

Reviewed by: Margaret L. Jones *M. L. Jones 4/2/87*  
Section III. Tox. Branch (TS-769C)  
Secondary reviewer: Marcia Van Gemert, Ph.D. *M. Van Gemert 4/13/87*  
Section III. Tox. Branch (TS-769C)

005841

#### DATA EVALUATION REPORT

STUDY TYPE: 2-Generation Reproduction TOX. CHEM. NO.: 862B  
ACCESSION NUMBER: 265205, 265206, 265207 MRID NO.:  
TEST MATERIAL: Triazolyl alanine  
SYNONYMS: triazole alanine  
STUDY NUMBER(S): CTL/P/1168 (Revised): RR0255  
SPONSOR: Ciba Geigy Corp., Agricultural Division (Jointly sponsored  
by ICI, Bayer AG, Ciba-Geigy, Rohm and Haas)  
TESTING FACILITY: Imperial Chemical Industries, PLC: Central  
Toxicology Laboratory, Alderley Park,  
Macclesfield, Cheshire, UK  
TITLE OF REPORT: Triazole Alanine: Two-Generation Reproduction  
Study in the Rat  
AUTHOR(S): Milburn, Birtley, Pate, Hollis, Moreland  
REPORT ISSUED: 19 August 1986  
CONCLUSIONS: No observed effect level for maternal toxicity =  
10000 ppm (highest dose tested).

No observed effect level for developmental toxicity =  
2000 ppm; lowest observed effect level for developmental toxicity =  
10000 ppm. Mean initial pup weights were lowered in F<sub>1B</sub> males and  
females at the high dose, and in F<sub>2A</sub> females at the high dose.

Classification: core-Supplementary. Summary incidence of pup  
abnormalities was not documented with number of litters showing  
abnormality or in individual pathology reports. Dose levels  
may not be sufficiently high to demonstrate parental toxicity.  
Statistical analysis of reproductive parameters was not  
performed. Table of males and females mated was not included.  
Historical control data for the observed abnormalities (eg.:  
blood clot on heart, imperforate vagina) were not included.  
Food consumption during pregnancy, lactation and weaning for  
parents was not reported.

Classification of the study may be upgraded following submission  
and evaluation of additional data.

005841

D. Discussion:

1. Dose selection: The rationale for dose selection was not given in the test report. The choice of doses was based on an unspecified "preliminary study". The doses were lower than previously completed studies:

1. Teratology/rats/0, 100, 300, 1000 mg/kg bw (0, 2000, 6000, 20000 ppm): No deaths were reported prior to sacrifice. (10/13/83)

2. Subchronic/rat/ HDT = 20000 ppm

The preliminary study should be submitted for consideration by the Agency, along with the rationale for dose selection. The report indicates the compound intake was somewhat less than 500, 2000, and 10000 ppm.

2. Animal numbers, brother-sister matings: The study used 15 males and 30 females per group. The Guidelines suggest 20 males and sufficient females to produce 20 pregnant females. Although an adequate number of pregnant females was produced, the use of only 15 males is marginally acceptable; particularly in view of the fact that a few brother-sister matings occurred due to error by the animal breeding laboratory. Male and female pups from the same litters were delivered by the lab. This resulted in 4 brother-sister matings (one per group) during the first mating (F<sub>1A</sub>). Three of the litters were normal. The abnormal litter was not identified. Two brother-sister matings occurred during the second mating (F<sub>1B</sub>) and both resulted in normal litters. F<sub>1</sub> parents were not selected from these litters. This reduced the number of litters from which to choose F<sub>1</sub> parents, a possible source of bias.

3. Statistical Analysis: Statistical analysis of reproductive performance data apparently was not performed. These data should be analysed using appropriate statistical methods since their interpretation is crucial to evaluation of the reproduction study.

4. Bodyweight Data: Tables of absolute bodyweights for animals throughout the study should be given. Instead, bodyweight gains and bodyweights in selected phases of the study were given. A summary table of parental mean absolute bodyweights in the premating and gestation/lactation/weaning phases of the study should be given.

5. Mating: A table of males and females mated was apparently not given. This information is necessary to distinguish familially related effects from compound related effects.

6. Food consumption: Food consumption after premating was not reported for F<sub>0</sub> or F<sub>1</sub> parents. There is no information on food consumption during pregnancy, lactation, or weaning. Compound intake cannot be verified for these phases of the study.

7. Incidence of pup abnormalities: Summary table of "Incidence of pup abnormalities" (Table 47, appended page 12) does not indicate number of litters showing the abnormalities and individual pathology reports (Appendix Z) do not reflect the findings of the summary table.

