Bladex 100101

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UNITED STATES ENVIRONMENTAL PROTECTION AGENCY

WASHINGTON, DC 20460

MAR-9 1990

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OFFICE OF
PESTICIDES AND
TOXIC SUBSTANCES

MEMORANDUM

SUBJECT: Review additional data submitted in support of a multigeneration study in rats with Bladex. EPA ID No. 352-475, EPA Record No. 246825, EPA MRID #. 411111@-01, HED PRoject No. 9-1636, Caswell No. 188C.

TO: Robert Taylor/Barnes (PM 25)
Herbicide-Fungicide Branch
Registration Division (H7505C)

FROM: Stephen C. Dapson, Ph.D. Stephen C. Dapson 3/7/90
Pharmacologist, Review Section I
Toxicology Branch - Herbicide, Fungicide, Antimicrobial Support/HED (H7509C)

THRU: Yiannakis M. Ioannou, Ph.D., D.A.B.T. J.M. Ioannou 3/7/90
Section Head, Review Section I
and
Marcia van Gemert, Ph.D.
Chief, Toxicology Branch - Herbicide, Fungicide, Antimicrobial Support
Health Effects Division (H7509C) M van Gemert 3/7/90

Registrant: E.I. du Pont de Nemours & Co. (Inc.)
Agricultural Products Department
P.O. Box 80038
Wilmington, DE 19880-0038

Action Requested: Review additional data submitted in support of a multigeneration study in rats with Bladex.

Recommendations: Upon review of the additional data submitted in support of a multigeneration study in rats with Bladex, the study is upgraded to Core-Minimum Data. The NOEL for reproductive toxicity is 3.8 mg/kg/day with a LOEL for reproductive toxicity of 11.2 mg/kg/day based on pup viability and decreased mean pup body weight during lactation of dams on a diet with 75 ppm. The LOEL for systemic toxicity is less than or equal to 1.8 mg/kg/day (LDT) based on decreased body weight of males (not statistically significant) and females ($p < 0.01$) of F1 adults at various time periods throughout the study.

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Background:

A 2-generation reproduction study in rats with technical Bladex (Cyanazine) was reviewed by TB-HFAS (Memo of S. Stolzenberg to R.Taylor, dated 2/16/88) and subsequently classified as Core-Supplementary Data. The study was considered upgradable if the following data were submitted to the Agency and found to be acceptable:

1.) Male Mating Index and Female Mating Index should be recalculated separately for each of the 4 matings. [original report combined F_{1a} with F_{1b} and F_{2a} with F_{2b}]

2.) All missing pups not included in the pathology data should be accounted for.

The registrant has provided this information in the present submission.

Discussion:

The following table presents the recalculated mating indices:

MATING PERFORMANCE SUMMARY

Dose:	Control	25 ppm	75 ppm	150 ppm	250 ppm
		F0-first mating			
Males	26/28	25/28	26/27	17/28*	24/28
%	93	89	96	61	86
Females	26/28	25/28	26/27	17/28*	24/28
%	93	89	96	61	86
		F0-second mating			
Males	25/28	23/27	23/27	18/28	22/27
%	89	85	85	64	81
Females	25/28	23/27	23/27	18/28	22/27
%	89	85	85	64	81
		F1-first mating			
Males	27/28	25/28	27/28	25/28	22/27
%	96	89	96	89	81
Females	27/28	25/28	27/28	25/28	23/28
%	96	89	96	89	82
		F1-second mating			
Males	22/27	22/28	25/28	26/28	22/27
%	81	79	89	93	81
Females	23/28	22/28	25/28	26/28	23/28
%	82	79	89	93	82

* = $p < 0.01$ compared to control using chi-square

The separate male and female mating indices for the F1 first and second matings reveal a statistically significant lower index for high mid dose males and females of the first mating and a slightly lower index for the second mating; however, this observation was not carried through the F2 first and second matings and is therefore, probably a chance occurrence.

The registrant also provided tables presenting the gross necropsy findings for the F1, first and second mating pups and the F2, first and second mating pups. No specific treatment related effects were noted. This presentation adequately accounts for the missing pups.

The study is upgraded to Core-Minimum Data.

The NOEL for reproductive toxicity is 3.8 mg/kg/day, with a LOEL for reproductive toxicity of 11.2 mg/kg/day based on pup viability and decreased mean pup body weight during lactation of dams on a diet with 75 ppm.

Systemic toxicity was observed at 1.8 mg/kg/day (LDT) based on decreased body weight of males (not statistically significant) and females ($p < 0.01$) of F1 adults at the time of terminal killing, around week 57 of the study and at various time periods throughout the study. This dosage (1.8 mg/kg/day) is based on intake of a diet containing 25 ppm of the test compound. Thus, the LOEL for systemic toxicity in adult rats exposed long-term to Bladex in the diet is considered to be less than or equal to 1.8 mg/kg/day (LDT).

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SECTION HEAD

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

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OFFICE OF
PESTICIDES AND TOXIC SUBSTANCES

MEMORANDUM

SUBJECT: Review of a Two-Generation Reproduction Study of Technical Bladex (Cyanazine) in Rats (Sprague-Dawley)

Caswell No.: 188C
TOX Proj. No. 8-0159

FROM: Sidney Stolzenberg, Ph.D.
Review Section V, Toxicology Branch
Hazard Evaluation Division (TS-769C

TO: Robert J. Taylor, PM 25
Fungicide-Herbicide Branch
Registration Division (TS-767C)

and

Joan Dizikes, PM 64
Special Review Branch
Registration Division (TS-767C)

THRU: Quang Q. Bui, Ph.D., D.A.B.T.
Acting Head, Review Section V
Toxicology Branch
Hazard Evaluation Division (TS-769C)

and

Theodore M. Farber, Ph.D., D.A.B.T.
Chief, Toxicology Branch
Hazard Evaluation Division (TS-769C)

Registrant: Agricultural Products Department
E.I. du Pont de Nemours & Company
Wilmington, DE

Action Requested:

Review the two-generation rat reproduction study with

cyanazine. The data are of particular concern because of developmental toxicity in tests with this compound has been previously observed in both rats and rabbits.

Background Information:

Anophthalmia and microphthalmia were observed at 25 mg/kg/day and at 75 mg/kg/day in 2 different studies with Fischer 344 rats and at 2 mg/kg/day in a study with New Zealand rabbits. Other frank indications of developmental toxicity were seen in both species, including dilated brain ventricles, cleft palate and diaphragm abnormalities in rats, domed cranium, dilated brain ventricles, thoracoschisis, alterations in skeletal ossification sites, decreased litter size, and increased postimplantation loss in rabbits. A NOEL for developmental toxicity was set at 5mg/kg/day in the rat and at 1 mg/kg/day in the rabbit (See reports by Q.Q. Bui of July 14 and August 14, 1987).

Conclusions and Recommendations:

In the present two-generation rat reproduction study with Sprague-Dawley rats, the doses of technical Bladex, admixed in the diet, were 0, 25, 75, 150, and 250 ppm. Based on food intake, the dosages in mg/kg/day throughout the study were generally similar for nonpregnant and pregnant F_0 and F_1 parents. During these stages, they averaged 0, 1.8, 5.3, 11.1, and 18.5 mg/kg/day, respectively. During lactation, these doses, based on food intake, were about twice as high and came to 0, 3.8, 11.2, 23.0, and 37.1 mg/kg/day, respectively, averaged for all four of the F_1 and F_2 lactational periods in the study.

The LEL for reproductive toxicity is considered to be 11.2 mg/kg/day, based on pup viability and decreased mean pup body weight during lactation of dams on a diet with 75 ppm. The NOEL found was 3.8 mg/kg/day (25 ppm).

Systemic toxicity, based on decreased body weight of F_0 and F_1 adults at various time periods and final body weight of males and females of F_1 adults prior to necropsy, was seen at 1.8 mg/kg/day (25 ppm). Dose-related increased toxicity based on decreased body weight and occasionally decreased food intake at all intervals during the course of this study was observed. Therefore, the LEL for systemic toxicity to the F_0 and F_1 adults is $\leq$1.8 mg/kg/day (LDT).

There was a 35% decrease in paired females that delivered a litter in the 150 ppm treated groups for both the F_{1a} and F_{1b} generations. A similar effect was not seen in the F_{2a} and F_{2b} generations nor was such an effect seen at the 250 ppm dose. The lower fertility in the 150 ppm group was considered a "random

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occurrence" by the applicant but since it occurred in two generations, we should not completely ignore the possibility of an effect.

