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

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OFFICE OF PESTICIDES AND TOXIC SUBSTANCES

1/11/82

MEMORANDUM

DATE: January 7, 1982

SUBJECT: EPA Reg.#524-316; Lasso (Alachlor); Three-Generation Rat Reproduction. CASWELL#11 Accession#070177

FROM: Amal Mahfouz, Toxicologist
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TO: Robert Taylor (25)
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THRU: Orville E. Paynter, Ph.D
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Action Requested:

Review of a 3-generation rat reproduction study in support of registration of Lasso (Alachlor: 2-chloro-2', 6' diethyl-N-(methoxymethyl)acetanilide) herbicide.

Recommendations:

The reproduction study is classified as Core-Minimum. NOEL for reproduction is 10 mg/kg/day and LEL is 30 mg/kg/day (HDL). Alachlor effect on kidneys is noted at 30 mg/kg/day dose level in F_2 generation and F_{3b} pups. This effect on kidneys is more remarkable in male animals.

Related Action:

EPA Reg. Nos. 524-285, -296, -314; PPs. 9F2144, 9F2156, 0F2313, 0F2338, 0F2348, 1F2447.

Review:

A three-generation reproduction study in rats with Alachlor, final report (Bio/dynamics Inc. No. 77-2066, BDN77-422, February 20, 1981) submitted on 6/29/81 by Monsanto Company, St. Louis, MO 63166.

I. Material and Methods

Test Material

Lasso technical (92.6% a.i.), Lot#XHI-167. The test material (a clear brown viscous fluid, solid at room temperature) was received from Monsanto on 1/5/78 and stored at room temperature.

Test Animals

Charles River Sprague-Dawley CD weanling rats (28 days old) were used in this study. One week prior to treatment, 48 males and 96 females were assigned to 4 groups (a control group and three treatment groups) so as to most nearly equalize, for each sex, the mean initial body weights. Groups were maintained for one week prior to treatment (baseline period). The baseline group mean weight (week 0) was 140.7 to 144.3 g for females and 189.5 to 196.4 g for males.

The animals were housed individually (except during mating and lactation) in elevated stainless steel wire mesh cages. During mating the rats were housed nightly in units of two females and one male until sign of mating was observed or until 15 days had elapsed with no evidence of mating. Nesting material (litter kleen hardwood shaving) were added to each dam's cage in a fitted stainless steel flour pan on day 19 of gestation through day 14 of lactation.

Throughout the study period, the rats were maintained on a 12-hour light/dark cycle, the room temperature was recorded twice daily (morning and afternoon). Control and test diet (untreated and test material admixed Purina Lab. Chow) and tap water were available ad libitum.

Dosage

Alachlor was administered in the diet to yield the following concentrations:

Group	Dosage mg/kg/day
I (Control)	0.0
II	3.0
III	10.0
IV	30.0

The test material was administered in the diet to the rats when 42 days old, (64 days prior to mating of the first generation, F_0) and continuously thereafter for three generations (the rest of the study period).

The test substance was warmed to 40-45°C prior to dispensing. Subsequently appropriate amounts of this material were dissolved in 100 ml acetone and mixed fresh weekly with the standard diet for groups II, III and IV. Acetone only (100 ml) was added to the control diet (group I). For week 1 of the F_0 growth period, test diets were prepared at dose levels of 0, 30, 100, and 300 ppm (equivalent to 0, 3, 10, and 30 mg/kg); thereafter, batch ratio for diet preparation were adjusted weekly during growth and rest periods to achieve appropriate dose levels.

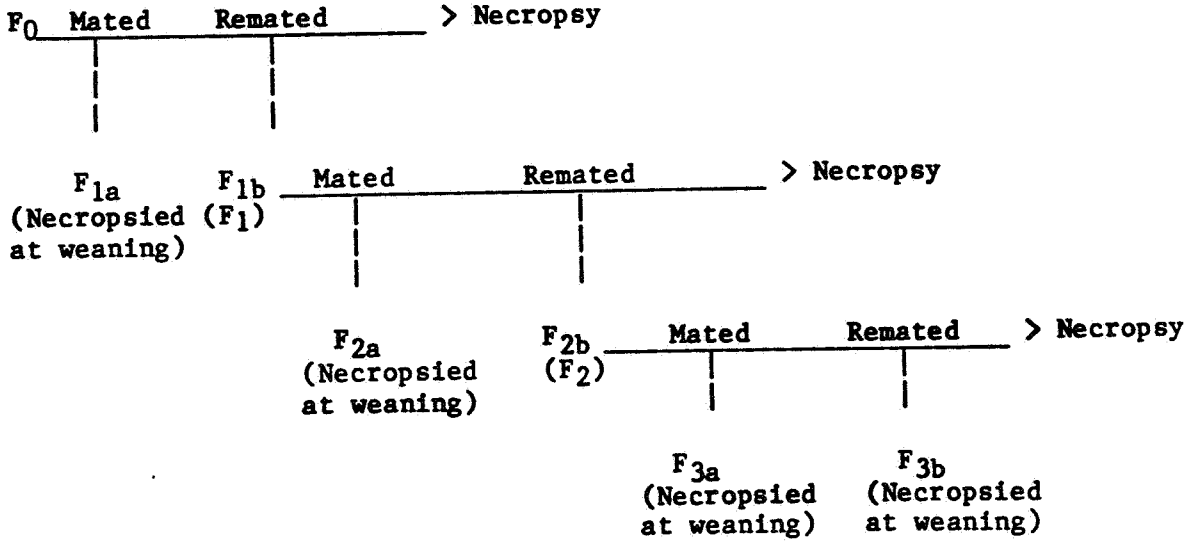
The test substance intake was calculated from individual body weight and food consumption data and reported as a group mean value (presented for weekly intervals during the growth and rest periods of all generations). Mean test substance intake values, as presented, were adjusted for 92.6% active ingredient concentration in the technical test material.

