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

MRIDOO15336

OFFICE OF
PESTICIDES AND TOXIC SUBSTANCES

MEMORANDUM

OCT 21 1986

SUBJECT: Assure (NC-302; ethyl 2-[4-(6-chloro-2-quioxalinyloxy)phenoxy]propionate): Evaluation of 2-generation reproduction study in rats.

Caswell No.: 215D
Accession No.: 074017
Project No.: 1162/1163

Action Code: 231
Record No. : 164169/164171

SUBMITTER: E. I. Du Pont De Nemours & Co.

TO: Robert J. Taylor
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FROM: Whang Phang, Ph.D. Whang Phang 9/25/86
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THRU: Marcia van Gemert, Ph.D. M. van Gemert 9.25.86
Section Head
and
Theodore M. Farber, Ph.D.
Chief
Toxicology Branch/HED (TS-769C)

Action Requested:
Review 2-generation reproduction study in rats to complete data base for registration and tolerance for soybeans and cotton.

Results:
The attached DER (EPA No. 68-02-4225; Dynamac No. 2061; Sept. 17, 1986) has been approved by Toxicology Branch. This 2-generation reproduction study in rats (unpublished study No. MR 7370-001 by Haskell Laboratory for Toxicology and Industrial Medicine, Newark, DE; Nov. 25, 1985) is classified Core Supplementary.

In this study, Assure was tested at concentrations of 0, 25, 100, and 400 ppm. At 400 ppm the body weights of both $F_0$ and $F_1$ males were decreased relative to controls. No additional compound related effects were observed in the parental animals. The LOEL for parental toxicity of Assure is 400 ppm.

For developmental effects, increased incidence of eosinophilic changes in the livers of $F_{2b}$ weanlings were observed at dose levels of 100 and 400 ppm. At 400 ppm, reductions of litter size, survival, body weights, and spleen weights were seen in offspring. At all concentrations of Assure tested, increased incidences of hematomas were found in $F_{1b}$ pups, and similar observations were made in $F_{2a}$ pups at levels of 100 and 400 ppm. Therefore, LOEL of developmental effect is 25 ppm, and NOEL for this effect could not be determined.

005547

CONFIDENTIAL BUSINESS INFORMATION
DOES NOT CONTAIN
NATIONAL SECURITY INFORMATION (EO 12065)

EPA: 68-02-4225
DYNAMAC No. 206I
September 17, 1986

DATA EVALUATION RECORD

ASSURE

Two-Generation Reproduction Study in Rats

STUDY IDENTIFICATION: Mullin, L. S. Two-generation reproduction study in rats with INY-6202. (Unpublished study No. MR 7370-001 by Haskell Laboratory for Toxicology and Industrial Medicine, Newark, DE, for E. I. duPont de Nemours and Company, Inc., Wilmington, DE; dated November 25, 1985.) Accession No. 074017.

APPROVED BY:

I. Cecil Falkner, Ph.D.
Department Manager
Dynamac Corporation

Signature: I. Cecil Falkner

Date: 9-17-86

005547

1. CHEMICAL: Assure; INY-6202; NC-302; INY-6202-15; propanoic acid, 2-[4-(6-chloroquinoxalin-2-yloxy)phenoxy]ethyl ester.

2. TEST MATERIAL: INY-6202 was described as an off-white powder of 99.1% purity.

3. STUDY/ACTION TYPE: Two-generation reproduction study in rats.

4. STUDY IDENTIFICATION: Mullin, L. S. Two-generation reproduction study in rats with INY-6202. (Unpublished study No. MR 7370-001 by Haskell Laboratory for Toxicology and Industrial Medicine, Newark, DE, for E. I. duPont de Nemours and Company, Inc., Wilmington, DE; dated November 25, 1985.) Accession No. 074017.

5. REVIEWED BY:

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7. CONCLUSIONS:

A. The LOEL for parental toxicity of INY-6202 is assessed at 400 ppm, the highest dose tested, and is based on decreased body weights and premating body weight gain for males when compared to controls. The NOEL for parental toxicity is 100 ppm.