In the tables which present the Male Mating Index and Female Mating Index, data for "both mating periods", i.e. F_{1a} with F_{1b} and F_{2a} with F_{2b} were combined. In its present form, these data are obviously higher than they would be if they were calculated separately for each mating and not combined for both matings. It would also give us a better perspective on the contribution of both of these parameters to the reproduction performances in each generation. The applicant should be requested to recalculate the Male and Female Mating Indices separately for each mating and not combined for two mating periods.

There is a question of reliability of the data on "Females with Evidence of Mating". Vaginal sperm or copulation plugs were not detected for a number of animals that became pregnant. It also appears likely that there were animals considered to have mated but actually did not, particularly if evidence for mating was based on copulation plugs under the cages instead of vaginal sperm. These data and other data derived from them should be considered unreliable.

In Tables 117 and 118 (volume 2 of the report) and Tables 152 and 153 (vol 4), the footnotes indicate "does not include pups found missing or cannibalized." In the F_{1a} generation, dams 23,703 and 23,748 lost all of their 8 pups between day 7-14 of lactation but only 2 or 3 of them are listed in the necropsy data which indicates the rest of the litter could be considered as missing. Pups that are missing may be due to experimental error or loss in handling. The applicant should be requested to account for all missing pups to the extent that this is possible (See page 12 of this DER).

Core Classification: Supplementary

This may be upgraded to Minimum if the following are submitted and considered satisfactory.

1). Male Mating Index and Female Mating Index should be recalculated separately for each of the 4 matings.

2). All missing pups not included in the pathology data should be accounted for.

Primary Reviewer: Sidney Stolzenberg, Ph.D.
Review Section V, Toxicology Branch
Hazard Evaluation Division (TS-769C)
Secondary Reviewer: Quang Q. Bui, Ph.D.
Review Section V, Toxicology Branch
Hazard Evaluation Division (TS-769C)

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DATA EVALUATION REPORT

Study Type: Reproduction: Two-Generation
Species: Rat (Sprague-Dawley)
Guidelines: 83-4

Study Title: Two-Generation Reproduction Study of Technical Bladex Herbicide (SD 15418) in Rats

EPA ID Nos.: 325-475, EPA Accession No. 403600-01, EPA Record No. 206097, Caswell No. 188C, Project No. 8-0159

Sponsor: Agricultural Products Department
E.I. du Pont de Nemours & Company
Wilmington, DE

Testing Laboratory: WIL Research Laboratories, Inc.
Ashland, OH 44805-9281

Compound Submitted By: Shell Development Company
Houston, TX

Study Nos.: SRO 15-87
WIL 93001

Study Period: August 29, 1985 to October 31, 1986

Date of Report: August 12, 1987

Study Author: Mark D. Hamec, B.S., Study Director. (The original study director was Dean Rodwell from initiation of study to issuance of draft for this final report, but he resigned.) Sponsorship of the study was transferred from Shell Development Company to DuPont on October 31, 1986.

Test Compound: Bladex technical, 100% (SD 15418, WRC 107F, Cyanazine) CAS No. 21725-46-2. Three batches of compound were used for this study. All received from Shell Development Company, Westhollow Research Center. Two were numbered TX 207 with an expiration date of April 1987. The third was numbered TX 213 with an expiration date of August 1987.

Doses: 0, 25, 75, 150, and 250 ppm.

Route of Administration: Admixed in diet.

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Quality Assurance:

A statement of compliance with GLP is signed by Deborah L. Little, Supervisor of Quality Assurance, dated August 12, 1987. Three deviations from GLP are indicated:

1. Five of each sex were not screened for ectoparasites and endoparasites prior to initiation of the study.

2. "There were slight deviations from protocol in the number of animals ordered and the age of animals at receipt."

3. Temperature and humidity "occasionally varied from the specified ranges."

It was concluded that these deviations did not affect the validity of the study.

A previous "initial" two-generation reproduction study with technical Bladex was initiated on August 7, 1985 but was aborted on August 28, 1985 due to suspicion of infection, possibly with a virus. The results of gross necropsies on 10 males and 10 females in control group and serological tests for 2 males and 2 females in control and high-dose groups did not reveal the cause of poor weight gain in the controls, which was the reason for aborting the study.

A summary of the initial study was presented in Appendix A. The procedure was identical to the main study. Initial starting weight of males was about 269 g and of females about 170 g in controls and in all 4 treated groups. Control males gained an average of 60.4 g the first week but lost 50.8 g the second week. Control females gained 13.9 g the first week but lost 18.8 g the second week. In contrast, all male and female treated groups gained weight the first and second weeks of the study and weighed significantly more than controls ($p < 0.01$). The decreased body weight gain in controls was accompanied by a significantly decreased food intake.

Necropsied animals at gross pathology (21st day) revealed no apparent abnormalities. Two controls and 2 high dose per sex were tested for viruses, including SDA/RCV, Sendai, PVM, Reo-3, KRV, H-1, GD-7, MAD, and LCMV. None were found positive to any virus.

Conclusion for Aborted Study:

The reason for poor performance of both the male and female control groups is unknown.

Diet Preparation and Analysis

Each test diet preparation was made by weighing out the proper amount of Bladex, dissolving in 150 mL acetone, then adding to 5 kg powdered rat chow as a premix. Diets were freshly prepared, usually at 3-week intervals. Appendix B listed dates of preparation for each diet and details of preparation.

Analytical support data pertaining to verification of compound by spectroscopy, its purity, and its composition in each dietary preparation was given in Appendix E. The Bladex preparation used in this two-generation study was entirely Sample No. 107F, also coded as Lot No. 06 AMK-5406. It was produced in 1979 by Shell Chemical Company "Mobile Plant." Analyses in 1980, April 1985, and August 1986 (the latter 2 date during the course of this rat study) all showed purities of 98 or 97.5 percent, considered "essentially unchanged" by the analytical chemist.

During the course of the main study, 25 mixings for each dietary dose were prepared. A stability check of dietary preparations of 25 and 250 ppm at 0, 2, 4, and 6 weeks of storage at 5 °C revealed virtually no change with time. A second diet stability test in which a 25 ppm diet was analyzed at 0, 3, 7, 24, and 27.5 h after preparation and stored at both 5 and 25 °C gave results suggesting slightly greater stability at 5 °C. Conclusion by the analytical chemist was that both the 25 and 250 ppm diet preparations were stable at 5 °C, refrigerator temperature. for at least 6 weeks (Table 1 and 2 of report). Storage of diets was therefore in the refrigerator.

Of the 25 mixes for each dietary dose level prepared during the course of this study, 5 were tested for homogeneity by taking samples from 3 different layers of the stored materials labeled as "top, middle, and bottom third." Variation was small, summarized as ± 9% at worst, "usually much less" (Tables 3, 4 and 6, 17, 26).

All 25 mixes for each dietary preparation were tested for analytical concentration of Bladex, usually after 2 to 3 days of refrigerated storage following its preparation. A number of them were analyzed 5 to 16 days after preparation. The vast majority of analyses fell below theoretical concentrations and the means came to -10.2, -11.5, -11.2, and -10.4 percent for the 25, 75, 150, and 250 ppm preparations. Occasionally, diets were as much as 25.2 or 33.2 percent below theoretical concentration.

The report on analysis of technical Bladex in rat chow is signed by J.R. Dawson, research chemist, dated February 20, 1987.

