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

OFFICE OF
PESTICIDES AND TOXIC SUBSTANCES

MEMORANDUM JUN 27 1983

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

THRU: William L. Burnam, Chief
Toxicology Branch/HED (TS-769)
and
Laurence Chitlik, Section Head
Toxicology Branch/HED (TS-769)

SUBJECT: Alachlor, EPA Reg.#524-316. Review of a 21-Day Dermal Toxicity with Rabbits. Report # MSL-2404, R.D.#434; 7/30/82. Accession#247960. CASWELL#11

This study was reviewed by Dynamac Corporation for the Alachlor Registration Standard. Extensive editing of Dynamac's final review was performed by the Toxicology Branch. A copy of the edited review is attached to this memo.

We note that a NOEL has not been demonstrated at 0.2 mg/kg/day(LDT) due to skin lesions and lung toxicity.

This study is graded as Core Supplementary due to the following reasons:

1. A relatively high incidence of animal mortality in the control group as compared to the treatment groups, i.e. 3/20 animals in the control group died as compared to 0/20, 2/20 and 3/20 animals in the low, mid and high dose groups respectively. This finding reflects poor animal husbandry for all animals in this study.

2. Incomplete histopathological examinations ,i.e. the lungs were only examined in a few animals; and the stomach, and thyroid were not examined microscopically, although gross necropsy data reflected coumpound-related lesions in these tissues.

6/24/83

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Attachment

ALACHLOR

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Signature John R. Strange
Date 9 March 1983

Richard L. Hebert, M.S.
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Date 3/10/83

ALACHLOR
(Technical)

Study Type: 21-Day dermal toxicity with rabbits.

Study Identification: IRDC#401-164 (IR-81-036), 5/13/82; Submitted by Monsanto as Report #MSL-2404, R.D. 434, 7/30/82

MRID Number: 000GS-02.

Sponsor: Monsanto Co., St. Louis, MO.

Contracting Laboratory: International Research and Development Corp. Mattawan, MI.

Date of Initiation: March 31, 1981.

Date of Termination: April 29, 1981.

Date of Submission: August 3, 1982

Purity of the Test Compound: Technical, 94.2% and 93.3% a.i. (90% stabilized).

Core Classification: Supplementary

Review

TEST ARTICLE

The test substance is a solid at room temperature. Shipments of test article labeled "90% stabilized technical for formulation of LASSO" were received on 2/20/81 and 4/16/81. The purity of each shipment was labeled as 94.2% and 93.3% a.i., respectively (Lot Nos. MBLT01-29-8 and MBLT04-14B, respectively).

ANIMALS AND HUSBANDRY

Sixty-five male and 67 female New Zealand white rabbits were obtained from H.A.R.E. (Hewwitt, NJ); 44 males and 44 females (weighing 2-3 kg) of these animals were randomly selected for use in this study after 17 days acclimation. The rabbits were housed individually in hanging wire-mesh cages in rooms controlled for temperature, humidity, and light (12 hours on/off cycle). Feed and water were available ad libitum. All animals were identified by a metal ear tag.

PROCEDURE

Four test groups, 10 rabbits/sex/group (5 abraded, 5 intact), were treated with Alachlor daily, five times a week for three consecutive weeks. The dosage levels used were 0, 0.2, 1.0, and 5.0 g/kg/day. However, 4 males and 4 females of the high dose group died during the first week of the study and were replaced; the high dose, 5.0 g/kg/day, was lowered to 4.0 g/kg/day for the remainder of the study period; and the replacement animals were dosed for a full 3-week period.

The test substance was applied to shaved skin areas (abraded or intact) which represented 5-10%, 20%, and 30% of the body surfaces for the low, mid and high dose groups, respectively. Before application, the test substance was melted in a water bath at 40°C and applied undiluted to the skin. The test site was then wrapped with gauze bandage, Saran wrap, and 75 mm Elastoplast tape. Ejay Saf-T shield collars were used to avoid oral ingestion of the test compound.

The test site was washed with tepid water after 6 hours of exposure.

Control animals were handled identically, except that they were not dosed (no vehicle was used in this study).