---

Page \_\_\_\_\_ is not included in this copy.

Pages 4 through 15 are not included.

---

The material not included contains the following type of information:

- ☐ Identity of product inert ingredients.
- ☐ Identity of product impurities.
- ☐ Description of the product manufacturing process.
- ☐ Description of quality control procedures.
- ☐ Identity of the source of product ingredients.
- ☐ Sales or other commercial/financial information.
- ☐ A draft product label.
- ☐ The product confidential statement of formula.
- ☐ Information about a pending registration action.
- ☒ FIFRA registration data.
- ☐ The document is a duplicate of page(s) \_\_\_\_\_.
- ☐ The document is not responsive to the request.

---

The information not included is generally considered confidential by product registrants. If you have any questions, please contact the individual who prepared the response to your request.

---

Special Review Criteria (40 CFR 154.7)

A. MATERIALS:

1. Test compound: Triazole alanine [2-amino-3-(1,2,4-triazol-1-yl) propionic acid] . Description off-white powder; Batch # TLB 1207/018-024 (Y01210/003/005), Purity 97.8% w/w, contaminants in CBI appendix (appended pages 1-2).
2. Test animals: Species: Rat, Strain: Alpk:AP Wistar-derived, Age: 28 days, Mean weight range among groups: 73.9-74.8 g. (males), 70.5-71.5 g. (females), Source: Alderley Park Breeding Unit, Imperial Chemical Industries, PLC, Alderley Park, Macclesfield, Cheshire, UK

B. STUDY DESIGN:

1. Animal assignment

F<sub>0</sub> parental animals were assigned randomly by litter to the following test groups. Appended page 3 shows the allocation of rats to F<sub>0</sub> parental groups. F<sub>1</sub> parents were chosen from F<sub>1B</sub> litters with 6-18 pups per litter. Thirty females and 15 males were selected from each group.

| Test Group  | Dose in diet (ppm)* | F <sub>0</sub> matings |        | F <sub>1</sub> matings |        |
|-------------|---------------------|------------------------|--------|------------------------|--------|
|             |                     | male                   | female | male                   | female |
| 1 Cont.     | 0                   | 15                     | 30     | 15                     | 30     |
| 2 Low (LDT) | 500                 | 15                     | 30     | 15                     | 30     |
| 3 Mid (MDT) | 2000                | 15                     | 30     | 15                     | 30     |
| 4 High(HDT) | 10000               | 15                     | 30     | 15                     | 30     |

2. Diet preparation

Diet was prepared monthly and stored at room temperature ("ambient conditions"). Samples of treated food from the 500 and 10000 ppm preparations from 21 May 1983 were analysed for stability on 27 May, 22 July, 22 August (500 ppm) and on 25 May, 27 July (10000 ppm). Homogeneity was determined from the same preparations, using 4 samples of each.

Results -

Mean concentrations ranged from 476 to 503 over the three month sampling interval and from 9114 to 9609 ppm over the two month sampling interval for stability determination. Deviations from the mean concentration ranged from -2.9 to +2.7 percent for the 500 ppm preparation, and from -4.1 to +3.1 percent for the 10000 ppm preparation.

16

005841

Triazole alanine was apparently homogeneously distributed in the feed and was stable over the period tested.

3. Animals received food ("control CT1 diet" from Special Diets Services, Stepfield, Witham, Essex, UK) and water ad libitum. Content of the diet is found in appended pages 4-5.
4. Statistics - The following procedures were utilized in analyzing the numerical data: see appended pages 6-8.
5. Quality assurance was signed by J.R. Pateman on 19 August 1986. Twelve inspections or audit of reports, protocols, etc., were made from 4 May 1983 to 14 August 1986.

## C. METHODS AND RESULTS

1. Mating Schedule - Four groups each containing 15 male and 30 female rats were designated the first parental generation in the study ( $F_0$ ). During the initial 12 weeks of the study, the males were housed individually and females were housed 2 (of the same group) per cage. During the mating period, each male was cohabited with 2 females of the same group. After mating the animals were individually housed. Pregnancy was initially diagnosed by a finding of sperm in vaginal smears examined daily. (Day 1 of pregnancy was the day sperm were observed in the smear.) Pregnancy was confirmed by abdominal enlargement and weight gain. Sperm-positive females which failed to become pregnant were remated as described above. The report did not specify if the same males were used in such rematings.

Six days after weaning litter A ( $F_{1A}$ ) the  $F_0$  females were mated to different males for litter B ( $F_{1B}$ ). From the  $F_{1B}$ , 30 females and 15 males were selected to become  $F_1$  parents.  $F_{1B}$  litters were separated from the parents at 29 days post partum but litters remained housed together until day 36 post partum.  $F_1$  parents were selected from litters with 6-18 pups.

