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HED File Code	**13000 Tox Reviews**
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HEALTH EFFECTS DIVISION
SCIENTIFIC DATA REVIEWS
EPA SERIES 361

011391

#295

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

DATE: **January 17, 1995**

MEMORANDUM

SUBJECT: ***DICAMBA:*** Reproductive Toxicity Study
Submitted in Response to the DCI **[Action: 627 Core Data].**

OFFICE OF
PREVENTION, PESTICIDES AND
TOXIC SUBSTANCES

FROM: Jess Rowland, M.S, Toxicologist Jess Rowland 1/17/95
Section II, Toxicology Branch II, Health Effects Division (7509C)

TO: **Walter Waldrop / Jane Mitchell**
Product Manager (71), Reregistration Division

THRU: K. Clark Swentzel, Head K. Clark Swentzel 1/17/95
Section II, Toxicology Branch II, Health Effects Division (7509C)
and
Marcia Van Gemert, Ph.D., Chief, Marcia Van Gemert 1/18/95
Toxicology Branch II, Health Effects Division (7509C)

TASK IDENTIFICATIONS: **P.C. Code: 029801** Submission: S459483 DP Barcode: D199958

REGISTRANT: Sandoz Agro, Inc., Desplains, ILL 60018

ACTION REQUESTED: Review the two-generation reproductive toxicity study in rats titled *"Technical Dicamba: A Study of the Effect on Reproductive Function of Two Generations in the Rat"* [MRID No. 431371-01] submitted in response to the Dicamba Acid DCI.

RESPONSE: A Data Evaluation Report for the above referenced study is attached. An Executive Summary is provided below:

In a two-generation reproduction study, Sprague-Dawley rats (32 or 28 per group) received Dicamba technical (86.5% a.i.) in the diet at dose levels of 0, 500, 1500, or 5000 ppm (0, 40, 122 or 419 mg/kg/day for males and 0, 45, 136 or 450 mg/kg/day for females, respectively). Systemic toxicity was observed at 5000 ppm. It was manifested as clinical signs in dams from both generations during lactation (tense/stiff body tone and slow righting reflex) and significantly increased relative liver to body weights (112% of control) in both generations and sexes, adults as well as weanlings. Relative kidney to body weights (107%) at 1500 and/or 5000 ppm were not considered to be toxicologically relevant since there were no gross or histopathological findings. **Based on these results, the NOEL and LOEL for Systemic Toxicity were 1500 and 5000 ppm, respectively.** Reproductive toxicity was observed at 1500 and 5000 ppm. It was manifested as significantly decreased pup growth in all generations and matings at 1500 ppm (86%-90% of control) and at 5000 ppm (74%-94% of control). In addition, delayed sexual maturation was noted in F_1 males (but not females) at 5000 ppm. **Based on these results, the NOEL and LOEL for Reproductive Toxicity were 500 and 1500 ppm, respectively.**

CORE CLASSIFICATION: **Minimum;** this study satisfies the Subdivision F Guideline Requirement [§83-4] for a two-generation reproductive toxicity in rats and is acceptable for regulatory purposes.

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PRIMARY REVIEWER: Jess Rowland, M.S, Toxicologist Jess Rowland 1/17/95
Section II, Toxicology Branch II

SECONDARY REVIEWER: Clark Swentzel, Head K. Clark Swentzel 1/17/95
Section II, Toxicology Branch II

DATA EVALUATION REPORT

STUDY TYPE: Multigeneration Reproduction - Rat (83-4)

P.C. CODE: 029801 **MRID NUMBER:** 431371-01

TEST MATERIAL: 3,6-Dichloro-o-anisic acid

SYNONYM: Technical dicamba

STUDY NUMBER: SNC 140/921437

SPONSOR: Sandoz Agro Inc., Des Plaines, IL

TESTING FACILITIES: Huntingdon Research Centre Ltd., Cambridgeshire, England

TITLE OF REPORT: *"A STUDY OF THE EFFECT ON REPRODUCTIVE FUNCTION OF TWO GENERATIONS IN THE RAT"*

AUTHOR: R.E. Masters (study director) **REPORT ISSUED:** October 20, 1993

EXECUTIVE SUMMARY: In a two-generation reproduction study, Sprague-Dawley rats (32 or 28 per group) received Dicamba technical (86.5% a.i.) in the diet at dose levels of 0, 500, 1500, or 5000 ppm (0, 40, 122 or 419 mg/kg/day and 0, 45, 136 or 450 mg/kg/day for females, respectively).