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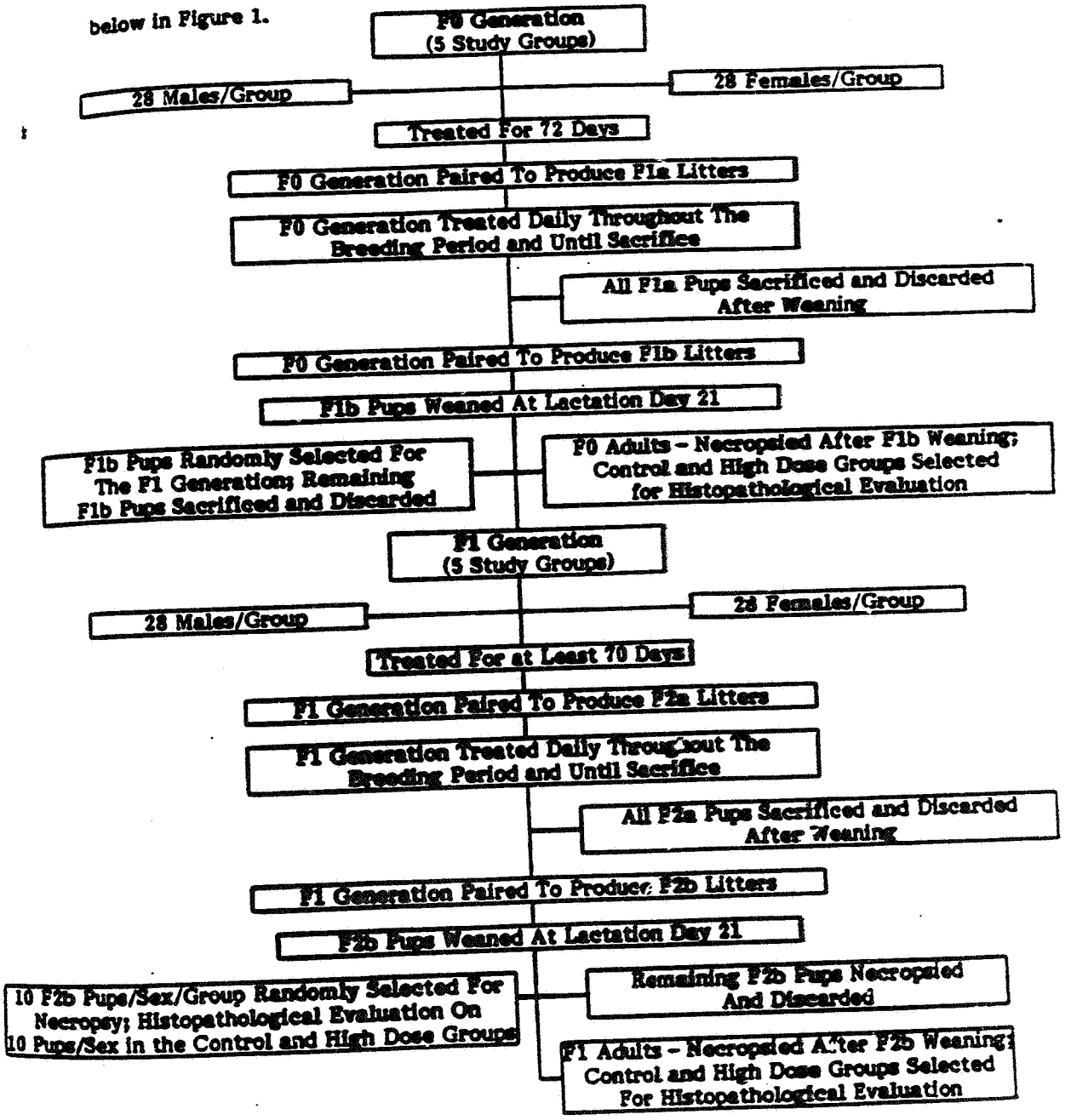
IV. STUDY DESIGN: (Figure 1)

A diagrammatic representation of the experimental design has been presented below in Figure 1.



Method

Sprague-Dawley Crl: COBS CD (SD) BR rats of Charles River Portage MI. 28 males and 28 females per group, starting at about 7 weeks of age, received 0, 25, 75, 150, and 250 ppm admixed in diet for a minimum of 72 days. Freshly mixed diets were prepared every 3 weeks. Dates of mating were determined by checking each day for vaginal sperm or copulation plugs. First F_0 mating occurred when the rats were about 17 weeks old. All females were allowed to give birth and all litters were culled on day 4 to 8 pups, half of each sex if possible. Offspring of F_1a litters that survived were all killed when weaned at 21 days of age. After a minimum of 10 days after weaning, F_0 parents were again bred (1 male to 1 female) to produce F_1b litters. F_1b pups were weaned at 21 days of age, when 28 of each sex per dose group were randomly selected as F_1 generation parents and the remainder were discarded. F_0 adults were necropsied at time of weaning the F_1b litters. Control and high-dose-treated F_0 adults were subjected to histopathology evaluation.

F_1 selected parents were treated for a minimum of 70 days. First mating at 16 to 17 weeks of age was around week 39 of the study to produce F_2a litters. F_2a litters were discarded at weaning, age 21 days, and about 10 days later the F_1 parents were again mated to produce F_2b litters. F_2b pups were weaned at 21 days of age when 10 pups of each sex per group were randomly selected for necropsy and gross pathology. Those on high dose and controls were subjected to histopathology evaluation. The remainder of the pups were discarded. All F_1 parents were necropsied after weaning the F_2b pups but only those in control and high-dose groups were selected for histopathology evaluation.

All litters of F_1 and F_2 generations were randomly culled to 8 pups maximum, 4 of each sex when possible, on day 4 postpartum. If a female did not mate, it was paired to another male at the same dose level a week later. Offspring dying during days 0 to 4 of lactation were subjected to a necropsy similar to the Staples technique. Normal gross necropsy was performed on those dying after day 4 and only tissues that appeared abnormal were preserved in formalin. No necropsies were performed on F_1a weanlings and nonselected F_1b weanlings. All F_2a weanlings and nonselected F_2b weanlings were also discarded without necropsy examination. Macroscopic pathology was performed on all F_0 and F_1 male and female parents, also the selected F_2b pups, which included all orifices and organs, external and cut surfaces of brain and spinal cord. Weights of liver, right and left testes, and ovary from adults were obtained. Histopathology was limited to grossly observed lesions and reproductive organs: in males testes, epididymides, prostate, and seminal vesicles, whereas in females ovaries, uterus and vagina.

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Statistics included Chi-square tests with Yates correction factor for F_0 and F_1 male and female gestation indices, also for stillbirths and pup sex ratios on day 1 lactation in all 4 reproduction studies. Dunnett's test was performed on mean viable pups, gestation length, survival indices, and litter weights on days 1, 4, 7, 14, and 21 of F_{1a}, F_{1b}, F_{2a}, and F_{2b} pups, parental weight and weight gains of F_0 and F_1 adults including before mating, during mating, gestation, and lactation; also food consumption, organ weights.

It should be noted that all exposures to compound admixed in diets were continuous for all F_0 and F_1 animals throughout the entire study. This also means that pups closer in age to weaning could feed on the diet or at an earlier age, particularly before growth of fur, could possibly absorb compound through the skin.

Results:

F_0 Generation:

Mortality - One male on 75 ppm dose during week 17, 2 females on 25 ppm during weeks 13 and 24, 3 females on 75 ppm during weeks 1, 24 and 29, 1 female at 250 ppm during week 15: another female on 150 ppm was killed in moribund state during F_{1b} delivery. Deaths were not attributed to treatment.

Clinical Observations - No compound-related effects.

Body Weight and Weight Gains - (Obtained weekly). Mean body weight at the initiation of the study before treatment was the same in controls and in all 4 treated groups. At weeks 1 and 2 weighings, body weight of all 4 treated male and female groups became higher than controls ($p < 0.01$) because of a 22 g lower increase in weight gain for controls of both sexes at week 1 versus the lowest dose 25 ppm groups. Nevertheless, there was clearly a dose-related decreased body weight gain with both sexes for the 3 highest dose groups if comparisons for them were made to low-dose-treated group. By week 8 in males and week 5 in females, 250 ppm highest dose became significantly lower than respective controls. Then, by week 9 in males and week 10 in females, the 150 ppm dose group became significantly lower than controls. The 2 highest dose groups remained lower, dose-related, than controls to week 30, the time of F_0 necropsy of males and females, signifying toxicity of the 2 highest doses, 150 and 250 ppm treated males and females. The dose-related trend in body weight decrease included the 75 ppm group, especially the females, where it became significant at weeks 8 to 10. Overall, from weeks 0 to 30, both males and females in the 3 highest dose groups showed a dose-related

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decreased weight. Throughout F_1a and F_1b gestation and lactation body weights were significantly lower than controls in the dams of the 2 highest dose treated groups at most time periods ($p < 0.05$ to 0.01). Generally, these were not due to further weight decrease in the 2 highest dose treated groups but were rather due to lower initial weights caused earlier by drug treatment, i.e., prior to gestation and lactation.

Food Consumption - (obtained weekly). Tables are presented both in terms of g/animal/day and g/kg/day, based on mean values for each dose group. During the first 2 weeks, the amount consumed by male and female controls was unusually low, reflecting poor weight gain. Therefore, food consumption, based on g/animal/day or g/kg/day, was higher in all 4 treated groups than in controls of both sexes ($p < 0.01$). The reason for this decreased food intake by controls during this time period could not be explained. From weeks 3 to 6 in males, all 4 treated groups had significantly lower feed intake than controls, based on g/kg/day, but in females, significantly lower feed intake by all 4 treated groups occurred during the third week, then during the fifth week. Feed consumption was usually lower in treated groups than in controls (based on mg/kg/day), especially at the 2 highest dose levels for both sexes. During the F_1a and F_1b gestation periods, there were no obvious effects of compound treatment on food intake. Diminished food intake was seen during both lactational periods, significant only during the F_1a lactation for the 250 ppm group.