$F_1$  parents were placed on a premating feeding period for 11 weeks after which they were bred to produce  $F_{2A}$  and  $F_{2B}$  litters as described above for the previous generation.

The mating schedule is shown in appended page 9. Reproductive performance and litter data are shown in part 5.

2. Observations: Toxicity, Mortality, and Interim Kills

Animals were inspected daily for signs of toxicity and mortality. Detailed examinations were made at the time of weighing. Mortality and interim kills are reported below with cause of death where apparent.

### Parental Observations

$F_0$  Premating (weeks 1-12)- Males showed scabs in 3/15 high dose, in 2/15 mid and low dose, v. 1/15 controls and chromodacryorrhea in 7/15 low dose v. 0/15 controls. Females showed hair loss in 6/30 at the high dose v. 1/30 controls.

$F_0$  Mating/Gestation/Lactation - Observations in males were similar to controls. Two control females and one low dose female were found dead following difficult parturition. Hair loss was observed in 6/30 females at the low dose, in 10/30 at the high dose, and in 4/30 controls. One female each in the low and mid doses was

killed after finding no vaginal opening. Imperforate vagina is a congenital anomaly of the Alpk:AP strain of Alderley Park rat, according to the test report, although no documentation was given to support this statement.

F<sub>1</sub> Premating (weeks 1-11)- Observations in males were similar to controls. Females showed coat staining in 5/30 low dose, in 6/30 mid dose and in 3/30 controls. One low dose male was killed in extremis in week 7. This male showed a jaw abnormality (malocclusion) and was hunched and ungroomed.

F<sub>1</sub> Mating/Gestation/Lactation- Males showed coat staining in 4/15 at the high dose and 2/15 controls. One low dose male and 2 mid dose males were killed in extremis: At 32 weeks the low dose male appeared to have a traumatic head injury, at 29 weeks one mid dose male appeared to have been attacked by a female and at histopathology examination the adrenals and pituitary were missing, at 26 weeks another mid dose male was found with hind legs paralyzed, bladder distended, pale kidneys, and minimal hepatitis.

One high dose female was found dead at 30 weeks. Dystocia was diagnosed. Another high dose female was killed at 20 weeks to investigate failure of parturition -18 pups were found in utero and dystocia was diagnosed. One control animal was killed at 28 weeks due to difficult parturition. No underlying cause of dystocia was discovered at pathology examination. Coat staining occurred in almost half of the females at each dose including controls.

### Litter Observations

F<sub>1A</sub> - A few litters showed minimal signs of toxicity such as bruising, small/thin pups, pale appearance and absence of milk in stomach.

F<sub>1B</sub>, F<sub>2A</sub>, and F<sub>2B</sub> showed similar findings with the exception of increased observations of chromodacryorrhea. There were no apparent increases in any of these findings in dose groups as compared to controls.

### 3. Body weight

Animals were weighed weekly through the premating periods. Males were then weighed every 4 weeks and females were weighed on days 1, 8, 15, and 22 of pregnancy. Day one of pregnancy was the day sperm were observed in the vaginal smear.



005841

## Results-

Parental mean absolute bodyweights during premating and pregnancy were similar in control and dose groups.

### Parental Bodyweight Gains

F<sub>0</sub> Premating- Males showed no significant differences from controls. Females showed several isolated instances of bodyweights significantly different from controls but the differences showed no pattern or dose relation.

F<sub>1</sub> Premating- Males showed mean bodyweight gains significantly reduced compared to controls during weeks 4-11 at the low and mid doses and during weeks 4-6 at the high dose. Mean bodyweight gains of females during the F<sub>1</sub> premating period were similar to controls.

### Selected Bodyweight Gains in F<sub>1</sub> Males - Premating (g)

|         | 0     | 500     | 2000    | 10000 (ppm) |
|---------|-------|---------|---------|-------------|
| week 4  | 209.9 | 195.5** | 196.0** | 199.3*      |
| week 5  | 244.0 | 227.5*  | 226.9** | 232.9       |
| week 6  | 272.9 | 252.2** | 252.5** | 258.3*      |
| week 7  | 293.9 | 275.9*  | 273.3** | 283.8       |
| week 8  | 313.7 | 295.2*  | 294.5*  | 302.8       |
| week 9  | 333.3 | 315.8*  | 313.0*  | 321.7       |
| week 10 | 351.7 | 333.5*  | 328.9** | 339.6       |
| week 11 | 363.7 | 347.6   | 343.6*  | 354.7       |

\* Statistically significantly different from controls at  $p < 0.05$ .