During the study treated diets were analyzed at preparation and at the end of the feeding period for weeks 1, 2, 3, 4, 6, 8, 12, 24, 36, 48, 60, 72, 88 and 94 to confirm the concentration and stability of alachlor in the diet preparations. Alachlor (near) was analyzed periodically to confirm its stability.

Breeding

The first generation (F_0) mated when 108 days old. Each parental generation (F_0, F_1, F_2) consisted of 10-12 male and 17-24 female rats. These parents were mated to produce two litters per generation. A 14-day rest period was allowed between weaning of the first litters (F_{1a} and F_{2a}) and initiation of the second mating period. Offspring from the second litters (F_{1b} and F_{2b}) were selected to be parents for the subsequent generation (See the following breeding diagram).

Shematic Diagram for 3-Generation Reproduction Study



Observation

Parental animals were observed daily for mortality or abnormal reactions. Once each week, animals were given a detailed physical examination. Body weights were measured weekly during growth and rest periods, on days 0, 6, 15 and 20 of gestation and on days 0, 4, 14, and 21 of lactation. Food consumption was determined weekly during the growth and rest periods. Pups were observed daily for mortality and were counted and weighed on days 0, 4, 14 and 21 of lactation. Specific observations for the reproduction aspects of this study included male and female fertility, litter size, number of pups (alive and dead) at birth, survival and lactation indices.

Necropsy

Parental animals were sacrificed at the time of weaning of their second litter. All F_2 parental animals were given a complete ophthalmic examination prior to sacrifice. All pups except those selected for production of succeeding generations were sacrificed at weaning. All animals were sacrificed by lethal exposure to ether.

All animals in the study were subject to a gross necropsy examination and abnormal tissues were saved in 10% neutral buffered formalin. Tissues were preserved from all parents and from 10 male and 10 female F_{3b} pups per group. For these animals, weights of adrenals, gonads, kidneys, brain, spleen, liver, heart and pituitary were recorded. Histopathological evaluation was conducted on the following tissues and organs from 10 male and 10 female control and high-dosage group parents (F_0, F_1 and F_2) and F_{3b} pups: adrenals, aorta, bone and bone marrow, brain, eyes with optic nerve and Harderian gland, gonads, heart, colon, duodenum, ileum, kidneys, liver, lungs with sciatic nerve, skin, spinal cord, spleen, stomach, thyroid, parathyroid, urinary bladder, uterus, prostate, thymus and any additional grossly observed lesions or tissue masses.

Statistical Analysis

Statistical analyses of data compared the treatment groups with the control group by various statistical methods. Effects were considered significant at $p < 0.05$.

II. Results

Alachlor Concentrations in Diet

Analysis of treated diets at the beginning of the feeding period indicated that the diet contained an average of 86 to 95% of the intended concentrations. Assays performed at the end of the weekly feeding periods indicated that 85 to 102% of the intended initial concentrations was detected in the diet samples. Analysis of the technical Alachlor (92.6% a.i.) during storage ranged from 81% to 100% with a mean value of 89.5%.

Thus the test material was stable on storage and in feed during the study period and the actual Alachlor concentration closely conformed to the intended dosages.

Mortality

F_0: No mortality occurred

F_1: One mid-dose male died on week 2 of growth
One high-dose male died on week 11 of growth

F_2: One low-dose male died early in mating period to produce F_{3a}; death occurred before mating with either of the two female partners.
One mid-dose female died on week 4 of growth

Death occurred spontaneously and Gross postmortem observations of the above animals reflected the following findings:

F_1 Mid-dose male - had an undersized spleen, hollow kidneys and the right kidney displaced posteriorly.