Mean compound intake in mg/kg/day based on food intake measurements were as follows:

Dose in ppm, F_0 Parents	0	25	75	150	250
	Compound Dosage (mg/kg/day)				
Females, excluding time of breeding, gestation, lactation	-	1.67	4.97	10.00	16.66
Females during F_1a gestation	-	1.83	5.25	10.65	18.50
Females during F_1b gestation	-	1.75	5.25	10.50	17.50
Females during F_1a lactation	-	2.98	9.15	19.80	26.50
Females during F_1b lactation	-	3.75	11.03	22.20	36.20
Males	-	1.43	4.32	8.80	15.26

Reproductive Performance

Data in the table that follows, Summary of Reproductive Performance, were compiled from 6 tables of the report (Tables 28 to 33). Males and females in the same dose groups were cohabited in pairs.

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There is some questions on reliability of the data on Females with Evidence of Mating in the table. Dates of insemination were missed for a number of females. It also seems possible to have mistakenly considered an animal to be inseminated particularly if based on a copulation plug under the cage rather than vaginal sperm.

Data on "Male Mating Index" and "Female Mating Index" were presented combined "for both mating periods", which we believe signifies for the F_{1a} and F_{1b} generations. In its present form these values would be higher than if they were not combined for the 2 mating periods and it is not possible to determine the contribution of these values to the reproductive performance of each group for each of the 2 generations.

The number of dams delivering a litter was reduced by 35% in the 150 ppm groups for both the F_{1a} and F_{1b} matings (See table which follows). The reason for the decreased number delivering a litter in the 150 ppm groups of both matings is unknown. It could have been due to decreased fertility, increased pregnancy terminations or other reasons. It may have been "a random occurrence" as suggested by the applicant, since it did not occur at the 250 ppm dose group, nor was it observed in the F_{2a} and F_{2b} generations. Since it occurred in 2 generations, we should not ignore the possibility of an effect.

There were no effects of treatment on length of the gestational period, sex ratio of pups at birth, or on mean gestation index (see footnote in table that follows) in the F_{1a} or F_{1b} litters In the F_{1b} part of the study not shown in the table that follows, 1 dam on 75 ppm died on day 22 of gestation, 1 dam on 150 ppm was killed in moribund state on day 23 of gestation, both of dystocia. It was claimed that dystocia was not a compound-related effect. There was no effect of compound on live litter size at birth and no increased number of dead pups at birth in the treated groups. There was an unusually large number of pups born dead or that died within the first 24 hours of birth in the control group of the F_{1b} generation, which was responsible for significantly lower number of dead pups in the treated groups.

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SUMMARY OF REPRODUCTIVE PERFORMANCE, F_1a and F_1b COMBINED

Dose Group (ppm)	0	25	75	150	250
Number of Females/GP° - F_1a	28	28	27	28	28
F_1b	28	27	27	28	27
Male Mating Index (%)+	100(28/28)	96(27/28)	100(28/28)	89(25/28)	100(28/28)
Female Mating Index (%)+	96(27/28)	100(27/27)	100(27/27)	86(24/28)	89(25/27)[c]
Females with Evidence of Mating# - F_1a (%)	100	89	96	93	89
- F_1b (%)	96	93	96	89	96
Number Delivering a Litter. F_1a	26	24	26	17	24
Number Delivering a Live Litter. F_1a	26	24	26	17	23
Gestation Length ± SD. F_1a (days)	22.2 ± 0.6	22.0 ± 0.7	21.9 ± 0.6	21.9 ± 0.3	21.9 ± 0.5
Number Delivering a Litter. F_1b	25	23	23	16	22
Number Delivering a Live Litter, F_1b	24	22	22	15	21
Gestation Length ± SD. F_1b (days)	22.8 ± 0.8	22.0 ± 0.7	22.0 ± 0.7	21.9 ± 0.6	21.8 ± 0.6
Male Ratio in Litter - F_1a (%)	49.5	53.6	51.5	53.8	45.3
- F_1b (%)	54.1	47.0	52.1	50.2	49.0
Gestation Index - F_1a (%)	100	96	100	100	100
- F_1b (%)	96	96	96	88	95
Mean Live Litter Size at Birth - F_1a	13.2	13.3	13.7	14.9	13.9
- F_1b	12.8	13.2	13.2	13.5	12.6
Total Number of Pups Born Dead in all Litters, Combined - F_1a	5	8	9	4	7
- F_1b	21	2**	2**	8	4**

° There were 28 males in every group during F_1a and F_1b.
+ Data for F_1a and F_1b were combined.
# Evidence of mating was based on vaginal sperm.
**$p < 0.01$ by Chi-Square test.

$$\text{Mating Index} = \frac{\text{Number mating that resulted in pregnancy}}{\text{Total number cohabited}} \times 100$$

$$\text{Gestation Index} = \frac{\text{Number giving birth to a live litter}}{\text{Number of pregnancies}}$$

[c] Includes 1 female that didn't deliver but had implantation scars at necropsy.

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Litter Performance

The tables on the 2 pages that follow are summaries of viability indices and body weights during lactation of F_1a and F_1b pups as an indication of the performance of the offspring.

A reduction in viability index was seen in the F_1a 250 ppm treated group by day 14 ($p < 0.01$) with reductions also seen for 75 and 150 ppm groups by day 21 but not statistically significant. Six dams in the 250 ppm group and 1 in the 150 ppm group had lost their entire litters whereas entire litter loss did not occur in dams of control or in lower dose treated groups. Sharply decreased mean pup weights were seen in the 150 and 250 ppm groups ($p < 0.05 - 0.01$) starting on day 4 and continuing throughout lactation. Decreased mean pup weights were also seen in the 75 ppm treated group by day 14 (n.s.) and on day 21 ($p < 0.05$) of lactation.

In the F_1b generation there was an unusually high level of mortality among pups in the control group especially between day 0 to 1 and again between days 14 to 21 of lactation. Based on the poor performance of the control group in this study. there were no effects of treatment on viability index and only the 250 ppm dose caused a decrease ($p < 0.01$) in body weight between days 14 to 21 of lactation.

SUMMARY OF PUP VIABILITY INDICES

F_1A Pups
Viability Index on Lactation Day (A)

Dose ppm	Day 1 %	Day 4 Before Culling %	Day 4 After Culling %	Day 7 %	Day 14 %	Day 21(B) %
0	99.8	98.7	100.0	100.0	100.0	99.5
25	98.3	97.5	100.0	98.9	98.9	98.9
75	99.5	98.5	100.0	99.5	97.7	92.9
150	98.1	95.1	100.0	99.3	98.6	89.8
250	94.9	93.2	100.0	99.5*	76.2**	75.0**

F_1b Pups
Viability Index on Lactation Day (A)

Dose ppm	Day 1 %	Day 4 Before Culling %	Day 4 After Culling %	Day 7 %	Day 14 %	Day 21(B) %
0	95.3	94.2	100.0	99.5	98.5	94.8
25	96.5	95.9	100.0	98.9	98.3	98.3
75	99.0	97.5	100.0	98.9	98.9	98.9
150	93.9	90.9	100.0	99.2	91.7	91.7
250	98.1	96.5	100.0	99.4	98.2	94.7

(A) = Mean values calculated using individual values.
(b) = lactation index.
Pup survival compared using Dunnett's test.
**p < 0.01 (Dunnett's test).