\*\* Statistically significantly different from controls at  $p < 0.01$ .

All of the weight differences indicated above were at most 8% lower than control bodyweight gains and showed no dose-related trend. The weight gain differences do not therefore appear to demonstrate significant toxicity.

F<sub>0</sub>/F<sub>1</sub> Gestation/Lactation/Weaning- Bodyweight gains were similar in controls and dose groups except for days 1-8 in F<sub>0</sub> low dose parents during the first mating (litter A). Bodyweight gain was significantly greater ( $p < 0.05$ ) than controls for this interval.

005841

# Litter and Pup Weights

Mean absolute pup weights in dosed animals did not differ significantly from controls, as shown below.

|                  |       | Mean Pup Weights (g) |       |       |       |       |       |           |       |
|------------------|-------|----------------------|-------|-------|-------|-------|-------|-----------|-------|
|                  |       | 0                    |       | 500   |       | 2000  |       | 10000 ppm |       |
|                  |       | M                    | F     | M     | F     | M     | F     | M         | F     |
| F <sub>1</sub> A | Day 1 | 5.67                 | 5.36  | 6.05  | 5.72  | 5.89  | 5.60  | 5.75      | 5.43  |
|                  | 5     | pages                | miss. | 9.40  | 9.15  | 9.29  | 8.83  | 9.14      | 8.46  |
|                  | 11    | 18.15                | 17.60 | 18.90 | 18.24 | 18.17 | 17.10 | 18.60     | 17.57 |
|                  | 22    | 41.89                | 40.58 | 43.01 | 40.72 | 41.90 | 39.30 | 43.27     | 41.07 |
|                  | 29    | 76.46                | 71.96 | 78.92 | 73.20 | 77.34 | 69.90 | 78.73     | 73.13 |
| F <sub>1</sub> B | Day 1 | 6.40                 | 6.03  | 6.30  | 5.83  | 6.25  | 5.83  | 5.91      | 5.57  |
|                  | 5     | 9.96                 | 9.51  | 9.74  | 9.30  | 10.00 | 9.42  | 9.30      | 8.77  |
|                  | 11    | 19.78                | 19.17 | 19.36 | 18.73 | 19.56 | 19.06 | 18.80     | 17.87 |
|                  | 22    | 45.43                | 43.30 | 44.08 | 42.08 | 44.98 | 42.18 | 43.56     | 41.44 |
|                  | 29    | 82.33                | 76.15 | 78.92 | 73.47 | 81.66 | 76.24 | 79.00     | 73.15 |
| F <sub>2</sub> A | Day 1 | 6.02                 | 5.70  | 6.28  | 5.81  | 6.28  | 5.87  | 5.60      | 5.29  |
|                  | 5     | 9.53                 | 9.32  | 9.66  | 9.02  | 9.72  | 9.24  | 8.88      | 8.55  |
|                  | 11    | 19.23                | 18.85 | 19.55 | 18.53 | 19.79 | 18.74 | 17.95     | 17.58 |
|                  | 22    | 42.68                | 41.45 | 43.30 | 41.09 | 44.67 | 41.99 | 40.64     | 39.67 |
|                  | 29    | 78.46                | 74.59 | 79.26 | 73.21 | 80.96 | 74.38 | 76.58     | 72.22 |
| F <sub>2</sub> B | Day 1 | 6.15                 | 5.83  | 6.62  | 6.35  | 6.34  | 5.98  | 5.91      | 5.53  |
|                  | 5     | 10.73                | 9.99  | 11.37 | 10.88 | 10.82 | 10.23 | 10.21     | 9.65  |
|                  | 11    | 22.09                | 21.02 | 22.89 | 21.89 | 21.50 | 20.57 | 19.88     | 19.32 |
|                  | 22    | 48.79                | 47.06 | 50.67 | 48.25 | 47.63 | 45.24 | 44.33     | 42.95 |
|                  | 29    | 87.35                | 81.83 | 90.55 | 83.89 | 85.92 | 78.92 | 81.44     | 76.12 |

Mean initial pup weights were similar in controls and dose groups, with the following exceptions.