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The animals were monitored for the following parameters:

Parameters	When Performed
1. Clinical observations (i.e., appearance, behavior, pharmacotoxic effects, visual estimates of food consumption, dermal irritation[a], and mortality	daily
2. Body weights	twice daily
3. Clinical laboratory tests[b] - hematology[c] - biochemistry[d]	4-11 days prior to study and on test day 21 (termination of dosing)

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[a]Erythema, edema, atonia, desquamation, coriaceousness, fissuring, were each scored according to the following scale:

0 = none
0.5 = doubtful or very slight
1.0 = slight
1.5 = slight to moderate
2.0 = moderate
2.5 = moderate to severe
3.5 = severe

[b]Thirty-five males and 35 females were randomly selected for clinical tests conducted prior to study initiation. Of these 70 animals, 40 were randomly selected for the study (5/sex/group). Blood was collected via the main ear artery. The animals were fasted overnight before blood collection.

[c]The following hematology parameters were measured: hemoglobin, hematocrit, erythrocyte count, total and differential leukocyte counts, platelet counts, reticulocyte counts, mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), and mean corpuscular hemoglobin concentration (MCHC).

[d]The following biochemistry parameters were measured: glucose, blood urea nitrogen (BUN), aspartate aminotransferase (SGOT), alkaline phosphatase, alanine aminotransferase (SGPT), total protein, albumin, globulin, calcium, cholesterol, bilirubin, creatinine, lactic dehydrogenase (LDH), sodium, potassium, chloride, and phosphorus.

Parameters	When Performed
4. Gross necropsy examination[e]	All deaths and sacrifices
5. Organ weights[f]	All deaths and sacrifices
6. Histopathology[g]	All deaths and sacrifices

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[e]Necorpsy consisted of verification of antemortem observation, external examination including all orifices, and examination of tissues and organs in situ and after removal. All animals necropsied at termination were sacrificed by an intravenous sodium pentabarbitol overdose.

[f]Weights were determined for the following organs: liver, kidneys, heart, testes, ovaries, adrenals, thyroid, parathyroid, and pituitary (fixed).

[g]The following tissues were processed and examined microscopically.

skin, treated (3 sections)
skin, untreated (3 sections)
liver
kidney
any lesions in any other tissues

Representative sections of the following tissues were preserved for possible future microscopic examination:

adrenal
brain (3 levels)
eye with Harderian gland
esophagus
stomach
small/large intestine
gonads
heart
lung with mainstem bronchi
lymph nodes (mesenteric, mediastinal, and regional if necessary)
mammary region
pancreas
pituitary
sciatic nerve
skeletal muscle (thigh)
spinal cord and vertebrae
spleen
sternum (bone and marrow)
thymic region
thyroid-parathyroid complex
trachea
urinary bladder
uterus (2 horns and cervix)
vagina
prostate and seminal vehicle
salivary gland, mandibular

The eyes were fixed with gluteraldehyde, the lungs were inflated with neutral formalin, and the remainder of the tissues were fixed in 10% formalin.

STATISTICS

Body weights, hematology and biochemistry parameters, and organ weights were compared by analysis of variance, Bartlett's test for homogeneity of variances, and t-tests using Dunnett's multiple comparison tables. Differences at the $p \leq 0.01$ and $p \leq 0.05$ were reported.

RESULTS

GENERAL OBSERVATIONS AND DERMAL IRRITATION

Clinical observations were generally comparable between control and treated groups. Purulent nasal discharge, soft stool, diarrhea, hyposensitivity, tremors, possible respiratory congestion, hair loss, and swollen eyes with or without purulent discharge were seen most often and with comparable frequencies in all groups (including the control group). Additional symptoms were noted in the 11 animals that died in the high-dose group,these symptoms were: ataxia, respiratory congestion, diarrhea, excessive salivation, and decreased limb tone. Respiratory congestion was also observed in one of the high dose survivors. Diarrhea and ataxia were observed in the control animals that died.

Dermal irritation was not observed at any time in untreated animals. Erythema, edema, atonia, desquamation, and fissuring were seen initially in treated animals between study days 2 and 5. Coriaceousness was seen first in treated groups between days 6 and 11, and was reported as very slight or slight. The frequency and severity of symptoms appeared to be dose-related. In the high dose group, the severity of erythema, edema, and atonia ranged from very slight to moderate; also desquamation ranged from very slight to moderate in the high dose group, and its severity increased with time. Fissuring was generally slight or moderate in the high dose group, with a few instances of marked fissuring. No differences were seen between groups with abraded skin and those with intact skin. No eschar or exfoliation was seen in any group.