Viability Index (%) Before Culling = $\frac{\text{No. Pups available per litter Day 4 before culling}}{\text{No. pups viable per litter Day 1}} \times 100$

Viability Index (%) After Culling = $\frac{\text{No. pups viable per litter on Day (N)}}{\text{No. pups viable per litter on Day 4 after culling}} \times 100$

SUMMARY OF MEAN PUP WEIGHTS DURING LACTATION

Dose (ppm)	0	25	75	150	250
N (on Day 0)	26	24	26	17	23
		F_1a Litters - Mean ± SD			
Day 1	6.6 ± 1.0	6.6 ± 1.0	6.6 ± 0.5	6.2 ± 0.6	6.1 ± 0.
4+	9.3 ± 1.5	9.3 ± 1.7	9.0 ± 0.8	8.1 ± 1.2*	8.2 ± 1.
4 -	9.3 ± 1.6	9.3 ± 1.6	9.0 ± 0.9	8.1 ± 1.3*	8.3 ± 1.
7	14.9 ± 2.3	15.4 ± 2.1	14.9 ± 1.5	12.8 ± 2.6*	11.5 ± 2.
14	24.1 ± 3.6	24.4 ± 3.8	24.9 ± 4.5	20.6 ± 3.8*	20.1 ± 4.
21	40.0 ± 6.0	37.9 ± 6.2	34.8 ± 6.9*	28.1 ± 5.2**	29.5 ± 6.
N (On Day 0)	24	22	22	15	21
		F_1b Litters - Mean ± SD			
Day 1	6.7 ± 0.8	6.6 ± 1.1	6.7 ± 1.1	6.3 ± 0.7	6.5 ± 0.
4+	9.3 ± 1.3	9.1 ± 1.9	9.4 ± 1.9	8.5 ± 1.3	9.0 ± 1.
4 -	9.3 ± 1.3	9.3 ± 1.8	9.4 ± 1.9	8.5 ± 1.3	9.0 ± 1.
7	14.6 ± 1.7	14.5 ± 2.9	14.8 ± 2.5	13.1 ± 2.9	13.2 ± 2.
14	26.2 ± 3.5	25.3 ± 5.2	25.5 ± 4.6	24.0 ± 3.0	23.9 ± 3.
21	37.3 ± 7.2	38.6 ± 6.3	38.2 ± 7.1	34.1 ± 3.4	30.5 ± 4.

N = Number of litters.

4+ Before culling on Day 4
4- After culling on Day 4

*$p < 0.05$ by Dunnett's test.
**$p < 0.01$.

Litter Losses During Lactation

For F_1a, 6 litters were entirely lost (100% dead) in 250 ppm group between days 7 to 14. For F_1b, 1 litter was entirely lost in 150 ppm group between days 7 to 14.

General Physical Condition and Mortality

This is a narrative summary of individual animal necropsy data from tables 117, 118, and 118A in volume 2. It includes all animals found dead in the F_1a and F_1b litters during the 21 days of lactation (Tables 117 and 118) and those found dead in F_1b litters between age 21 to 30 days, prior to selection of F_1 parents. These tables obviously include among the dead those that were missing and not actually found dead. For example, in the F_1a high dose group, dam #23703 lost all her pups between days 7-14 of lactation but only 3 of them are accounted for in the necropsy data of Table 117. Dam #23748 also lost all 8 pups between days 7-14 of lactation but only 2 of them are accounted for. These are only two examples which require accountability for missing pups.

In F_1a litters, there were 8 control deaths, each of them from a different litter. One male had external anasarca and brachydactyly. At 25 ppm, there were 7 deaths in 1 litter and 5 additional deaths in separate litters. One female had distended bilateral ureters. At 75 ppm there was a total of 22 deaths in 10 litters, of which 8 occurred in a single litter, 2, 3, and 3 deaths in 3 other litters, 1 in each of 6 other litters. No remarkable findings were found. At 150 ppm, there were 25 dead pups within 8 litters, of which there was 5, 7, 6, 2, and 2 deaths in litters with more than 1 death. No remarkable findings occurred. At 250 ppm, there was a total of 61 deaths within 15 litters. Multiple deaths (i.e., 2 or more) in 11 litters occurred numbering 9, 3, 2, 2, 2, 7, 3, 2, 8, 8, and 3. No remarkable findings were observed. A large number of pups on 250 ppm dose, at least 30, were small in size, but otherwise "not remarkable."

In the F_1b litters, controls had 29 deaths in 10 litters with multiple deaths in 7 litters, in which there were 3, 4, 3, 3, 7, 3, and 2 deaths; no remarkable findings in gross pathology. In 25 ppm, there were only 3 deaths all occurring within 3 separate litters; no remarkable findings in gross pathology. At 75 ppm, there were only 3 deaths in 3 separate litters; no remarkable findings. At 150 ppm, there were 6 deaths in 4 litters, 2 of the litters with 2 deaths. At 250 ppm, there were only 8 deaths in 5 litters, 4 of them occurring in 1 litter; no remarkable observations at gross pathology examination.

Of the F_1b rats that were being held to day 30 for possible use as F_1 generation adults that died, 3 controls from 3 separate litters were found. At 25 ppm, 3 from 3 litters were found. At 75 ppm, only 1 died. At 150 ppm, only 1 died. At 250 ppm, 34 from 10 litters died. No remarkable findings were reported for the necropsy results.

Pathology of F_0 Parents

Necropsy of Those Dying During the Study (From Table 36. Vol. 1 and from Table 119 in Vol 3).

No controls died. At 25 ppm 2 females died 1 on day 96 of study (day 20 estimated gestation) of renal failure the second on day 168 of undetermined cause. At 75 ppm. 1 male on day 124. 3 females on days 8. 171. and 208 of undisclosed causes. At 150 ppm. 1 female died during late pregnancy on day 132. At 250 ppm. 1 female died on day 109 of study of undetermined cause. It is claimed that no compound related deaths were evident.

Scheduled Necropsy (Week 30 days 210 and 211)

The table that follows clearly illustrates the dose-related decreased body weights in the treated 75. 150. and 250 ppm males and females on the day of scheduled necropsy.

Final Body Weight + SD at Terminal Sacrifice Week 30

Dose (ppm)	0	25	75	150	250
Males	534.0 + 40.7	550.1 + 54.5	522.4 + 45.4	474.3 + 36.5**	450.8 + 40.3**
(Number)	(28)	(28)	(27)	(28)	(28)
Females	314.9 + 24.1	309.6 + 22.2	301.3 + 25.9	282.9 + 15.2**	272.6 + 27.6**
(Number)	(28)	(26)	(25)	(27)	

**$p < 0.01$ Dunnett s test.

Organ Weights (from Table 38 in Vol. 1 of report)

In the table below. the decreased absolute weights of liver of both sexes occured only in the 250 ppm dose treated groups of males and females. However. there was no effect on relative liver weight at any dose level for either sex. There were also no effects on absolute weights of testes or ovaries nor on relative weights of ovaries. The increased relative weights of testes in the 2 highest dose groups was considered by the investigators as being not biologically meaningful since it was not accompanied by macroscopic or microscopic changes. The conclusion was that there were no effects on liver or gonadal weights in either sex.

Organ Weights at Necropsy in Males (From table 40 of report)

Dose (ppm)	Absolute (g ± SD) 0	25	75	150	250	Relative (g ± SD) 0	25	75	150	250
Liver	20.1 ± 2.6	20.8 ± 3.6	19.6 ± 3.1	18.3 ± 2.9	17.7 ± 2.5*	3.76 ± 0.40	3.78 ± 0.50	3.75 ± 0.43	3.86 ± 0.53	3.93 ± 0.37
Left Testis	1.72 ± 0.14	1.72 ± 0.17	1.75 ± 0.16	1.71 ± 0.21	1.60 ± 0.15	0.32 ± 0.03	0.31 ± 0.03	0.34 ± 0.04	0.36 ± 0.04**	0.40 ± 0.04**
Right Testis	1.73 ± 0.14	1.71 ± 0.17	1.84 ± 0.43	1.73 ± 0.18	1.77 ± 0.18	0.33 ± 0.03	0.31 ± 0.03	0.35 ± 0.08	0.37 ± 0.03**	0.40 ± 0.05**

Organ Weights at Necropsy in Females (From table 39 of report)

Dose (ppm)	Absolute (g ± SD) 0	25	75	150	250	Relative (g ± SD) 0	25	75	150	250
Liver	12.8 ± 1.49	12.5 ± 1.2	12.6 ± 1.3	12.0 ± 1.9	11.6 ± 2.1*	4.07 ± 0.31	4.05 ± 0.20	4.18 ± 0.31	4.24 ± 0.47	4.25 ± 0.54
Left Ovary	0.042 ± 0.012	0.046 ± 0.014	0.042 ± 0.011	0.039 ± 0.015	0.040 ± 0.014	0.014 ± 0.004	0.015 ± 0.005	0.014 ± 0.004	0.014 ± 0.005	0.015 ± 0.005
Right Ovary	0.042 ± 0.010	0.040 ± 0.012	0.042 ± 0.012	0.037 ± 0.014	0.038 ± 0.012	0.014 ± 0.003	0.013 ± 0.005	0.013 ± 0.003	0.013 ± 0.004	0.014 ± 0.004

* $P < 0.05$ by Dunnett's test

** $P < 0.01$

Gross Necropsy of F_0 Parents (From Table 37 and 119 of the report).