|                  |                | Selected Mean Initial Pup Weights (g) |                   |      |                   |
|------------------|----------------|---------------------------------------|-------------------|------|-------------------|
|                  |                | 0                                     | 500               | 2000 | 10000 (ppm)       |
| F <sub>1</sub> A | Males          |                                       |                   |      |                   |
|                  | Initial Weight | 5.7 <sup>+</sup>                      | 6.2 <sup>**</sup> | 5.9  | 5.8               |
|                  | Females        |                                       |                   |      |                   |
|                  | Initial Weight | 5.3                                   | 5.8 <sup>**</sup> | 5.6  | 5.4               |
| F <sub>1</sub> B | Males          |                                       |                   |      |                   |
|                  | Initial Weight | 6.5                                   | 6.3               | 6.3  | 6.0 <sup>**</sup> |
|                  | Females        |                                       |                   |      |                   |
|                  | Initial Weight | 6.1                                   | 5.9               | 5.9  | 5.6 <sup>**</sup> |
| F <sub>2</sub> A | Females        |                                       |                   |      |                   |
|                  | Initial Weight | 5.7                                   | 5.8               | 5.9  | 5.3 <sup>*</sup>  |
| F <sub>2</sub> B | Females        |                                       |                   |      |                   |
|                  | Initial Weight | 5.8                                   | 6.3 <sup>*</sup>  | 6.0  | 5.6               |

\* Statistically significantly different from controls at  $p < 0.05$ .  
 \*\* Statistically significantly different from controls at  $p < 0.01$ .  
 + Mean values have been adjusted for standard deviation, as part of statistical analysis.

21

Mean pup weight gains between day 1 and day 29 were similar in controls and dose groups.

Total litter weights in the F<sub>1A</sub> generation were significantly lower than controls at the high dose group throughout lactation and weaning and in the mid dose group only initially. Litter weights were similar between controls and dose groups in the F<sub>1B</sub> generation. In the F<sub>2A</sub> generation there were significant differences between controls and mid and high dose groups through lactation and weaning and in the F<sub>2B</sub> generation at the high dose through lactation and weaning, as shown below. Although the differences shown are statistically significant, total litter weight is not considered a sensitive indicator of toxicity since it can vary according to the number of pups and/or litters surviving.

| Selected Mean Total Litter Weights (g) |       |               |             |        |
|--|-------|---------------|-------------|--------|
|  | 0     | 2000          | 10000 (ppm) |        |
| F <sub>1A</sub>                        |       |               |             |        |
| Initial                                | 63.7  | 55.7 (12.5%)+ | 55.3        | (13%)  |
| Day 5                                  | 89.5  | 84.1 (6)      | 76.9        | (14)   |
| 11                                     | 177.7 | 169.9 (4)     | 158.6       | (11)   |
| 22                                     | 406.4 | 369.1 (9)     | 363.4       | (11)   |
| 29                                     | 709.8 | 658.6 (7)     | 649.4       | (8.5)  |
| F <sub>2A</sub>                        |       |               |             |        |
| Initial                                | 64.2  | 58.7 (11.7%)  | 61.3        | (7.8%) |
| Day 5                                  | 99.7  | 85.5 (14)     | 91.2        | (8.5)  |
| Day 11                                 | 202.3 | 172.0* (15)   | 182.6       | (10)   |
| Day 22                                 | 445.4 | 380.1* (15)   | 413.0       | (7)    |
| Day 29                                 | 812.5 | 689.7* (15)   | 770.3       | (5)    |
| F <sub>2B</sub>                        |       |               |             |        |
| Initial                                | 64.2  | 64.9          | 51.8*       | (19%)  |
| Day 5                                  | 105.7 | 107.6         | 88.5*       | (16)   |
| Day 11                                 | 210.2 | 213.4         | 168.5**     | (20)   |
| Day 22                                 | 462.7 | 468.8         | 372.4**     | (20)   |
| Day 29                                 | 817.7 | 839.3         | 671.5*      | (18)   |

\* Statistically significantly different from controls at p<0.05.

\*\* Statistically significantly different from controls at p<0.01.

+ Number in parentheses shows change from control value.

#### 4. Food consumption and compound intake

Method: Consumption was determined for F<sub>0</sub> and F<sub>1</sub> parental animals during their respective premating periods only. Compound intake was measured from diet samples and was calculated in ppm. Efficiency was calculated from food consumption and body weight gains for weeks 1-4, 5-8, and 9-12 (F<sub>0</sub>) or 9-11 (F<sub>1</sub>) for the premating period.

22

### Compound Intake

Concentration of triazole alanine in the diet was measured in ppm. The conversion to mg/kg/day appears in the following table as the average of several measurements.

| Mean Intake of Triazole Alanine (ppm) |              |        |
|---------------------------------------|--------------|--------|
| Theoretical ppm                       | Measured ppm | mg/kg+ |
| 0                                     | 0            | 0      |
| 500                                   | 477          | 23.85  |
| 2000                                  | 1956         | 97.80  |
| 10000                                 | 9586         | 479.30 |

+ Calculated from ppm as reported.