The LOEL for developmental effects is assessed at 25 ppm based on increased incidences of hematomas in pups at all dose levels, increased liver weights and increased incidence of eosinophilic changes in the livers of offspring from the 100- and 400-ppm groups, and reductions in litter size, survival, body weights, and spleen weights of 400-ppm offspring. The NOEL for developmental toxicity could not be determined.

B. This study is classified Core Supplementary.

8. RECOMMENDATIONS:

In the event that further work is conducted, the following steps are recommended:

A. To clearly establish the NOEL for developmental toxicity of INY-6202 in rats it may be necessary to test at a dose level lower than 25 ppm.

B. Individual data are needed for length of mating period, length of gestation, and pup clinical observations.

C. The potential effects of INY-6202 on pup hematologic parameters and their possible relationship to the incidence of hematomas in pups should be investigated.

9. BACKGROUND:

In a 13-week feeding study with a 6-week recovery period, male and female rats were fed diets containing 0, 40, 128, or 1280 ppm INY-6202. Significantly decreased testicular weights and atrophy and/or suppression of spermatogenesis were reported for the males receiving 1280 ppm.

It was reported that in a 2-year dietary study with dose levels of 0, 25, 100, and 400 ppm, no compound-related testicular effects were observed.

Item 10--see footnote 1.

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[1] Only items appropriate to this DER have been included.

11. MATERIALS AND METHODS (PROTOCOLS):



A. Materials and Methods: (See Appendix A for details.)

1. Test Material: INY-6202 from lot No. 8002 was described as an off-white powder of 99.1% purity. The test material was mixed with ground Certified Purina Laboratory Chow #5002 to produce concentrations of 0, 25, 100, and 400 ppm. Diets were prepared weekly and refrigerated until use.

   Test material concentrations in the diet were determined from diet samples taken five times throughout the study. Additional samples were taken at two of those sampling times for determination of homogeneity of the mixtures. Stability of the test material was assayed for preparations stored for 10 and 17 days at room temperature.

2. Test Animals and Study Design: Male and female Crl:CD(SD)BR rats were obtained at the age of 22 days from Charles River Breeding Laboratories, Kingston, NY, and individually housed. Following a 13-day pretest period, 23 males and 23 females were assigned to each of the four treatment groups and designated as $F_0$ parental animals. Upon randomization, mean body weights were approximately equal between all groups of each sex. All rats were fed their respective group's diets throughout the study.

   Following a premating period of approximately 70 days, $F_0$ rats were paired, one male with one female of the same group, for up to 7 days. If a copulatory plug was not observed, the female was rehoused with a proven male for up to 7 days. The female was returned to her assigned cage after the 14-day mating period or when a copulatory plug was observed (designated day 0 of gestation).

   Approximately 1 week after the resulting $F_{1a}$ litters were weaned on day 21 postpartum, $F_0$ females were rebred (to different males) to produce $F_{1b}$ litters.

   From the $F_{1a}$ litters, 23 male and 23 female weanlings were randomly selected to comprise the $F_1$ parental generation. Approximately 80 days after weaning, the $F_1$ rats were bred in the same manner as their parents to produce $F_{2a}$ and $F_{2b}$ litters. Sibling pairings were avoided.

3. Parameters Measured: Animals were observed in their cages at least twice daily and individually handled and examined for abnormal benavior and appearance once per week.

Body weights were determined weekly for all parental animals. Females with confirmed copulation were weighed on days 0, 7, 14, and 21 of gestation and lactation. Food consumption was determined weekly during the $F_0$ and $F_1$ premating periods. Gestational food intake was recorded on days 0, 7, 14, and 21; however, data for days 14-21 were not reported due to early deliveries and food spillage.

The pups of each litter were sexed and weighed collectively by sex on days 0, 4, 7, and 14 postpartum. On day 4, litters were reduced by random selection to eight pups, retaining an equal number of male and female offspring when possible. Day 4 litter weights were determined before and after culling. Individual pup weights were recorded on day 21.

Necropsies were conducted on parental animals that died or were euthanized. Surviving parental males and females were killed after siring or weaning their second litters. For both generations, 10 animals per sex group were selected for gross necropsy and microscopic examination of reproductive organs (i.e., testes, epididymides, and prostate or ovaries, uterus, and vagina).