Systemic toxicity was observed at 5000 ppm. It was manifested as clinical signs in dams from both generations during lactation (tense/stiff body tone and slow righting reflex) and significantly increased relative liver to body weights (112% of control) in both generations and sexes, adults as well as weanlings. Relative kidney to body weights (107%) at 1500 and/or 5000 ppm were not considered to be toxicologically relevant since there were no gross or histopathological findings. Based on these results, **the NOEL and LOEL for Systemic Toxicity were 1500 and 5000 ppm, respectively.**

Reproductive toxicity was observed at 1500 and 5000 ppm. It was manifested as significantly decreased pup growth in all generations and matings at 1500 ppm (86%-90% of control) and at 5000 ppm (74%-94% of control). In addition, delayed sexual maturation was noted in F_1 males (but not females) at 5000 ppm. Based on these results, **the NOEL and LOEL for Reproductive Toxicity were 500 and 1500 ppm, respectively.**

CORE CLASSIFICATION: Minimum. This study satisfies the minimum requirements for a reproduction study (83-4) in rats.

ED_005172C_00001728-00003

I. INTRODUCTION

This Data Evaluation Report describes an experiment performed to assess the effect of Dicamba on the growth and reproductive performance of the rats when administered continously and at fixed concentrations in the diet for two consecutive generations.

II. MATERIALS AND METHODS

1. Test Material

Description:	Pink/cream powder
Batch number:	52103810
Purity:	86.9% a.i.

2. Test Animals

Species:	Rat
Strain:	Crl:CD (SD) BR VAF/Plus
Age:	Four weeks upon arrival
Weight:	Males--69-115 g; Females--40-95 g upon arrival
Source:	Charles River UK Limited, Margate, Kent
Housing:	Premating--four (same sex) per cage Mating--one male and one female per cage Postmating--four males per cage Gestation and lactation--one female per cage
Temperature:	21°C
Humidity:	55%
Air changes:	Not reported
Photoperiod:	12-hour light/dark cycle
Acclimation:	Two weeks

3. Diet Preparation and Analysis

Diets were prepared weekly by mixing the test material with a small amount of diet. This premix was then used to prepare the desired concentrations for all dose levels. The report did not state whether adjustments were made for purity. Analyses for homogeneity and stability up to 18 days at room temperature were conducted on duplicate samples at 500 and 12000 ppm prior to study initiation. Analyses for concentration of the test compound in the diet were conducted on samples from all dose levels during weeks 1, 11, 14, 17, 29, 34, 40, and 42.

4. Mating Procedure

F_0 males and females were mated in a ratio of one to one for 20 days regardless of when mating was confirmed by vaginal smear which were taken daily or by a plug. The day on which mating was confirmed was defined as gestational day (GD) 0. F_1 animals were mated in the same manner as the F_0 animals. Sibling matings were avoided. A second F_1 mating between different partners was conducted because of low pregnancy rates in the first mating.

5. Mating Schedule

F_0 animals were given test diets for 10 weeks prior to mating. Following weaning on lactation day (LD) 21, 28 males and 28 females F_1 pups were randomly selected as parents for the F_2 litters. F_1 animals were given test diets for 12 weeks prior to the first mating.

6. Dose Selection Rationale

Doses were selected based on a range-finding study (SNC 120/911395). The following results were reported: *"treatment at 12000 ppm was associated with a notable response in F_0 animals and offspring manifested as reduced adult body weight gain and marked effects on post natal survival of the pups with 10/10 litter losses. Treatment at 6000 ppm elicited less severe responses in the adult with only slightly increased pup mortality and reduced pup growth, and at 3000 ppm there was a marginal effect on F_0 adults and slightly reduced pup growth. On the basis of these findings, dietary inclusions of 0 (control), 500, 1500 and 5000 ppm were selected for this main investigation"*.

7. Animal Assignment

F_0 animals were assigned to the test groups shown in Table 1 using computerized stratified randomization based on body weight. F_1 animals were randomly selected using random numbers.

TABLE 1. ANIMAL ASSIGNMENT

			No. of Animals per Group			
			Males		Females	
Group	Treatment	Dose (ppm)	F_0	F_1	F_0	F_1
1	Control	0	32	28	32	28
2	Low-Dose	500	32	28	32	28
3	Mid-Dose	1500	32	28	32	28
4	High-Dose	5000	32	28	32	28

8. Observation Schedule

A. Parental Animals

Animals were checked regularly for mortality, moribundity, and clinical signs of toxicity. Body weight data were recorded weekly during premating for all animals; daily during mating and gestation for females (but only reported weekly); and on GDs and LDs 0, 7, 14, and 21 for females. Food consumption data were recorded weekly during premating. Water consumption data were recorded during the first two weeks and last two weeks of premating.