### Food Consumption

F<sub>0</sub> Premating period- Food consumption was similar in dose groups and controls for both males and females except for males at 2000 ppm. During week 6 consumption for these males was significantly greater than controls. This apparently isolated instance does not appear to be toxicologically meaningful.

F<sub>1</sub> Premating period- Food consumption was similar in dose groups and controls for males and females except at 500 ppm for females where consumption was significantly lower during weeks 7,8, and 11. Total food consumption during the premating period did not appear to differ among dose groups as appears in the following table.

| Total Food Consumption/Premating Period (g/rat) |      |      |      |             |
|---|------|------|------|-------------|
|   | 0    | 500  | 2000 | 10000 (ppm) |
| F <sub>0</sub> males                            | 2430 | 2448 | 2474 | 2462        |
| F <sub>0</sub> females                          | 1698 | 1675 | 1690 | 1682        |
| F <sub>1</sub> males                            | 2366 | 2313 | 2353 | 2324        |
| F <sub>1</sub> females                          | 1571 | 1522 | 1564 | 1585        |

### Food Utilization

Premating- Efficiency of food utilization was significantly different from controls in several intervals which did not appear to follow a pattern related to dose, as shown in the following table.

| Selected Food Utilization (g food/g growth) |       |         |        |             |
|---|-------|---------|--------|-------------|
|   | 0     | 500     | 2000   | 10000 (ppm) |
| F <sub>0</sub> parents                      |       |         |        |             |
| Males- wk 1-4                               | 3.56  | 3.61    | 3.60   | 3.69*       |
| wk 9-12                                     | 14.49 | 12.57** | 13.98  | 13.81       |
| F <sub>0</sub> parents                      |       |         |        |             |
| Females-wk 9-12                             | 25.77 | 22.33** | 23.65  | 24.26       |
| F <sub>1</sub> parents                      |       |         |        |             |
| Males- wk 1-4                               | 3.78  | 3.93*   | 3.97*  | 3.91        |
| Females-wk 9-11                             | 25.74 | 27.17   | 39.90* | 29.76       |

\* Statistically significantly different from controls at p<0.05.

\*\* Statistically significantly different from controls at p<0.01.

23

When food utilization was calculated for the overall pre-mating period there were no significant differences between controls and dose groups.

Food consumption during gestation/lactation/weaning was apparently not reported. (See Section D. Discussion.)

# 5. Reproductive Performance and Litter Data

The following table shows the summary of reproductive performance and litter data for both matings in both generations.

Summary of Reproductive Performance and Litter Data  
(Adapted from Tables 13A,13B,14A,14B,25A,25B,26A,26B)

|                               | Dose (ppm)                |     |      |       |                           |     |      |       |
|-------------------------------|---------------------------|-----|------|-------|---------------------------|-----|------|-------|
|                               | F <sub>0</sub> generation |     |      |       | F <sub>1</sub> generation |     |      |       |
|                               | 0                         | 500 | 2000 | 10000 | 0                         | 500 | 2000 | 10000 |
| Males                         | 15                        | 15  | 15   | 15    | 15                        | 15  | 15   | 15    |
| Females                       | 30                        | 30  | 30   | 30    | 30                        | 30  | 30   | 30    |
| <u>First Littering</u>        |                           |     |      |       |                           |     |      |       |
| No. females paired            | 29                        | 29  | 29   | 30    | 30                        | 30  | 30   | 30    |
| No. w. positive vaginal smear | 28                        | 25  | 27   | 28    | 27                        | 29  | 24   | 29    |
| No. that littered             | 26                        | 20  | 26   | 25    | 28                        | 29  | 28   | 29    |
| No. viable litters at birth   | 26                        | 20  | 26   | 25    | 28                        | 29  | 28   | 28    |
| No. viable litters at day 1   | 26                        | 20  | 26   | 25    | 28                        | 29  | 28   | 28    |
| No. litters stillborn         | 0                         | 0   | 0    | 0     | 0                         | 0   | 0    | 1     |
| No. litters viable at weaning | 24                        | 20  | 24   | 24    | 27                        | 29  | 28   | 28    |
| No. pups born live            | 278                       | 213 | 254  | 246   | 312                       | 320 | 280  | 323   |
| dead                          | 19                        | 2   | 3    | 2     | 15                        | 3   | 11   | 8     |
| Total pups born               | 297                       | 215 | 257  | 248   | 327                       | 323 | 291  | 331   |

24

Reproductive Performance (continued)

