.1 ml	0.25 ml	0.5 ml

29

(4) Cytotoxicity Assay: Cytotoxicity was determined by a reduction of colony-forming ability of cells after treatment with the test material at doses of 0.33, 1.0, 3.33, 10, 33.3, 100, 333, and 1000 µg/ml. The assay was performed without activation and with S9 activation at S9 concentrations that were 1, 2, 5, and 10% of the treatment volume. Five hours after treatment, the liquid cultures were washed and incubated for 19 hours and the cells plated and incubated for 7-8 days. Colonies were then counted and the three dose levels which yielded 10, 50, and 100% cell survival were used to determine a dose-response range for the mutagenicity assay.

(5) Mutagenicity Assay: Cultures of cells seeded at 5×10^5 cells were prepared and treated with appropriate levels of test material, solvent or positive [dimethylnitrosamine (DMN) and ethylmethanesulfonate (EMS)] control with or without S9 activation.

For mutant selection, the cells were plated at a density of 2×10^5/100 mm plate (5 plates) in hypoxanthine-free medium containing 10 µm 6-thioguanine. Cloning efficiency was assessed in hypoxanthine-free medium, by seeding with 200 cells/plate, in triplicate. After 7 days of incubation the cells were stained and counted for total number of mutant clones; cloning efficiency and the mutation frequency were calculated.

(6) Evaluation criteria: The assay was considered to be positive if a) the mutation frequencies of at least one of the 3 highest test material concentrations, with a mean survival rate of at least 10%, were significantly ($p < 0.01$) greater than the solvent control, b) the change in the mean mutation frequency with increasing test material concentrations showed a significant ($p < 0.01$) linear component of the dose-response relationship up to maximum toxicity level of 90%.

(7) The statistical method used was that of Snee and Irr.[2] Appendix A, page 8.

(8) The assay was conducted according to the method of O'Neill, et al.[3]

(9) A preliminary mutagenicity assay was conducted with the test material at doses of 33, 100, and 333 µg/ml without activation, and using 1, 2, or 5% S9 mix for activation. The test material was also assayed at doses of 500, 750, and 1000 µg/ml using 10% S9 mix for activation. The results

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[2] Snee, R.D. and Irr, J.D., Mutation Research 85, 77-93, 1981.

[3] O'Neill, J.P., et al., Mutation Research 45, 91-101 and 103-109, 197[illegible]

30

were not definitive due to the increased mutation frequencies in some solvent controls and the relatively low cloning efficiencies in many of the control and alachlor-treated cultures. Cytotoxicity was reported at a dose level of 333 µg/ml for assays using 1 or 2% S9 mix for activation, and in the nonactivated assays. Likewise, at doses of 750 and 1000 µg/ml using 10% S9 mix for activation there was cytotoxicity. However, no mutation induction was indicated in any of the S9-activated assays.

For details of Materials and Methods, please see Appendix A.

B. Protocol: For details, please see Appendix A.

9. REPORTED RESULTS:

Cytotoxicity Assay: In the cytotoxicity assay all cells were killed by 1000 µg/ml of test material without activation and when either 1 or 2% S9 mix was used for activation. The relative cell survival (RCS) values were 1 and 34% at the 1000 µg/ml dose using 5 and 10% S9-mix for activation, respectively. The RCS values at the 333 µg/ml dose were 10, 5, 19, and 69% when 1, 2, 5, and 10% S9 mix was used for activation, respectively. In the nonactivated assay at 333 µg/ml, the RCS value was 8%. The RCS values at 100 µg/ml dose were 101, 46, 98, and 50% when 1, 2, 5, 10% S9 mix was used for activation. In the nonactivated assay at 100 µg/ml the RCS value was 65%.

Mutagenicity Assay: Based on the preliminary cytotoxicity and mutagenicity studies, the test material was assayed at doses of 15, 30, 60, 100, and 150 µg/ml without activation and using 2% S9 activation (standard concentration) at dose levels of 15, 30, 60, 100, and 200 µg/ml. In the nonactivated assay, the RCS values ranged from 10 to 91%, and from 13 to 84% in the S9 activated assays. There were no statistically significant increases in the mutation frequencies induced by the test material, at any of the doses, compared to the solvent control either with or without S9 activation (see Table 1, page 6).

Additional assays were conducted on the test material at doses of 30, 60, 100, 200, and 330 µg/ml using 5% S9 mix for activation. Cytotoxicity was evident at the highest dose; RCS values ranged from 21% to 90.7 at doses between 30 to 200 µg/ml. All cells were killed at the 330 µg/ml dose. The data from these assays showed that there were no significant increases in the mutation frequencies induced by the test material when compared to the solvent control. Representative results are presented in Table 2, page 7.