B. Reproductive Performance

Parental reproductive performance was assessed from breeding and parturition records of animals in the study. A mating was considered successful if sperm was observed in a lavage. The following indices were calculated:

$$\text{Mating index} = \frac{\text{No. of mated animals}}{\text{No. of paired animals}} \times 100$$

$$\text{Fertility index} = \frac{\text{No. of pregnant females}}{\text{No. of mated females}} \times 100$$

$$\text{Gestation index} = \frac{\text{No. of females with live pups}}{\text{No. of pregnant females}} \times 100$$

9. Litter Observations

The following litter observations were made:

TABLE 2. F_1/F_2 LITTER OBSERVATIONS

	Time of Observation (Lactation Days)						
Parameter	Birth	Daily	4	8	12	16	21
Clinical signs	x						
Number of Live pup	x						
Number of Dead pups	x	x	x[a]				
Pup body weight	x		x	x	x	x	x
Externa alteration	x						
Sex of each pup	x						

a = On Day 4 post partum, where possible, the litter was standardized to a toal litter size of eight pup, four male and four female, if available, by computer generated random number selection for each sex. No pups were culled from litters of eight or less regardless of sex ratio.

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In addition, the following parameters were recorded for physical develop-ment: females from LD 28, vaginal opening; males from LD 35, balanopreputial skinfold.

The following F_1 and F_2 indices were calculated:

$$\text{Live birth index} = \frac{\text{Total pups born alive}}{\text{Total pups born}} \times 100$$

$$\text{Viability index} = \frac{\text{Total live pups on day 4 pre-cull}}{\text{Total live pups born}} \times 100$$

$$\text{Lactation index} = \frac{\text{Total live pups on day 21}}{\text{Total live pups on day 4 post-cull}} \times 100$$

10. Necropsy

A. Parental Animals

All animals were subjected to post mortem examinations. Gross necropsy on females included a count of implantation sites. The reproductive tissues listed below were preserved in 10% buffered formalin and processed for histological examination. Tissues from the control and high-dose groups as well as from animals from all groups suspected of infertility or found dead/sacrificed were evaluated for histopathology.

- Prostate gland
- Testes
- Epididymides
- Vagina
- Coagulating gland
- Pituitary gland
- Ovary
- Uterus

The following organ weights were recorded:

- Adrenals
- Kidneys
- Pituitary
- Epididymides
- Brain
- Liver
- Prostate
- Thymus
- Heart
- Lungs
- Testes

The adult male reproductive system was evaluated by collecting sperm from both vas deferens and analyzing for motility, count, and morphology. The left testis was used for counts.

B. Offspring

All F_1 and F_2 offspring found dead during the study were subjected to post mortem examination. Offspring not selected as the F_1 perental generation were sacrificed after weaning and subjected to post mortem examination; sex was confirmed by gonadal inspection. Pups with anomalies were preserved in 10% buffered formalin. One pup per sex and litter representing the median body weight per sex on LD 21 was selected for organ weight analysis and tissue preservation.

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11. Statistical Analyses

The following tests were used:

- Body weight, food and water consumption, length of pregnancy, litter data, sexual maturation, sperm analyses, and organ weights--ANOVA and William's test (parametric data) or Kruskal-Wallis and Shirley's test (nonparametric data)

- 75% or more of the values being the same for a given value--Fisher's Exact test

12. Regulatory Compliances

A signed statement of No Data Confidentiality Claim was provided that was dated 12/6/93.

A signed statement for Potential Adverse Effects, signed and dated 2/11/94 indicated that this study neither meets nor exceeds any of the applicable criteria stipulated in 40 CFR 158.34.

A signed statement dated 1/4/94 indicated that this study was conducted in accordance with the principles of EPA's GLP [40CR.160] as promulgated in Fed. Reg. 48, 1983.

A Quality Assurance Statement was provided that was dated 10/20/93.

III. RESULTS

Analytical Chemistry Results

Homogeneity: Mean ranges were 90%-103% of target.
Stability: Mean ranges were 91%- 94% of target.
Concentration: Mean ranges were 95%-112% of target.