005841

|                               | Dose (ppm)                      |     |      |       |                                 |     |      |       |
|-------------------------------|---------------------------------|-----|------|-------|---------------------------------|-----|------|-------|
|                               | <u>F<sub>0</sub> generation</u> |     |      |       | <u>F<sub>1</sub> generation</u> |     |      |       |
|                               | 0                               | 500 | 2000 | 10000 | 0                               | 500 | 2000 | 10000 |
| Males                         | 15                              | 15  | 15   | 15    | 15                              | 15  | 15   | 15    |
| Females                       | 30                              | 30  | 30   | 30    | 30                              | 30  | 30   | 30    |
| <u>Second Littering</u>       |                                 |     |      |       |                                 |     |      |       |
| No. females paired            | 28                              | 29  | 29   | 30    | 30                              | 30  | 30   | 30    |
| No. w. positive vaginal smear | 28                              | 29  | 27   | 24    | 28                              | 28  | 27   | 26    |
| No. that littered             | 27                              | 27  | 27   | 24    | 27                              | 28  | 27   | 26    |
| No. viable litters at birth   | 27*                             | 26  | 27   | 24    | 27                              | 28  | 26   | 26    |
| No. viable litters at day 1   | 27                              | 26  | 27   | 24    | 27                              | 28  | 26   | 26    |
| No. litters stillborn         | 0                               | 1   | 0    | 0     | 0                               | 0   | 1    | 0     |
| No. litters viable at weaning | 26                              | 25  | 27   | 23    | 24                              | 27  | 26   | 24    |
| No. pups born live            | 278                             | 282 | 285  | 260   | 257*                            | 255 | 275  | 221   |
| dead                          | 2                               | 12  | 5    | 8     | 33                              | 5   | 5    | 43    |
| Total pups born               | 280                             | 294 | 290  | 268   | 290                             | 260 | 280  | 264   |

\* Includes one litter with one viable pup but female died with remaining pups in utero.

+ Excludes one litter not weighed on day 1.

There were decreases in the number of pups born live in the F<sub>1A</sub> (12% less than controls) and in the F<sub>2B</sub> (14% less than controls) generations at the high dose only. These differences were apparently due to one less viable litter at the high dose than in controls in each of these generations.

The proportion of fertile animals in the low dose group in the F<sub>0</sub> generation at first mating (Litter A) was 74%. This finding does not appear significant since fertility during the second mating of this group (Litter B) was 93%. Other reproductive parameters in dosed animals were similar to controls.

25

005841

# 6. Histopathology Examination

The examination excluded females with abnormal breeding records, which are reported separately in appended pages 10-11. Controls and high dose animals were examined. Low and mid dose animals were not examined unless gross abnormalities were found.

## Histopathology: Microscopic Findings in Parents (no. observations/no. examined)

|                                       | Males |     |      |       | Females |     |      |       | ppm |
|---------------------------------------|-------|-----|------|-------|---------|-----|------|-------|-----|
|                                       | 0     | 500 | 2000 | 10000 | 0       | 500 | 2000 | 10000 |     |
| <u>F<sub>0</sub> Parents</u>          |       |     |      |       |         |     |      |       |     |
| Liver                                 |       |     |      |       |         |     |      |       |     |
| Inflammatory cell infiltration        | 1/15  | 0/0 | 0/0  | 3/15  | 1/30    | 0/4 | 0/2  | 2/30  |     |
| Pituitary cysts                       | 1/15  | 0/0 | 0/0  | 1/15  | 2/29    | 0/0 | 0/0  | 4/30  |     |
| Lungs                                 |       |     |      |       |         |     |      |       |     |
| alveolar histiocytosis                | 0/0   | 0/0 | 0/0  | 0/0   | 0/1     | 2/3 | 0/0  | 0/0   |     |
| Kidneys                               |       |     |      |       |         |     |      |       |     |
| Nephrocalcinosis                      | 0/15  | 0/2 | 0/2  | 1/15  | 29/30   | 3/3 | 3/3  | 30/30 |     |
| Tubular dilatation                    | 3/15  | 0/2 | 1/2  | 6/15  | 0/30    | 0/3 | 0/3  | 1/30  |     |
| Hyaline casts                         | 1/15  | 0/2 | 1/2  | 4/15  | 0/30    | 0/3 | 0/3  | 1/30  |     |
| Tubular basophilia                    | 2/15  | 0/2 | 1/2  | 3/15  | 0/30    | 1/3 | 0/3  | 0/30  |     |
| Ovaries                               |       |     |      |       |         |     |      |       |     |
| Cystic follicle(s) (follicular cysts) | -     | -   | -    | -     | 5/26    | 0/3 | 0/5  | 3/20  |     |
| <u>F<sub>1</sub> Parents</u>          |       |     |      |       |         |     |      |       |     |
| Liver                                 |       |     |      |       |         |     |      |       |     |
| Hepatitis                             | 6/15  | 1/2 | 1/2  | 8/15  | 1/30    | 0/0 | 0/1  | 0/30  |     |
| Bile duct                             |       |     |      |       |         |     |      |       |     |
| prolif./fibrosis                      | 4/15  | 1/2 | 1/2  | 5/15  | 0/30    | 0/0 | 0/1  | 0/30  |     |
| Kidneys                               |       |     |      |       |         |     |      |       |     |
| Nephrocalcinosis                      | 3/15  | 0/3 | 1/4  | 1/15  | 29/30   | 1/1 | 1/1  | 30/30 |     |
| Nephropathy                           | 6/15  | 0/3 | 1/4  | 7/15  | 0/30    | 0/1 | 0/1  | 1/30  |     |
| Ovaries                               |       |     |      |       |         |     |      |       |     |
| Follicular cysts (cystic follicle(s)) | -     | -   | -    | -     | 5/25    | 0/2 | 0/2  | 5/28  |     |
| Luteal cysts                          | -     | -   | -    | -     | 2/25    | 0/2 | 0/2  | 4/28  |     |
| Simple cysts                          | -     | -   | -    | -     | 0/25    | 0/2 | 0/2  | 2/28  |     |