A summary table for gross pathology observations at terminal kill for all males and females was presented but no effects of treatment appeared obvious in any organ.

Microscopic Pathology of F_0 Parents (From Table 41)

Lesions observed at necropsy in all animals and reproductive organs from all on highest (250 ppm) dose and controls were included. No compound related effect was observed.

Results with F_1 Parents

Mortality of F_1 Adults - One male control, 1 female 75 ppm dose, one female 150 ppm dose found dead weeks 45, 57, and 53, respectively.

Clinical Observations - No compound-related effect was obvious throughout the study at any dose level.

Body Weight and Body Weight Gains (Weeks 28 to 59 of the study). Decreased body weight was already apparent at week 28 for the 75 ppm (n.s.), 150 ppm ($p < 0.05$), and 250 ppm ($p < 0.01$) doses when F_1 parent exposure was just starting after weaning and mean control weight was 57.8 g. By week 33, mean weight of 75 ppm males and females were significantly less than controls and those on 25 ppm were also decreased (n.s.) but the decreased body weight for the 25 ppm dose became significant for males at about 45 weeks and after, for females at 34 weeks and after. Total body weight gains for the entire period between weeks 28 to 59 were significantly depressed in all 4 treated male and female groups in a dose-related manner.

During both the F_{2a} and F_{2b} gestations, mean body weights of dams were significantly lower than controls (dose related) in all 4 treated groups at every time period, including days 0, 6, 12, 15, 18, and 20. Total body weight gain throughout the F_{2a} gestational period including days 0 to 20 were significantly depressed only for the 150 and 250 ppm groups and for the F_{2b} generation only for the 250 ppm group.

During lactation, body weights were significantly depressed in a dose-related manner from day 1 of lactation on to day 21 in both F_{2a} and F_{2b} generation. However, there was generally no significant decrease is body weight gain due to treatment during lactation.

Food Consumption - Obtained weekly. When based on g/animal/day, food intake for F_1 males was decreased for the 75, 150, and 250 ppm dose groups from weeks 28 to 59 of the study ($p < 0.01$ at every time period throughout the study for the 2 highest doses). When converted to g/kg/day, mean food intake was no longer less than controls in the treated groups; in fact, it tended to be significantly higher ($p < 0.01$) at most of the time periods for the 250 ppm group, sometimes higher ($p < 0.05$ to 0.01) for the 150 ppm group. Obviously, the higher food intake based on g/kg/day is due to a correction for higher mean body weights: body weight was highest for controls and inversely dose related in the treated groups. The females, during non-gestation or non-lactation periods, similarly had decreased food intake at most of the time periods for the 75, 150, and 250 ppm doses, if based on g/animal/day. When converted to g/kg/day, there were similarly no differences in food intake for the 25, 75, and 150 ppm doses, but it was generally increased for 1/3 of the time periods with the 250 ppm dose. Mean food consumption during the F_{2a} and F_{2b} gestational and F_{2a} lactation periods were lower than controls for most time periods, especially for the 75, 150, and 250 ppm doses only when based on g/animal/day but was no longer apparent if based on g/kg/day. Differences in treated animal food intake were not apparent during F_{2b} lactation whether based on g/animal/day or g/kg/day.

The following was an estimation of dosage as mg/kg/day at various stages of the study, based on feed consumption measurements.

Feed Content of Bladex (ppm)	0	25	75	150	250
	Compound Dosage (mg/kg/day)				
Males and females, excluding breeding, gestation, lactation	-	2.01	6.04	12.26	21.08
During F_{2a} gestation (mg/kg/d)	-	1.90	5.55	11.25	20.25
During F_{2b} gestation (mg/kg/d)	-	1.73	5.10	12.20	17.25
During F_{2a} lactation (mg/kg/d)	-	4.23	12.60	24.30	42.00
During F_{2b} lactation (mg/kg/d)	-	4.13	11.93	25.80	43.75

Reproductive Performance, F_1 Rats

Males were cohabited with females that had received the same dosage of compound for about 70 days, starting after weaning. F_{2a} mating occurred during weeks 40 to 43, F_{2b} mating occurred at 50 to 53 weeks of the study.

Data in the table which follows were compiled from Tables 69-73 of the report, which basically is similar in presentation as the previous data on Reproductive Performance. We are similarly

requesting that the data which were combined for the Male and Female Mating Indices be separated for the F_{2a} and F_{2b} generations. There is again the question of results on Females with Evidence of Mating and the data on any Reproductive Indices which may have been derived from these results.

Based on the data in the table on Reproductive Performance which follows, there was no reduction in females delivering a litter in any treated group, no evidence of increased number of dead litters or of dystocia at parturition. Although pregnancy duration was 22.2 days for control groups of both the F_{2a} and F_{2b} pregnancies, gestation length was apparently reduced ($p < 0.05$ to 0.01) in all 4 treated groups by 0.5 to 0.8 days of the F_{2a} but no such effect for the F_{2b} pregnancy. The applicant considered the decreased gestation period in the F_{2a} pregnancies as not biologically meaningful. Gestation index was 100 percent in virtually all control and treated groups for F_{2a} and F_{2b} pregnancies. In addition, there were no effects on live births per litter. Although total number of pups born dead was increased for the 75 ppm group of the F_{2b} litters ($p < 0.05$), it was considered a sporadic effect due to 2 litters with all or almost all pups dead on day 0. Sex ratio at birth was similar in control and treated groups for litters of both generations.

MARY OF REPRODUCTIVE PERFORMANCE; F_{2a} and F_{2b}

e Group (ppm)	0	25	75	150	250
ber in Group; each sex	28	28	28	28	27=
ing Index, Males (%)+	96(27/28)	96(27/28)	100(28/28)	100(28/28)	93(25/27)
ing Index, Females (%)+	96(27/28)	96(27/28)	96(27/28)	96(27/28)	93(26/28)
ales with Evidence of Mating# - F_{2a} (%)	82	93	96	100	96
- F_{2b} (%)	93	93	96	96	100
ber Females Delivering (F_{2a})	27	25	27	25	23
ber Delivering Live Litter, (F_{2a})	23	24	26	25	23
ation Length ± SD, F_{2a})	22.1 ± 0.6	21.5 ± 0.6**	21.4 ± 0.5**	21.3 ± 0.5**	21.6 ± 0.5*
er Females Delivering F_{2b})	23	22	25	25	23
er Delivering Live itter, (F_{2b})	22	21	24	24	23
ation Length ± SD, F_{2b})	22.1 ± 0.4	22.1 ± 0.6	21.9 ± 0.7	22.0 ± 0.4	21.8 ± 0.5
ation Index - F_{1a} (%)	100	100	100	96	100
- F_{1b} (%)	100	100	100	100	100
Litter Size F_{2a}	12.4	13.7	12.7	12.3	11.0
Litter Size F_{2b}	13.0	13.2	12.8	13.4	13.2
. Dead Pups at Birth					
all Litters - F_{2a}	11	5	7	21	5
- F_{2b}	10	4	28$	4	7
Ratio in F_{2a} (%)	45.6	51.6	55.6	48.5	43.4
Ratio in F_{2b} (%)	50.7	50.9	50.8	50.0	50.3

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ludes both mating periods. Apparently, F_{2a} and F_{2b} were combined.
males, 28 females were included.
ed on vaginal sperm in paired female or copulation plug.
0.05 by the Chi-Square test (comparing total) number of dead in all litters
bined per group.
0.05 by Dunnett's test.
0.01 by Dunnett's test.

litter size = total number of pups/number of litters.

g Index = $\frac{\text{Number mating that became pregnant}}{\text{Total number cohabited}} \times 100$

ion Index = $\frac{\text{Number giving birth to live litter}}{\text{Number of pregnancies}} \times 100$

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Litter Performance

Summaries of mean live litter size based on viability indices and of mean pup body weights throughout lactation are given in the 2 tables that follow as indications of performance of the offspring.

Viability index was decreased only on days 1 and 4 before culling in the 150 and 250 ppm groups of the F_2a generation. No significant reduction in viability index was seen after culling in any treated groups of both the F_2a and F_2b generations. However, decreased pup weights were clearly evident during lactation even after culling on day 4 in a dose-related manner for the 2 highest doses in the F_2a generation and for the 3 highest doses in the F_2b generation.

There was no effect of compound treatment at any dose level on number of lactating dams that lost the entire litters.