A. SYSTEMIC (PARENTAL TOXICITY)

1. Mortality and Clinical Signs

No compound-related mortality was observed. Incidental deaths are reported below.

In the F_0 generation, one female at 500 ppm and another at 5000 ppm were killed after weaning and on LD 0, respectively.

In the F_1 generation among males, two (weeks 10 and 30), one (week 32), one (week 25), and one (week 9) animal(s) died at 0, 500, 1500, and 5000 ppm, respectively. Among females, one animal from the control group was killed during premating and two were killed at 500 ppm, one on GD 16 (first mating) and one on LD 2 (second mating).

Tense/stiff body tone and slow righting reflex observed at 5000 ppm in F_1 dams during late lactation (9/19 in the first mating; 12/19 in the second mating). Since similar signs had been noted in the range-finding study, they were considered to be compound related.

2. Body Weight/Weight Gain, Food Consumption, and Water Consumption

A summary of body weight and food consumption data during premating is presented in Table 3.

No effects were noted on body weight or weight gain in the F_0 generation. In the F_1 generation among males at 5000 ppm, body weight decreased significantly during weeks 4 (84% of control) and 8 (91% of control); among females, body weight also decreased significantly during weeks 4 (83% of control) and 8 (94% of control). Although these decreases were considered to be treatment related, they were not toxicologically relevant. Body weight gains for the premating period were similar in all dose groups for both sexes and generations. For dams during gestation and lactation, body weight gains were similar for all matings.

No compound-related effects were noted on food consumption (g/rat/week; Table 3), food conversion ratios (food consumption/body weight gain; data not shown), or water consumption (g/rat/week; data not shown).

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TABLE 3. MEAN BODY WEIGHT (G) AND MEAN FOOD CONSUMPTION (G/RAT/WEEK) DURING PREMATING

Parameter/Sex/Week	Dose (ppm) 0	500	1500	5000
F_0 GENERATION - MALES				
Body Weight				
Week 0	223 ± 20	221 ± 16	220 ± 17	223 ± 19
3	391 ± 33	387 ± 27	388 ± 33	390 ± 31
6	500 ± 49	492 ± 38	490 ± 45	497 ± 40
10	595 ± 66	581 ± 53	586 ± 57	584 ± 52
Weight Gain Weeks 0-10	373 ± 54	360 ± 46	366 ± 45	360 ± 41
Food consumption				
Week 0	203 ± 7	192 ± 8	198 ± 8	200 ± 8
3	211 ± 11	207 ± 9	207 ± 8	207 ± 9
6	222 ± 14	218 ± 8	216 ± 9	220 ± 11
10	214 ± 11	210 ± 9	210 ± 11	212 ± 12
Total Weeks 1-10	2096 ± 98	2062 ± 75	2049 ± 82	2059 ± 93
F_0 GENERATION - FEMALES				
Body Weight				
Week 0	161 ± 13	161 ± 11	161 ± 11	161 ± 11
3	239 ± 19	235 ± 20	236 ± 17	245 ± 20
6	286 ± 25	280 ± 25	281 ± 22	295 ± 24
10	327 ± 34	322 ± 36	324 ± 30	336 ± 35
Weight Gain Weeks 0-10	166 ± 31	161 ± 30	163 ± 25	175 ± 28
Food consumption				
Week 0	142 ± 8	148 ± 8	148 ± 7	143 ± 9
3	149 ± 9	147 ± 10	145 ± 6	142 ± 5
6	163 ± 12	163 ± 10	164 ± 14	158 ± 7
10	145 ± 4	143 ± 10	150 ± 6	145 ± 7
Total Weeks 1-10	1471 ± 62	1451 ± 67	1482 ± 63	1437± 51
F_1 GENERATION - MALES				
Body Weight[b]				
Week 4	95 ± 17	100 ± 12	100 ± 13	80 ± 16**
8	342 ± 35	359 ± 28	362 ± 38	311 ± 47**
12	513 ± 52	536 ± 39	544 ± 66	488 ± 53
16	612 ± 63	641 ± 54	649 ± 88	592 ± 68
Weight Gain Weeks 4-16	516 ± 55	541 ± 47	549 ± 79	511 ± 59
Food consumption[b]				
Week 5	120 ± 6	126 ± 4	125 ± 7	112 ± 5
8	205 ± 8	217 ± 7	220 ± 13	193 ± 12
12	220 ± 16	231 ± 8	232 ± 17	224 ± 9
16	216 ± 10	223 ± 11	221 ± 22	221 ± 14
Total Weeks 5-16	2415 ± 115	2530 ± 80	2543 ± 165	2396 ± 91
F_1 GENERATION - FEMALES				
Body Weight				
Week 4	92 ± 13	91 ± 11	90 ± 11	76 ± 10**
8	230 ± 27	232 ± 20	228 ± 20	216 ± 21*
12	306 ± 40	307 ± 33	305 ± 29	297 ± 39
16	349 ± 48	352 ± 45	350 ± 34	344 ± 46
Weight Gain Weeks 4-16	257 ± 45	261 ± 43	260 ± 30	268 ± 44
Food consumption				
Week 5	108 ± 6	110 ± 2	112 ± 5	102 ± 4
8	152 ± 14	150 ± 6	152 ± 6	143 ± 7
12	158 ± 11	158 ± 9	154 ± 6	154 ± 10
16	146 ± 10	147 ± 10	143 ± 7	144 ± 11
Total Weeks 5-16	1776 ± 107	1763 ± 68	1786 ± 68	1718 ± 76