MORTALITY

Eight animals (4/10 males and 4/10 females) that were treated with 5 g/kg/day Alachlor died within 3 to 5 days of treatment; these animals were replaced, and the dosage was lowered to 4 g/kg/day. Three mortalities were reported in the adjusted high dose group (4 mg/kg/day), two in the mid dose (1 g/kg/day), zero in the low dose (0.2 g/kg/day) and three in the control group. However, the control mortalities occurred at a later date than the mortalities in the treatment groups, see the table below:

Dosage level (g/kg/day)	Sex	Abraded (A) or Intact (I) skin	No. of deaths/ Total No. Animals	Day of death (test day)
Control				
(0.0)	M	A	1/5	21
		I	0/5	--
	F	A	1/5	17
		I	1/5	21
Low Dose				
(0.2)	M	A	0/5	--
		I	0/5	--
	F	A	0/5	--
		I	0/5	--
Mid Dose				
(1.0)	M	A	1/5	11
		I	0/5	--
	F	A	0/5	--
		I	1/5	21
Adjusted High Dose				
(4.0)	M	A	0/5	--
		I	0/5	--
	F	A	1/5	11
		I	2/5	6,9
Initial High Dose				
(5.0)	M	A	3/5	4,4,5
		I	1/5	4
	F	A	2/5	3,4
		I	2/5	3,4

Data for the 5 g/kg/day animals were reported separately and were not considered in the statistical and comparative analyses.

The cause of death (excluding 5 g/kg/day group) was reported as follows: control male, acute enteritis; 3 high-dose females and the mid-dose female, pneumonia; all others, undetermined.

NOTE: The control data in this study reflected a relatively high incidence of mortality; the cause of death in this group was reported for only 1/3 dead animals; and 2/3 control deaths occurred on day 21 (the last day of the study period). Hence, this reviewer questions the animal husbandry conditions in this testing facility.

BODY WEIGHTS AND FOOD CONSUMPTION

No significant differences in the mean body weight changes were observed at any dose level in the abraded skin animals of both sexes as compared to the respective control groups.

Significantly lower ($p < 0.05$) terminal body weights were noted in the treated males at all dose levels with intact skin. However, the initial mean body weights of the low and mid dose male (intact) groups were lower than the control group, and the mean body weight changes in these 2 groups were not statistically significant from the control. Mean body weight changes of the high dose intact males and mid dose intact females were significantly lower ($p < 0.05$) than the mean changes in the respective control groups. No significant effect was noted in the low or high dose female (intact) groups. The variability in these data do not appear to be dose-related, and may be due to other undetermined factors in this study.

Food consumption data were not determined quantitatively, hence, could not be evaluated.

CLINICAL LABORATORY TESTS

No compound-related effects were noted in the hematological or biochemical parameters. The variability in these data appeared to be within the inherent biological variability.

GROSS NECROPSY EXAMINATIONS

Dose-dependent skin lesions (i.e., thickening and encrustation) were noted in all treated groups at the sites of Alachlor application. A dose response effect was also noted in the lungs, i.e., the incidences of lung congestion, consolidation, and emphysema increased in the treated groups. These effects were further noted upon microscopic examination, see Histopathology section below. The table below reflects the gross findings:

	Incidences per dose level (g/kg/day)[a]				
	0	0.2	1.0	4.0	5.0
No. examined:	20	20	20	20	8
Organ or tissue — No. within normal limits:	9	1	0	0	0
Lung	1	7	5	13	6
Skin (application site)	3	24	28	36	13
Stomach	1	0	1	2	3
Thymus	0	0	0	0	4
Thyroid	0	1	0	0	2
Trachea	0	0	0	3	2

[a]There were 10 animals/sex/group. At initiation, the high dose level was 5 g/kg/day, and 8 animals from this group died between days 3 and 5. The animals were replaced, and the dose was lowered to 4 g/kg/day.