SUMMARY OF PUP VIABILITY INDICES

F_2A Pups
Viability Index on Lactation Day (A)

Dose ppm	Day 1 %	Day 4 Before Selection %	Day 4 After Selection %	Day 7 %	Day 14 %	Day 21(B) %
0	98.9	98.6	100.0	99.6	97.7	97.7
25	99.7	98.9	100.0	99.5	99.5	99.5
75	98.0	97.3	100.0	100.0	100.0	99.6
150	83.2**	82.1**	100.0	99.5	99.5	99.5
250	85.6*	83.6*	100.0	98.4	96.3	90.9

F_2b Pups
Viability Index on Lactation Day (A)

Dose ppm	Day 1 %	Day 4 Before Selection %	Day 4 After Selection %	Day 7 %	Day 14 %	Day 21(B) %
0	99.7	99.7	100.0	100.0	100.0	99.0
25	97.3	96.1	100.0	98.3	98.3	98.3
75	95.0	92.3	100.0	98.5	92.3	90.2
150	97.2	96.4	100.0	99.5	98.0	92.6
250	94.7	89.6	100.0	96.8	95.7	94.6

(A) = Mean values calculated using individual values.
(B) = lactation index.
Pup survival compared using Dunnett's test.
*p $\leq$ 0.05.
**p < 0.01 (Dunnett's test).

Viability Index (%) Before Culling = $\frac{\text{No. pups viable per litter Day 4 before culling}}{\text{No. pups viable per litter Day 1}} \times 100$

Viability Index (%) After Culling = $\frac{\text{No. pups viable per litter on Day (N)}}{\text{No. pups viable per litter on Day 4 after culling}} \times 100$

SUMMARY OF MEAN PUP WEIGHTS DURING LACTATION

Dose (ppm)		0	25	75	150	250
N (on day 0)		27	25	27	22	22
			F_2a Litters· Mean ± SD			
Day	1	6.6 ± 0.7	6.1 ± 0.5	6.1 ± 0.8	6.0 ± 0.9	6.1 ± 1.1
	4+	9.5 ± 1.2	8.4 ± 0.8*	8.5 ± 1.2	8.3 ± 1.6	8.1 ± 1.8**
	4 -	9.5 ± 1.2	8.4 ± 0.8*	8.5 ± 1.2	8.3 ± 1.7*	8.1 ± 1.9**
	7	15.1 ± 1.5	14.1 ± 1.3	13.9 ± 1.8	13.0 ± 2.8*	12.2 ± 3.1**
	14	28.8 ± 3.1	28.2 ± 2.6	28.7 ± 3.7	26.9 ± 3.7	23.6 ± 5.0**
	21	43.6 ± 3.8	41.1 ± 2.8	41.7 ± 5.6	39.4 ± 5.3	36.4 ± 6.7**
N (On day 0)		23	22	25	25	23
			F_2b Litters· Mean ± SD			
Day	1	6.6 ± 0.7	6.4 ± 0.7	6.2 ± 0.8	6.4 ± 0.6	5.7 ± 0.9**
	4+	9.6 ± 1.3	9.1 ± 1.2	8.7 ± 1.2	8.6 ± 1.0	7.5 ± 1.5**
	4 -	9.6 ± 1.3	9.1 ± 1.2	8.7 ± 1.2	8.6 ± 1.0*	7.5 ± 1.5**
	7	15.7 ± 1.7	15.0 ± 1.6	13.7 ± 2.6**	13.7 ± 1.9**	11.0 ± 2.5**
	14	31.6 ± 3.1	29.6 ± 3.0	27.4 ± 5.1**	25.4 ± 5.3**	23.8 ± 4.9**
	21	46.3 ± 4.9	44.2 ± 3.5	42.8 ± 4.1	38.5 ± 7.0**	36.1 ± 6.9**

N = Number of litters.

4+ Before culling
4- After culling

*p < 0.05 by Dunnett's test.
**p < 0.01.

Litters Lost During Lactation

For F_2a, 1 litter was entirely lost (100% dead) in 250 ppm group between days 7 to 14 of lactation. For F_2b, 1 litter was entirely lost in 75 ppm group only between days 14 to 21.

Physical Appearance and Mortality of Pups

This is summarized narratively from the individual animal necropsy tables of pups. It includes only those found dead and "does not include pups found missing or cannibalized," as stated in tables 151 and 152, pages 1515 to 1529 of the report. Examination of the tables on Individual Litter Viability (Tables 145 and 147) and the data on necropsy in Tables 151 and 152 revealed no serious problem in accounting for missing pups.

In the narration, it is claimed that the pups in the 75, 150, and 250 ppm groups of the F_2a generation were small. Surprisingly, this small appearance of pups in treated groups is not claimed for F_2b pups even though the table above for mean pup weights clearly shows decreased weight of pups in the 3 highest dose treated groups.

In the F_2a generation, the only discovered malformation was in a 25 ppm group female pup that died on day 0, which had an agnathia. In the F_2b generation, 1 male on 75 ppm had hydrocephaly. With 150 ppm, 1 male had hydrocephaly, a female in the same litter had an ocular defect thought to be anophthalmia and also had anasarca.

In the F_2a generation control group, 18 dead pups were found in 7 litters with 4 deaths in 2 litters, 3 deaths in 2 litters, 2 deaths in 1 litter, and 2 litters each with 1 death. At 25 ppm, there were 9 deaths occurring in 5 litters with 3 deaths occurring in 2 of them. At 75 ppm, there were also 9 deaths in 5 litters. At 150 ppm, there were 40 dead pups in 13 litters, 1 of them with 12, another with 8 dead pups. In the 250 ppm group, there were 31 deaths in 12 litters, multiple deaths of 5 or more occurring in 2 of them.

Mortality of pups in the F_2b generation included 10 controls in 8 litters, 5 at 25 ppm all in the same litter. At 75 ppm there were 40 deaths in 14 litters, 2 of which had 5 or more deaths. At 150 ppm there were 19 deaths in 9 litters, 2 of them with 5 deaths. At 250 ppm there were 18 deaths in 12 litters, all with 3 deaths or less in a litter.

Gross Necropsy of Pups that Died:

Complete gross necropsies were obviously performed, most with "no remarkable observations," a number of them autolyzed. No discernible compound effect was evident.

Pathology of F_1 Adults

Final Body Weights

Dose-related decreases were seen in males and females.

Final Body Weights ± SD at Terminal Sacrifice, Week 30

Dose (ppm)	0	25	75	150	250
Males (g)	611.7 ± 65.4	575.7 ± 63.8	533.4 ± 50.6**	488.3 ± 62.4**	447.4 ± 46.9**
(Number/group)	(27)	(28)	(28)	(28)	(27)
Females (g)	337.9 ± 32.4	310.8 ± 28.2**	301.7 ± 31.1**	278.7 ± 21.6**	271.4 ± 17.3**
(Number/group)	(28)	(28)	(27)	(27)	(28)

**p < 0.01 by Dunnett's test.

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Organ Weights

Liver weights in both males and females were decreased ($p < 0.01$) in a dose-related manner when based on absolute weight, but these decreases were no longer evident when based on relative weight (corrected for body weight). In fact, relative liver weights were increased in the 150 and 250 ppm female groups. The biological significance of this organ change is unknown. There were no changes in absolute weight of testes or ovaries but on a relative basis there was a tendency for a dose related increase in both the male and female gonads.

Males

		Absolute Weight(g)					Relative to Body Weight				
	GROUP:	0 PPM	25 PPM	75 PPM	150 PPM	250 PPM	0 PPM	25 PPM	75 PPM	150 PPM	250 PPM
LIVER											
	MEAN	22.36	20.55	19.69**	18.30**	16.24**	3.660	3.564	3.689	3.741	3.623
	S.D.	3.325	3.211	2.596	2.978	2.214	0.4176	0.3531	0.3127	0.2999	0.2345
	N	27	28	28	28	27	27	28	28	28	27
LEFT TESTIS											
	MEAN	1.78	1.83	1.84	1.88	1.86	0.294	0.321	0.346**	0.390**	0.419**
	S.D.	0.198	0.170	0.160	0.144	0.242	0.0399	0.0350	0.0306	0.0518	0.0612
	N	27	28	28	28	27	27	28	28	28	27
RIGHT TESTIS											
	MEAN	1.83	1.84	1.85	1.88	1.86	0.301	0.321	0.348	0.391**	0.419**
	S.D.	0.169	0.160	0.156	0.145	0.255	0.0345	0.0329	0.0357	0.0505	0.0656
	N	27	28	28	28	27	27	28	28	28	27