* Significantly different from control, p≤0.05
** Significantly different from control, p≤0.01

3. Test Substance Intake

Based on food/compound-intake values, doses expressed as mg test substance/kg body weight during the premating period are presented in Table 4.

TABLE 4. TEST SUBSTANCE INTAKE[A]

	Dose (ppm)					
	Males			Females		
Week	500	1500	5000	500	1500	5000
			F_0 GENERATION			
1	52.0	156	494	52.7	161	509
3	40.3	122	403	46.7	137	436
6	32.6	97	327	42.4	128	391
10	26.2	78	263	32.6	100	312
Mean	37.8	113.3	371.8	43.6	131.5	412.0
S.D	11.1	33.7	99.6	8.5	25.2	82.5
			F_1 GENERATION			
5	69.0	207	765	69.4	215	765
8	47.6	143	488	49.2	152	507
12	31.8	95	339	37.7	110	379
16	25.2	74	270	30.3	88	303
Mean	43.4	129.8	465.5	43.4	141.3	488.5
S.D	19.5	59.0	219.5	16.5	55.9	202.6

[A]Data extracted from Study No. SNC 140/921437, Table 6

4. Reproductive Performance

As shown in Tables 5 and 6, no treatment-related effects were observed on parental reproductive performance. The effects on offsprinng litter size, pup viability and pup body weights are presented and discussed in more detail in Tables 8 and 9. In the second generation, pregnancy rates were low and a second mating was therefore conducted. Low pregnancy rates including that of the control group, persisted and were judged to be independent of the test compound. The increased number of pup deaths at 5000 ppm in the F_1 first mating was mainly due to complete litter loss (14 pups) by one dam and loss of 14/17 pups by another dam. This was not considered to be treatment related since a similar effect was not observed in the other matings at this dose level. Similarly, the high pup death at 500 ppm in the F_1 second mating was due to complete litter loss by two dams with 8 and 11 pups each.

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TABLE 5. REPRODUCTIVE PERFORMANCE - F_0 GENERATION[A]

Observation	Dose (ppm) 0	500	1500	5000
Precoital interval (days)	2.5	2.0	2.0	2.0
Males				
Number on study	32	32	32	32
Number mated	30	30	31	29
Number fertile	27	28	29	27
Fertility not determined	5	4	3	5
Intercurrent deaths	0	0	0	0
Females				
Number on study	32	32	32	32
Number mated[b]	30	30	31	29
Number pregnant	27	28	29	27
Fertility not determined	5	4	3	5
Intercurrent deaths	0	1[c]	0	1[d]
Gestation interval (days)	22.0	21.9	22.0	21.6
No. of live litters at birth	26	28	29	26
Total litter losses	1	0	0	0
Mean litter size (day 0)	13.2 (26)[e]	14.0 (28)	14.6 (29)	13.7 (26)
Mean litter size (day 21)	7.7 (25)[g]	7.5 (28)	7.8 (29)	7.4 (26)
No. of live pups (day 0)	342	391	424	355
No. of pups culled (day 4)	138	157	182	146
Pup deaths (days 0-21)[f]	12 (0.48)[h]	24 (0.85)	17 (0.58)	17 (0.65)
No. of live pups (day 21)	192	210	225	192
Mean pup wt (g) (day 0)	6.3	6.3	6.3	5.9*
Mean pup wt (g) (day 21)	59.6	57.8	57.3	45.4**