There were possible compound-related effects on the stomach, trachea, thymus, and thyroid in the high dose group: 1) The stomach mucosae of 3/4 females that were dosed at 5 g/kg/day, and 1/10 females that were dosed at 4 g/kg/day (which died during the study) were hyperemic or had petechiae; 2) One male and three females dosed at 5 g/kg/day showed one or more of the following symptoms in the thymus: hemorrhage, congestion, and petechiae; 3) Congested, hyperemic, or hemorrhagic mucosa of the trachea were seen in 1/4 males and 1/4 females of the 5 g/kg/day, group and in the three (replacement) females of the 4 g/kg/day group that died during the study; 4) One male of the 5 g/kg/day group had a hemorrhagic thyroid, and another had an enlarged (2x) thyroid.

It is unfortunate that histopathological examinations of the stomach,and thyroid were not performed, and that only the trachea of 3 high-dose animals (which died in the study) were examined, see the Histopathology section below.

There were scattered observations among treated groups for kidneys, lymph nodes, and external/skin/subcutis (nonapplication) sites. These did not appear to be treatment related. There was a high incidence of the following observations in the liver of the low and mid dose male groups: gray/yellow/tan foci, surface fissuring, enlargement, and accentuated lobulation. However, there was no apparent treatment-related effect in the high dose males and in all the female groups.

ORGAN WEIGHTS

No significant differences were noted in the absolute or relative organ weights with the exception of the pituitary gland. The mean absolute and mean relative weights of this gland in the high dose male group were significantly higher ($p < 0.01$) than the control group. This effect appears to be treatment-related (the only exceptional value in the group was an especially low absolute weight value of 21 mg and relative weight value of 0.81×10^{-3}).

HISTOPATHOLOGY

Microscopic observations of the liver, kidney, treated skin, and nontreated skin were conducted on all animals. The results are summarized in the histopathology table below:

Histopathology Table

Tissue: Observation	No. of Animals with Finding — MALES			
	0 g/kg/day	0.2 g/kg/day	1.0 g/kg/day	4.0 g/kg/day
Kidney	(10)[a]	(10)	(10)	(10)
chronic nephritis	3	6	6	6
Liver	(10)	(10)	(10)	(10)
vacuolar degeneration	10	8	6	4
pericholangitis	3	10	9	9
necrosis	0	1	2	2
cholangitis	0	3	3	0
hemorrhage	0	0	0	0
hematopoeisis	0	0	0	1
adhesion	0	0	1	0
Skin, Treated	(10)	(10)	(10)	(10)
dermatitis, chronic, (trace to mild)	6	6	1	0
dermatitis, chronic or ulcerative (mod. to severe)	1	4	9	10
Skin Untreated	(10)	(10)	(10)	(10)
dermatitis, chronic	8	5	6	6
necrosis	0	1	1	2
ulceration	1	1	0	0
Lung	(1)	(4)	(4)	(5)
pneumonia	1	3	3	3
lymphoid aggregates	0	4	3	2
congestion, severe	0	1	1	3
hemorrhage, multifocal	0	0	1	0
alveolar exudates	0	0	1	0
necrosis	0	0	0	0
Trachea	(0)	(0)	(0)	(0)
tracheitis, chronic	0	0	0	0
hemorrhage, focal	0	0	0	0

[a]Values in parentheses are the numbers of animals examined for particular tissue.

Table continue on next page.

Histopathology Table (continued)

Tissue: Observation	No. of Animals with Finding — FEMALES			
	0 g/kg/day	0.2 g/kg/day	1.0 g/kg/day	4.0 g/kg/day
Kidney	(10)	(10)	(10)	(10)
chronic nephritis	6	4	5	3
Liver	(10)	(10)	(10)	(10)
vacuolar degeneration	7	8	8	6
pericholangitis	10	10	10	9
necrosis	1	0	4	4
cholangitis	2	2	1	1
hemorrhage	0	0	1	0
hematopoeisis	0	0	1	0
adhesion	0	0	0	0
Skin, Treated	(10)	(10)	(10)	(10)
dermatitis, chronic, (trace to mild)	6	6	1	0
dermatitis, chronic or ulcerative (mod. to severe)	0	4	9	10
Skin Untreated	(10)	(10)	(10)	(10)
dermatitis, chronic	7	8	8	8
necrosis	2	1	1	1
ulceration	1	0	1	0
Lung	(0)	(2)	(1)	(6)
pneumonia	0	0	0	2
lymphoid aggregates	0	1	1	5
congestion, severe	0	1	1	5
hemorrhage, multifocal	0	0	0	0
alveolar exudates	0	1	1	2
necrosis	0	0	0	1
Trachea	(0)	(0)	(0)	(3)
tracheitis, chronic	0	0	0	3
hemorrhage, focal	0	0	0	1

[a]Values in parentheses are the numbers of animals examined for particular tissue.