Females

	GROUP:	0 PPM	25 PPM	75 PPM	150 PPM	250 PPM	0 PPM	25 PPM	75 PPM	150 PPM	250 PPM
LIVER											
	MEAN	13.14	12.00*	11.58**	11.70**	11.43**	3.899	3.860	3.839	4.189**	4.213**
	S.D.	1.493	1.452	1.438	1.682	1.098	0.3662	0.3051	0.3211	0.4040	0.3157
	N	28	28	27	27	28	28	28	27	27	28
LEFT OVARY	MEAN	0.0458	0.0428	0.0515	0.0475	0.0448	0.014	0.014	0.017*	0.017*	0.016
	S.D.	0.01426	0.01232	0.01323	0.01168	0.01327	0.0041	0.0045	0.0044	0.0045	0.0047
	N	28	28	27	27	28	28	28	27	27	28
RIGHT OVARY	MEAN	0.0438	0.0426	0.0471	0.0490	0.0473	0.013	0.014	0.016	0.018**	0.017**
	S.D.	0.01424	0.01020	0.01328	0.01268	0.01620	0.0040	0.0040	0.0041	0.0046	0.0055
	N	28	28	27	27	28	28	28	27	27	28

* p<0.05 by Dunnett's test
** p<0.01

Pathology F_1 Male and Female Adults

Gross pathology was performed on all animals on test (Tables 77, 78 and 155). Histopathology included lesions found in organs of all groups when seen at necropsy. Reproductive organ histopathology included all animals in the control and the 250 ppm treated groups. No compound related effect was evident.

Pathology of F_{2b} Pups

Gross pathology and limited histopathology was performed for 10 of each sex per group in controls and 250 ppm dose. No compound-related effect was apparent.

Final body weights were as follows:

Dose (ppm)	0	25	75	150	250
Males (g ± SD)	46.2 ± 6.8	44.2 ± 5.5	43.2 ± 5.8	37.5 ± 7.2**	35.9 ± 8.5**
Females (g ± SD)	43.6 ± 2.3	42.7 ± 4.3	41.4 ± 3.6	38.4 ± 7.7	35.7 ± 6.6**

** $P < 0.01$ by Dunnett's test.

This indicates generally dose-related decreases in final body weights of the pups at the 2 highest dose levels.

There were also no effects on liver weights or female reproductive organs. There was a decrease in absolute and relative testes weight at the 150 and 250 ppm dose groups.

Summary and Evaluation

Doses selected in the present two-generation reproduction rat study were 0, 25, 75, 150, and 250 ppm in the diet which came to an average of 0, 1.8, 5.3, 11.1, and 18.5 mg/kg/day, respectively, for the 5 dose levels in nongravid animals and during gestation. These were about twice as high in lactating females; i.e., an average of 0, 3.8, 11.2, 23.0, and 37.1 mg/kg/day, respectively. F_0 and F_1 parents were treated for at least 70 to 72 days prior to the first pairing. Histopathology was performed only on control and high-dose-treated F_0 and F_1 adults but only 10 of each sex of control and high-dose-treated F_{2b} pups at weaning. Histopathology was limited to reproductive organs of both sexes, but also included areas of grossly observed lesions in organs of animals of all treated groups. Organ weights included liver and reproductive organs.

Manifestations of toxicity to male and female F_0 and F_1 parents were seen as dose-related decreased body weight gain and decreased food intake at 75, 150, and 250 ppm doses. In addition, final body weights at the time of terminal sacrifice was lower in the 25 ppm treated male (n.s.) and female ($p < 0.01$)

F_1 parents. There was a tendency for decreased liver weights particularly at the 3 highest dose levels. No effects on gross pathology or histopathology due to treatment were evident in the F_0 and F_1 males or females.

In both the F_{1a} and F_{1b} generations there was a 35% decrease in number of paired females that delivered a litter in the 150 ppm treated group. Such an effect was not seen for either generation of the 250 ppm treated group nor for any treated group of the F_{2a} and F_{2b} generations. The lower fertility in the 150 ppm group was considered a "random occurrence" by the investigator but since it occurred in two successive matings we should not ignore the possibility of an effect.

For the "Male Mating Index" and "Female Mating Index", data for "both mating periods"; i.e. F_{1a} with F_{1b} and F_{2a} with F_{2b}, were combined. These data should be recalculated and presented separately for each mating period. In its present form, the data appear misleading and are higher than they should really be.

There is also the question on the reliability of the data on "Females with Evidence of Mating". Vaginal sperm or copulation plugs were missed for a number of animals on test. It also appears likely that a similar number of animals were considered to have mated but did not, particularly if evidence of mating was based on copulation plugs under the cages rather than vaginal sperm.

In the 4 tables on pathology of the pups (Tables 117, 118, 152 and 153), there are footnotes "Does not include pups found missing or cannbalized". In the F_{1a} generation, dams 23,703 and 23,748 each lost all 8 of their pups between days 7-14 of lactation but on 2 or 3 or them are accounted for with each of the 2 litters. The applicant should account for all missing pups.

There were slight decreases in gestation length in all 4 treated groups if the F_{2a} litters ($P<0.05$-0.01; 22.1 days for controls 21.3 to 21.6 days for treated). The latter effect is also difficult to explain but it did not occur in the F_{2b}, F_{1a} or F_{1b} generations. It was not accompanied by dystocia or other pregnancy irregularity and was considered not a compound effect.

There were obviously no compound related decreases in live litters at birth, no decreases in live litter size or increase in dead pups at birth, no effect on sex ratio or on Gestation Index (live litter births/total pregnancies). Gestation Index was slightly decreased in all 4 treated groups of the F_{1a} generation, but since it did not occur with any other generation, it was considered sporadic by the applicant.

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Viability of pups during lactation was decreased ($p \leq 0.05$ to 0.01) as early as days 1 and 4 of lactation in the 150 and 250 ppm treated groups of the F_{2a} generation. There was a decrease in pup viability in the 150 and 250 ppm dosed groups of the F_{1a} generation, which became evident by day 14 of lactation. However, dose-related decreased pup weight by day 21 of lactation was evident in the 75, 150, and 250 ppm treated groups in the F_{1a} and F_{2b} generations ($p < 0.01$). Decreased pup weight may be a clearer indication of toxicity to the pups than decrease in viability index.

Results of necropsies of pups found dead are given. Although anomalies were found in a few pups, all of which were subjected to necropsy if not autolyzed, there is no indication of increased incidence of anomalies with dosing of compound. A large number of pups in the 250 ppm dose treated group of the F_{1a} generations prior to discarding them were observed to be small in size, but otherwise "not remarkable." In the F_{2a} generation, pups were visually observed to be small in the 75, 150, and 250 ppm treated groups prior to discarding after weaning, but otherwise "not remarkable."

Ten pups of each sex per group in the F_{2b} generation were examined by gross and microscopic pathology at time of weaning. No compound effect was noted. Final body weights were lower in the pups from the 150 and 250 ppm treated male and female groups. No effect was seen on liver weights of pups, but a decrease was noted in testes weight in the 150 and 250 ppm groups based both on absolute and relative weights, which could suggest retarded testicular development of the pups in the 2 highest dose groups. However, there was obviously no impairment in reproduction function in males based on reproduction indices of F_1 males.

At the terminal killing of adults, parent body weights were decreased for F_0 males and females at the 3 highest doses and in F_1 males and females at all 4 doses, dose-related. There were no effects on relative liver weights of either sex nor on absolute testis or ovarian weights. No effects on gross pathology performed on organs of all animals on test was observed. A histopathology report, signed by Robert G. Geil, D.V.M., Diplomate, American College of Veterinary Pathologists, dated January 19, 1987, indicates that no microscopic lesions related to treatment were seen in F_0 and F_1 parental rats or F_{2b} weanling rats from the 250 ppm group. Histopathology was limited to organs of the reproductive tract of both the males and females of controls and 250 ppm group and lesions of other organs in animals of all groups.

In conclusion, systemic toxicity was observed in the present two-generation rat study with Bladex at 25 ppm in the diet (LDT), based on decreased body weight of males (n.s.) and females

($p < 0.01$) of F_1 adults at the time of terminal killing, around week 57 of the study and at various time periods throughout the study. This dosage is equivalent to about 1.8 mg/kg/day based on food intake. Thus, 1.8 mg/kg/day (LDT) is considered the LEL for systemic toxicity in rats due to long-term oral intake of Bladex.

The LEL for Reproductive Toxicity is considered to be 11.2 mg/kg/day based on pup viability indices and decreased body weight gain of pups during lactation with a diet containing 75 ppm Bladex or higher. The NOEL is 3.8 mg/kg/day (25 ppm).