[a] Data extracted from Study No. SNC 140/921437, Tables 1 and 8-11 and individual data
[b] Confirmed pregnant retrospectively without showing plug or sperm
[c] Killed following weaning
[d] Killed on LD 0
[e] Number of litters included in calculation within parenthesis
[f] Includes pups dying, missing, and/or cannibalized
[g] Total litter loss by dam #148 on Day 4 post-partum
[h] Pup deaths/litter
* Significantly different from control, $p \leq 0.05$
** Significantly different from control, $p \leq 0.01$

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TABLE 6. REPRODUCTIVE PERFORMANCE - F_1 GENERATION[a]

Observation	Dose(ppm) 0	500	1500	5000
FIRST MATING				
Precoital interval (days)	6.0	4.0	>20	2.5
Males				
Number on study	27	28	28	27
Number mated	19	22	18	27
Number fertile	15	17	13	21
Females				
Number on study	27	28	28	28
Number mated[b]	19	22	18	27
Number pregnant	15	17	13	21
Gestation interval (days)	22.4	22.4	22.2	22.1
No. of live litters at birth	15	17	12	20
Total resorptions	0	0	0	1
Total litter losses	0	0	1[c]	1[d]
Mean litter size (day 0)	12.3 (15)[e]	13.8 (17)	12.5 (12)	12.8 (20)
Mean litter size (day 21)	7.7 (15)	7.6 (17)	7.8 (12)	7.5 (19)
No. of live pups (day 0)	185	235	150	255
No. of pups culled (day 4)	65	98	55	78
Pup deaths (days 0-21)[f]	5 (0.33)[g]	7 (0.41)	2 (0.17)	34[h] (1.7)
No. of live pups (day 21)	115	130	93	143
Mean pup weight (g) (Day 0)	6.6	6.4	6.4	6.1*
Mean pup weight (g) (Day 21)	65.0	62.5	58.4*	47.9**
SECOND MATING				
Precoital interval (days)	9.0	3.0	4.0	4.0
Males				
Number on study	27	27	28	27
Number mated	19	18	17	23
Number fertile	15	18	15	19
Fertility not determined after second mating	3	3	6	2
Intercurrant deaths including both matings	2	1	1	1
Females				
Number on study	27	27	28	27
Number mated[b]	19	18	17	23
Number pregnant	15	18	17	19
Fertility not determined after second mating	7	6	7	4
Intercurrent deaths including both matings	1	2	0	0
Gestation interval (days)	21.9	22.1	22.1	22.0
Number of live litters at birth	15	18	14	19
Total resorptions	0	0	2	0
Total litter losses	1[i]	1[j]	1[k]	0
Mean litter size (day 0)	13.5 (15)[e]	13.4 (18)	11.4 (14)	12.1 (19)
Mean litter size (day 21)	7.6 (14)	7.8 (18)[l]	7.6 (14)	7.2 (19)
Number of live pups (day 0)	202	242	160	229
Number of pups culled (day 4)	61	92	54	75
Pup deaths (days 0-21)[f]	14 (0.93)[g]	38[m] (2.11)	0	18 (0.94)
Number of live pups (day 21)	107	122	106	136
Mean pup weight (g) (Day 0)	6.6	6.6	6.7	6.1
Mean pup weight (g) (Day 21)	61.9	59.8	52.9*	43.2**

[a] Data extracted from Study No. SNC 140/921437
[b] Confirmed pregnant retrospectively without showing plug or sperm
[c] Total litter loss by dam #437 on Day 0 post partum
[d] Total litter loss by dam #463 on Day 4 post partum.
[e] No. of litters included in calculation.
[f] Includes pups dying, missing, and/or cannibalized
[g] Pup deaths/litter
[h] Loss of 14 pups each by two dams #463 & 470
[i] Total litter loss by dam #383 on Day 6 post partum
[j] Total litter loss by dam #399 on Day 7 post partum.
[k] Total litter loss by dam #439 on Day 0 post partum
[l] Total litter loss by dam #403 on Day 2 post partum.
[m] Included 8 & 11 pups each by dams #399 & 403
* Significantly different from control, $p \leq 0.05$
** Significantly different from control, $p \leq 0.01$

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5. Necropsy Results

Organ Weights

A summary of organ weight data is presented in Table 7. Compound-related effects were observed on relative liver and kidney weights at 1500 and/or 5000 ppm. Relative liver weights increased significantly at 5000 ppm in F_0 females (113% of control) and F_1 males (110%). Relative kidney weights increased significantly in F_0 males and females and in F_1 males at 1500 and 5000 ppm (107%). Differences from controls observed on various other organ weights were not considered to be compound related.