31

TABLE 1. Results of the CHO Forward Gene Mutation Assay with Alachlor Technical

Substance	Dose µg/ml	S9 Activation[a]	Average RCS (%)[b]	Avg. Cloning Efficiency (%)[b]	Mutation Frequency x 10^{-6}
Untreated Control		-	101.2	95.6	4.9
		+	93.1	90.5	7.5
Solvent Control					
Ethanol	10	-	88.5	90.6	3.3
		+	92.9	85.0	4.4
Positive Controls					
Ethylmethanesulfonate	200	-	48.9	71.2	257.1
Dimethylnitrosamine	100	+	26.0	38.7	371.1
Test Material					
Alachlor	15	-	85.7	81.3	0.8
	30	-	74.6	85.9	5.4
	60	-	54.1	78.6	7.5
	100	-	39.9	80.2	3.3
	150	-	13.1[c]	80.3[c]	0.0
	15	+	79.5	89.5	3.0
	30	+	65.9	83.5	3.7
	60	+	58.5	85.6	6.8
	100	+	47.7	87.8	2.1
	200	+	18.9	78.8	1.0

[a] Standard (2%) S9-mix concentration

[b] The results were averaged by the reviewers from triplicate plates (CBI pp. 16-18).

[c] Results averaged by reviewers from duplicate plates.

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TABLE 2. Representative Results of the CHO Forward Gene Mutation Assay with Alachlor Technical

Substance	Dose µg/ml	5 % S9 Activation	Average RCS (%)[a]	Avg. Cloning Efficiency (%)[a]	Mutation Frequency x 10^{-6}
Untreated Control		-	100	96.8	4.2
		+	92.4	89.6	7.1
Solvent Control					
Ethanol	10	-	99.3	83.7	5.1
		+	96.8	83.5	5.9
Positive Controls					
Ethylmethanesulfonate	200	-	43.1	69.2	304.4
Dimethylnitrosamine	100	+	20.5	44.0	376.1
Test Material					
Alachlor[d]	30[b]	+	91.1	91.2	8.4
	200[c]	+	20.3	84.3	8.3

[a]The results were averaged by the reviewers from triplicate plates.

[b]Lowest dose tested.

[c]Highest (non-lethal) cytotoxic dose.

[d]Results of the remaining dose levels (60 and 100 µg/ml) were also similar to the solvent control and the low dose.

7/18/86

33

10. STUDY AUTHORS' CONCLUSIONS/QUALITY ASSURANCE MEASURES:

A. The authors concluded that "The results for the test article, Alachlor Technical, were negative in the CHO/HGPRT Mammalian Cell forward Gene Mutation Assay at the dose levels tested both with and without activation (S9) preparation according to the criteria of the test protocol."

B. A quality assurance statement was present, signed and dated July 19, 1984.

11. REVIEWERS' DISCUSSION AND INTERPRETATION OF STUDY RESULTS:

Preliminary Mutagenicity Assay: Our assessment is that mutation frequency increases occurred in the nonactivated assay; these frequencies were 15.2 and 20.9 x 10^6 in the Alachlor-treated cells at doses of 33 and 100 µg/ml compared to the solvent control mutation frequency of 10.3 x 10^6. These results were not definitive because of the low cloning efficiencies in the untreated (27.7%) and solvent (33.2%) controls.

Primary Mutagenicity Assay: It is our assessment that the test material did not cause a statistically significant increase in the mutation frequency at any of the dose levels tested, 15 to 150 µg/ml nonactivated or 15 to 200 µg/ml with 2% S9 activation (see Table 1, page 6). The cloning efficiency of the untreated and solvent controls was above 50% (with and without S9 activation) as was stipulated in the authors' protocol.

The mutation frequency of the solvent control was within 2 standard deviations of historical range as established by the authors' laboratory: Untreated cultures 7.47/10^6 ±6.44 without S9 and 7.44/10^6 ±6.57 with S9 activation (see Table 1, page 6).

The results of the positive controls, EMS at 200 µg/ml (-S9) and DMN at 100 µg/ml (+S9), showed that the assay was adequately sensitive to detect a mutagenic response (see Table 1, page 6).

12. One-Liner Form for Study: Appendix B contains the completed one-liner form for this study.

13. CBI APPENDIX: Appendix A, Materials and Methods (Protocol), CBI pp. 2-9.

34

APPENDIX A

(Materials and Methods)

ALACHLOR

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____ Description of the product manufacturing process.

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APPENDIX B

(One-Liner Form for this Study)