The above table reflects a dose-related chronic or ulcerative dermatitis in the treated male and female skin at the lowest dose tested and above. However, no effect was noted in the untreated skin of the treated animals as compared to the control animals. The incidence of chronic nephritis of the kidneys, relative to controls, was higher in treated males, but it was lower in treated females. No apparent treatment-related effects were observed in the liver. Although the incidence of liver necrosis, relative to controls, was higher in the mid and high dose females, it was lower in treated males,see table above.

The lungs were microscopically examined only in a few animals. In these animals, a high incidence of pneumonia, severe congestion, and lymphoid aggregates was observed in all treated males and the high dose females.

At necropsy, three high dose females had congested, hyperemic, or hemorrhaging trachea. These were also examined microscopically: all had tracheitis and one had a focal hemorrahage.

The results of microscopic observations of the animals that were dosed at 5 g/kg/day and died were similar to the results for the other high dose animals. In addition, hemorrhage, petechiae, or congestion of the thymus were noted in 4/8 animals at necropsy in this group; this was confirmed histologically as multifocal hemorrhaging.

Although hemorrhaging and petechia were seen in stomachs of several high dose animals (see gross necropsy section), none was examined microscopically. Similarly, there were no histologic examination of the hemorrhagic and enlarged thyroids seen in two of the 5 g/kg/day animals that died before this dose was adjusted to 4 g/kg/day.

The histopathology was so incomplete that to draw any far-reaching conclusions would be misleading. However, skin lesions and lung toxicity appear to be dose-related at 0.2 g/kg/day (LDT) and above.

CONCLUSIONS:

The NOEL in this study is lower than 0.2 g/kg/day (LDT) based on skin lesions and lung toxicity:

1. Dose-related skin erythema, edema, desquamation, and fissuring were noted in this study; in addition, a coriaceous appearance was noted in the high dose group. Gross necropsy observations revealed treatment-induced encrustation and thickening of the treated skin; microscopic observations indicated that chronic or ulcerative dermatitis occurred in all treatment groups; and the severity of these lesions was dose related.

2. Gross observations of the lungs and trachea revealed certain effects in all treatment groups for the lungs and in the high dose group for the trachea. The findings most often encountered were hemorrhaging, petechiae, and congestion. Histopathological examinations of the lungs and trachea of a few animals reflected high incidences of pneumonia, lymphoid aggregates, and severe congestion of the lungs, and trachitis. However, an insufficient number of tissues were examined microscopically to determine the extent of the incidence of these lesions in all animal groups (see the histopathology table on pages 10 and 11).

Additional effects were noted at the high dose level:

3. Gross necropsy observations revealed possible treatment-related effects on the stomach, thymus, and thyroid. However, stomach and thyroid tissues were not examined microscopically, and the thymus was only examined in four of the animals that died in the 5 g/kg/day group. Hence,the study did not fully demonstrate any auxilliary site(s) at which Alachlor might exert its toxic effects.

4. The mean absolute and mean relative pituitary gland weights of the high dose males were significantly greater ($p < 0.01$) than those of the control group. However, the increase in the mean relative pituitairy weight may be partially due to the decrease in the mean body weight in this group.

5. The mortality rate in this study was reported as 3/20, 0/20, 2/20 and 3/20 animals in the control, low, mid and high dose groups respectively. In addition, the initial high dose, 5 mg/kg/day, was adjusted to 4 g/kg/day after the first three days of testing due to death of 40% of the animals (4/10 females and 4/10 males).

Due to the relatively high mortality in the control group, the health and the animal husbandry conditions for all animals in this study are questionable.

CORE CLASSIFICATION: Supplementary

- °Questionable conditions of animal health and husbandry in this testing facility which is reflected in the relatively high mortality rate in the control group as compared to the treatment groups.

- °Incomplete histopathology examination as demonstrated by the insufficient number of animals examined for lung lesions, and the absence of any microscopical examination of the gross lesions noted in the stomach and thyroid.