TABLE 7. RELATIVE ORGAN TO BODY WEIGHT DATA[A]

	Dose (ppm)							
	Males				Females			
Organ	0	500	1500	5000	0	500	1500	5000
				F_0 GENERATION - Adults				
Liver	28	29	27	29	16	15	16	18**
Kidneys	5.3	5.5	5.5	5.6*	3.0	3.1	3.2*	3.2*
				F_1 GENERATION				
Liver	28	27	29	31*	NR	NR	NR	NR
Kidneys	5.4	5.6*	5.7*	5.8*	NR	NR	NR	NR

[A]Data extracted from Study No. SNC 140/921437, Table 14
* Significantly different from control, $p \leq 0.05$
**Significantly different from control, $p \leq 0.01$
NR = Not reported

6. Pathology

No compound-related gross or microscopic findings were observed.

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B. REPRODUCTIVE (OFFSPRING) TOXICITY

1. Pup Viability and Clinical Signs

No compound-related effects were observed. Data are presented in Table 8.

TABLE 8. PUP VIABILITY DURING LACTATION[A]

	Dose (ppm)			
Observation/Lactation Day	**0**	**500**	**1500**	**5000**
	F_1 Litters			
Mean % surviving in each litter				
Days 1-4 (viability index)	94	94	96	98
Days 4-21 (lactation index)	99	100	99	96
No. of litters with all pups surviving to Day 21 /Total no. of litters	20/26 (0.71)	19/28 (0.68)	6/29 (0.55)	17/26 (0.65)
	F_{2A} Litters			
Mean % surviving in each litter				
Days 1-4 (viability index)	98	98	98	90
Days 4-21 (lactation index)	100	99	100	99
No. litters with all pups surviving to Day 21 /Total no. litters	11/15 (0.73)	15/17 (0.88)	10/12 (0.83)	14/20 (0.70)
	F_{2B} Litters			
Mean % surviving in each litter				
Days 1-4 (viability index)	92	93	100	96
Days 4-21 (lactation index)	93	94	100	95
No. litters with all pups surviving to Day 21 /Total no. litters	10/15 (0.66)	10/18 (0.56)	14/14 (1.0)	12/19 (0.63)

[A]Data extracted from Study No. SNC 140/921437, Table 1 and individual data

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2. Pup Body Weight

A summary of pup body weight is presented in Table 9. Compound-related effects were observed in all three matings at 1500 and/or 5000 ppm. Among F_1 litters, pup body weight was significantly decreased throughout lactation at 5000 ppm (LD 0, 94% of control; LD 4, 90%; LD 8, 84%; LDs 12 and 16, 81%; and LD 21, 76%). Among F_{2A} litters, pup body weight was significantly decreased throughout lactation at 5000 ppm (LD 0, 92%; LD 4, 90%; LD 8, 84%; LD 12, 80%; LD 16, 77%; and LD 21, 74%) and on LD 21 at 1500 ppm (90%). Among F_{2B} litters, pup body weight was significantly decreased on LD 8 (81%), LD 12 (76%), and LDs 16 and 21 (70%) at 5000 ppm and on LDs 12 (88%) and 21 (86%) at 1500 ppm.

TABLE 9. MEAN PUP BODY WEIGHT[A]

	Dose (ppm)			
Lactation Day	0	500	1500	5000
		F_1 Litters		
Day 0	6.3	6.3	6.3	5.9*
Day 8	19.0	18.6	19.1	16.0**
Day 21	59.6	57.8	57.3	45.4**
		F_{2A} Litters		
Day 0	6.6	6.4	6.4	6.1*
Day 8	21.6	20.2	19.4	18.1**
Day 21	65.0	62.5	58.4*	47.9**
		F_{2B} Litters		
Day 0	6.6	6.6	6.7	6.1
Day 8	20.1	19.1	18.2	16.3**
Day 21	61.8	59.8	52.9*	43.2**

[A]Data extracted from Study No. SNC 140/921437, Table 11

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3. Sexual Maturation

Sexual maturation among male pups in the F_1 generation was significantly delayed at 5000 ppm as evidenced by the mean number of days that males showed balanopreputial skinfold cleavage. At 0, 500, 1500, or 5000 ppm the number of days were 43.7, 43.3, 43.4, 45.6, respectively. Similar effect was not seen in females.