High-dose male - had black foci on the stomach glandular portion, dark red meninges and dark red pituitary

F_2 Low-dose male - had encrustation around nares and eyes, gas distention of the gastrointestinal tract, red mesentric lymph nodes and harderian glands, and scatted red foci over the lungs (all lobes and surfaces).

Mid-dose female - had foamy white discharge from nose and red discharge from mouth, slightly thickened and red urinary bladder, dark red adrenals, dark red lungs (all lobes and surfaces), and several tan foci on surface of both kidneys.

For surviving animals, the author indicated that the physical observation data were similar between control and treated groups.

Food Consumption and Body Weight

No significant difference was noted in food consumption between treated groups and control groups during the study. A trend towards slightly higher mean food consumption data was evident in the mid- and high-dose groups (males and females) of the F_1 and F_2 generations. Thus diet treatment with Alachlor did not cause any food avoidance.

Body weights for treated female rats were comparable or slightly higher (10 to 14%) than controls for the 3 generations (F_0, F_1, F_2) at the end of the growth period, especially at the high-dose level. However initial body weights (week 1 of growth period) for F_1 and F_2 were 10 to 12% lower than the controls at the mid- and high-dose levels.

Body weights for male rats of the mid- and high-dose groups of F_0 were slightly lower (5 to 7%) than the control group during most of the growth period and remained low (7 to 13%) for these two groups in the second generation (F_1). The same trend (14 to 15% lower than control) was also noted in the third generation (F_2). However, the difference in the mean weight change for treated males in the three generations was not remarkable (2 to 7%) as compared to the control groups.

Reproduction Data

•Parental Data

No statistically significant effects were noted on male fertility or female pregnancy rates. However, only 54-58% of F_1 females in each treatment group (3, 10 and 30 mg/kg) mated and delivered F_{2b} litters as compared to 79% in the control group. The following table (#1) reflects these data:

% (No. of pregnant females/Total No. of females initiated per group)

		Control	3 mg/kg	10 mg/kg	30 mg/kg
F_0	F_{1a}	75(18/24)	83(20/24)	95(21/24)	91(22/24)
	F_{1b}	100(18/18)	80(16/20)	86(18/21)	82(18/22)
F_1	F_{2a}	71(17/24)	75(18/24)	95(21/24)	63*(15/24)
	F_{2b}	79(19/24)	58*(14/24)	54*(13/24)	58*(14/24)
F_2	F_{3a}	71(17/24)	95(22/24)	91(21/23)	75(18/24)
	F_{3b}	94(16/17)	95(21/22)	76(16/21)	83(15/18)

*Remarkable reduction in the total reproductive activities (mating and/or pregnancy) in treated females as compared to the control females.

Table (#1) demonstrates that the maximum negative effect on reproduction (mating and pregnancy) is noted in F_1 generation second mating (F_{2b}). This effect is not dose-related i.e. reductions of 24%, 25% and 21% are noted at the low-, mid- and high-dose levels respectively as compared to the control groups. The reduction in pregnancy rates is also remarkable (12% lower than the control group) in the first mating (F_{2a}) at the high-dose level only. However, it is not clear if this effect noted in F_1 generation is compound-related or epizootic especially that parasitic infections were reported in F_{2b} pups.

°Litter Data

No adverse effects were noted in any generation for the ratio of live to dead pups at birth, for litter survival indices, lactation indices, or for pup body weights in this three-generation reproduction study.

Necropsy Data

°Gross pathology of the parental generations (F_0, F_1, F_2) and their litters (F_{1a}, F_{1b}, F_{2a}, F_{2b}, F_{3a}, F_{3b}) reflected compound-related effects on the kidneys at the high dose level in all parent generations and F_{3b} pups. The author reported that kidney's discoloration was noted in all groups (including controls) and that the incidence of this observation was higher for the high-dosage F_2 group than the other animals. Microscopic examination of 10 animals/sex of this F_2 high-dose group revealed a high incidence of chronic nephritis in males (8/10 animals) as compared to the control group (1/10 animals) but no difference was noted between the high-dose females and the respective control group.

No microscopic finding was noted for the high-dose F_0 and F_1 adults or F_{3b} female pups; however F_{3b} male pups reflected 20% increase over the control group in kidney pathology (1/10 animals had chronic nephritis and 1/10 animals had hydronephrosis).

No significant histopathological examination was done at the two lower dose levels.

In addition to the above findings, the high-dose male F_{3b} pup kidney weights were slightly elevated (5%) but not significantly as compared to the control group, and the kidneys/body weight ratio in this group was also elevated (16%, $p < 0.05$). This effect was not remarkable in F_{3b} female pups at any dose level. No data were reported on F_{1a}, F_{1b}, F_{2a}, F_{2b}, F_{3a} pups.