26

005841

Histopathology: Microscopic Findings in Offspring  
(no. observed/no. examined)

|                                     | Males |     |      |       | Females |     |      |       | ppm |
|-------------------------------------|-------|-----|------|-------|---------|-----|------|-------|-----|
|                                     | 0     | 500 | 2000 | 10000 | 0       | 500 | 2000 | 10000 |     |
| <u>F1A Offspring</u>                |       |     |      |       |         |     |      |       |     |
| Kidneys                             |       |     |      |       |         |     |      |       |     |
| Hydronephrosis                      | 4/4   | 1/1 | 0/0  | 1/1   | 1/1     | 0/0 | 1/1  | 0/0   |     |
| <u>F1B Offspring/Full necropsy</u>  |       |     |      |       |         |     |      |       |     |
| Kidneys                             |       |     |      |       |         |     |      |       |     |
| Nephrocalcinosis                    | 0/5   | 0/0 | 0/0  | 0/4   | 1/5     | 0/0 | 0/0  | 2/6   |     |
| <u>F1B Offspring/Gross necropsy</u> |       |     |      |       |         |     |      |       |     |
| Kidneys                             |       |     |      |       |         |     |      |       |     |
| Hydronephrosis                      | 4/4   | 0/0 | 3/4  | 3/3   | 3/3     | 0/0 | 0/0  | 1/1   |     |
| <u>F2A Offspring/Gross necropsy</u> |       |     |      |       |         |     |      |       |     |
| Kidneys                             |       |     |      |       |         |     |      |       |     |
| Hydronephrosis                      | 2/2   | 1/1 | 3/3  | 2/2   | 2/2     | 2/2 | 3/3  | 1/1   |     |
| <u>F2B Offspring/Full necropsy</u>  |       |     |      |       |         |     |      |       |     |
| Kidneys                             |       |     |      |       |         |     |      |       |     |
| Hydronephrosis                      | 1/10  | 1/1 | 1/1  | 3/10  | 0/10    | 1/1 | 0/0  | 0/10  |     |
| Nephrocalcinosis                    | 0/10  | 0/1 | 0/1  | 0/10  | 0/10    | 0/1 | 0/0  | 4/10  |     |
| <u>F2B Offspring/Gross necropsy</u> |       |     |      |       |         |     |      |       |     |
| Kidneys                             |       |     |      |       |         |     |      |       |     |
| Hydronephrosis                      | 0/0   | 1/2 | 2/2  | 1/1   | 2/3     | 0/0 | 0/0  | 0/0   |     |

Histopathology findings were similar in controls and dose groups. A frequent finding was kidney histopathology including nephrocalcinosis and hydronephrosis. However, this observation was of similar frequency in controls and the high dose group except for offspring in the F2B in which nephrocalcinosis was described in 4/10 high dose females and 0/10 controls.

The abnormal breeding records (appended pages 10-11) show similar findings between controls and dose groups. The table of significant pup abnormalities (appended page 12) summarizes abnormalities in each of the four litters produced in the study. In the high dose F2B litters there were 14 pups with ureters kinked and/or dilated. Ten of the 14 pups were from one litter, and the control incidence was 3. In addition, in the high dose F2B litters there were 4 pups with a blood clot on the heart. The significance of this finding and the finding of kinked and/or dilated ureters cannot be evaluated since the individual pathology reports for pups at this dose do not permit verification of the findings in the summary table (appended page 12).

27