4. Necropsy Results

A summary of selected relative organ to body weight data in weanlings is presented in Table 10. Significant differences compared to controls were observed at 1500 and/or 5000 ppm in relative heart, lungs, liver, and/or kidneys weights. The increased liver weights were the only consistent effect across sexes and generations and appeared to be dose related. However, the biological significance of any of these weight changes is questionable since no compound-related gross or microscopic findings were observed.

TABLE 10. RELATIVE ORGAN TO BODY WEIGHT DATA[A]

	Dose (ppm)							
Observation	0	500	1500	5000	0	500	1500	5000
	Males				Females			
F_1 Generation - Weanlings								
Heart	0.41	0.40	0.39	0.36**	0.39	0.38	0.37	0.34**
Lungs	0.81	0.73	0.66*	0.65*	0.73	0.67	0.62*	0.60*
Liver	2.88	3.02	3.07*	3.66**	2.71	2.83	2.88*	3.45**
Kidneys	0.85	0.85	0.85	0.81	0.83	0.82	0.82	0.78*
F_{2A} Generation - Weanlings								
Heart	0.40	0.39	0.36	0.37	0.37	0.37	0.34*	0.33*
Lungs	0.77	0.73	0.76	0.66	0.88	0.79	0.71	0.57**
Liver	3.00	2.99	3.21	4.05**	2.88	2.90	3.08	3.96**
Kidneys	0.90	0.85	0.86	0.80**	0.87	0.83	0.84	0.81
F_{2B} Generation - Weanlings								
Heart	0.40	0.38	0.37	0.36*	0.36	0.37	0.34	0.35
Lungs	0.89	0.79	0.81	0.73	0.81	0.78	0.74	0.58*
Liver	2.67	2.84*	2.88*	3.63**	2.58	2.65	2.72	3.51**
Kidneys	0.83	0.87	0.83	0.78	0.79	0.81	0.81	0.77

[A] Data extracted from Study No. SNC 140/921437, Table 15
Significantly different from control: * = $p \leq 0.05$; ** = $p \leq 0.01$

IV. DISCUSSION

A. SYSTEMIC TOXICITY

Systemic toxicity was observed at 5000 ppm as evidenced by clinical signs in dams for a few days during the latter part of lactation. The decreased body weight in both F_1 sexes at 5000 ppm during weeks 4 and 8 did not impact on the overall weight gain during premating and was not considered to be toxicologically relevant. Significant increases in relative liver weights at 1500 and/or 5000 ppm were not accompanied by gross or histopathologic changes. While these increases were considered to be physiologic adaptations to the test compound at 1500 ppm (increases were <10%), they were considered to be toxicologically relevant at 5000 ppm (increases were >10%). Increases in relative kidney weights were also noted at 1500 and 5000 ppm but, again, were without accompanying gross or histopathologic changes, were of a small magnitude (<10%) and, therefore, considered to be unrelated to treatment.

B. REPRODUCTIVE TOXICITY

Reproductive toxicity was observed at 1500 and 5000 ppm as evidenced by impaired pup growth during lactation. Effects were also noted on selected organ weights in pups and on sexual maturation in F_1 males. The effects on organ weights (increased liver weights being the only consistent obser-vation across sexes and generations) were not accompanied by gross or histopathological findings. Parental reproductive performance was unaffected by the test compound.

C. STUDY/REPORTING DEFICIENCIES

The low fertility rates in the second generation matings were statistically analyzed (by the study author) and correlated with a slightly increased body weight in these females. A comparison with the laboratory's historical control data would have been a more appropriate approach in order to sort out the cause for this decreased fertility which also occurred in control groups.

The number of litters available for evaluation at the highest dose level in both matings of the second generation were sufficient according to the guidelines (although at other dose levels they were too low). Therefore, the study has been classified as Minimum in spite of the lower numbers in the other dose groups.

VIII. CONCLUSIONS

Under the conditions of this study, the following NOELs and LOELs are established for systemic and reproductive toxicity:

Systemic NOEL = 1500 ppm
Systemic LOEL = 5000 ppm based on clinical signs in dams during lactation

Reproductive Toxicity NOEL = 500 ppm
Reproductive Toxicity LOEL = 1500 ppm based on impaired pup growth during lactation

VII. CORE CLASSIFICATION: Minimum. This study meets the Subdivision F Guideline requirement (83-4) for a two-generation reproduction study in rats.