Kidney weights were also elevated for the high-dose F_2 males as compared to F_2 male controls (18%, $p < 0.05$) including the kidneys/body weight ratio (9%, $p < 0.01$), and for mid-dose F_2 females (5%, $p < 0.01$) including the kidneys/body weight ratio (10%, but not statistically significant). A trend towards increased kidney weights was not remarkable at the lower doses in this generation or at any dose level in F_0 and F_1 generations.

°Lower ovary weights were noted in the high dosage females in each parent generation and F_{3b} pups. This decrease was maximal (17%) and significant ($p < 0.05$) in the F_0 generation. This decrease was also associated with 17% decrease in the ovaries to body weight ratio in this group. A trend towards decreased ovary weights (not statistically significantly) was also noted at the lower dose levels in F_0 adults and F_{3b} pups, and at the mid-dose level of F_2 generation. However, this effect cannot be successfully correlated with the reduction in reproductive activities since the reduction was maximal in the F_1 generation and not in the F_0 generation. No microscopic changes were reported.

°Ocular effects were mainly noted in F_{1b} and F_{2a} pups. These effects ranged from simple eye irritation (especially in controls) to iritis, keratitis and corneal opacity. The following table represents the number of litters and pups affected per each of these 2 groups:

	F_{1b}		F_{2a}	
Dosage (mg/kg)	# of litters affected	# of pups affected/ # affected litters	# of litters affected	# of pups affected # affected litters
0.0	2/18	2/2	0/17	-
3.0	2/16	5/2	3/18	12/3
10.0	2/18	3/2	4/21	10/4
30.0	0/18	-	2/15	8/2

Ocular lesions are noted above at all levels tested in F_{1b} or/and F_{2a} litters including the F_{1b} control group. However this effect may not likely be compound-related since it is only apparent in F_{1b} and F_{2a} pups and not found in other generations. The progeny of F_1 adults (F_{2b} pups) were found to be infected with parasites; thus, it is like that the F_1 parent generation (originally F_{1b} pups) was also infected. Consequently the ocular effects noted in the above table may be epizootic.

Gross pathology of F_1 parents reflected eye effects at the low- and mid-dose groups (2/36 animals (1M + 1F) of the low-dose group and 2/36 animals (1M + 1F) of the mid-dose group had inverted eye lashes and/or closed eye lids). No eye lesions were noted in F_0. These data indicate that these ocular effects may not be compound related because the presence of these effects was not noted in other generations.

In addition, to the above findings ophthalmoscopic examination of F_2 parents at termination did not reflect remarkable eye injury (focal retinopathy in 1/12 males in each of the low- and high-dose groups and 1/24 females at the low-dose level).

°Parasites and worm infections were noted in F_{2b} pups of the control and treatment groups. No explanation was provided concerning any changes in sanitary or housing conditions that may have contributed to this infection especially that this problem was not present in litters of other generations (F_0 and F_2).

°Lungs Interstitial pneumonitis was noted in animals histopathologically examined (control and treatments). High incidence of this finding was reported in the male control groups of F_0 and F_2 parent generations (6/10 animals in each of these two groups were affected). No explanatation was provided.

III. Conclusions:

Alachlor did not cause significant adverse effects on the reproduction of adult rats at the three dose levels tested (3, 10 and 30 mg/kg) in this 3-generation reproduction study, (a 21 to 24% reduction in the second mating/pregnancy of F_1 generation was noted at the three dose levels tested as compared to the control group, however this effect was not dose-related and may be epizootic in origin). Compound-related effects were not remarkable on the litter size, survival and lactation indices, or the pup body weights.

However, gross pathology examinations indicated compound-related effects on kidneys (in parents and progeny) of the high-dose group males and females especially in the F_2 parent generation and F_{3b} pups i.e., kidneys discoloration and significant increases (5 to 18%, $p < 0.05$ at least) in kidney weights and kidney/body weight ratios (see necropsy section pages 7). These adverse effects were further confirmed microscopically by the presence of chronic nephritis in 8/10 animals of the F_2 high-dose males (compared to 1/10 animals in the control groups) and as healing infarct in 1/10 F_{3b} male pups in addition to hydronephrosis in another F_{3b} male pup (total of 2/10 animals as compared to none in the control group).

Lower ovary weights were also noted in the high-dose females of each parent generation and F_{3b} pups. This decrease was maximal in the F_0 generation (i.e. 17% decrease ($p < 0.05$) in ovary weights was noted). This decrease was also associated with 17% decrease ($p < 0.05$) in the ovaries to body weight ratio in this F_0 high-dose females.

Finally, NOEL for reproduction is 10 mg/kg and the LEL is 30 mg/kg (HDL) based on the above noted effects on the kidneys in both F_2 adults and F_{3b} pups at 30 mg/kg dose level. This effect on kidneys is more remarkable in male animals.

Classification: Core-Minimum

TS-769:th:TOX/HED:AMahfouz:9-21-81:card 3:Retype 12-15-81