Ten $F_{2b}$ weanlings per sex per group were randomly chosen for gross examination and determination of selected organ weights. Selected tissues of weanlings in the control and high-dose groups were examined microscopically. Livers and gross lesions of weanlings from all dose groups were examined microscopically.

4. Statistical Methods: Parental body weight, weight change, food consumption, and organ weight were evaluated by one-way analysis of variance (ANOVA), Bartlett's test for homogeneity of variance, and a test for linear trend. Significant differences detected by ANOVA were further evaluated using the least significant difference and Dunnett's tests.

   Incidences of clinical findings were analyzed by Fisher's exact test with a Bonferroni correction and the Cochran-Armitage test for trend.

   Reproductive indices, litter data, and mean pup weights were evaluated using Fisher's exact, Kruskal-Wallis, and Mann-Whitney U tests and Jonckheere's test for trend.

B. Protocol: See Appendix B.

12. REPORTED RESULTS:

A. Diet Analyses: All diet samples were within 94-114% of the nominal concentrations of the test material. Results of analyses indicated that the diet preparations were homogeneous and that the test material remained stable in the diet over the 10- and 17-day periods tested.

B. Parental Effects: One control $F_0$ female was found dead on lactation day 14; autolysis precluded a postmortem examination. A low-dose $F_0$ female with a skull fracture was euthanized. A pregnant $F_0$ female of the high-dose group died after failing to deliver; uterine torsion was noted at necropsy. None of these deaths were regarded to be compound related and no other deaths occurred in $F_0$ or $F_1$ parental rats.

Incidences of clinical observations were not significantly different between dose groups and controls.

Body weights of high-dose male rats were significantly lower than controls at various intervals of both generations (Table 1). The total premating weight gain was significantly decreased for high-dose $F_0$ males and nonsignificantly decreased for high-dose $F_1$ males. The body weight gain of mid-dose $F_0$ males was significantly lower than controls from days 21-28.

Premating body weights and weight gain were generally comparable between control and compound-treated $F_0$ females. In the $F_1$ generation, high-dose females weighed significantly less than controls for the first 21 days on study; however, total premating weight gains were comparable for control and dosed females.

Total body weight gains during days 0-21 of gestation were generally comparable between the controls and dose groups of both generations (Table 2). The mean body weight of high-dose $F_1$ females was significantly lower than controls on day 21 of gestation for the $F_{2b}$ litters; however, a dose-related trend was not evident at this interval.

No adverse compound-related effects were reported for body weight changes during lactation of any litter interval. During the second lactation period for $F_0$ females, significant dose-related trends of increased weights on days 0, 7, 14, and 21 and of smaller total weight losses during days 0-21 were reported. Body weights of mid- and high-dose $F_0$ females were significantly higher than controls on lactation days 14 and 21 ($F_{1b}$ interval). During lactation of the $F_{2b}$ litters, the total weight gain of the high-dose females was significantly greater than for controls, which lost weight over the 21-day period.

TABLE 1. Mean Premating Body Weights and Body Weight Changes (g±SD) of Rats Fed INY-6202 for Two Generations

| Dose Level (ppm) | Day 0 | Day 7 | Day 21 | Day 42 | End of Premating | Premating Weight Change |
|---|---|---|---|---|---|---|
| | | | $F_0$ Males | | | |
| 0 | 133.5± 9.9 | 194.5±11.3 | 306.0±14.6 | 428.8±21.1† | 521.4±31.9† | 387.9±33.3† |
| 25 | 133.1± 9.1 | 195.5±11.2 | 310.4±17.6 | 430.3±24.5 | 520.2±29.7 | 387.1±26.1 |
| 100 | 132.5± 8.4 | 193.3± 9.8 | 304.2±17.0 | 420.6±30.2 | 513.9±44.3 | 381.4±44.1 |
| 400 | 133.0±12.7 | 195.9±15.9 | 304.9±21.4 | 412.9±29.0 | 493.1±38.9* | 360.1±35.6* |
| | | | $F_0$ Females | | | |
| 0 | 116.0± 9.1 | 151.3±11.5 | 197.0±14.3 | 248.1±19.0 | 285.1±20.9 | 169.1±19.4 |
| 25 | 115.9± 8.7 | 155.0±12.4 | 207.1±17.6 | 260.2±24.4 | 301.9±30.3 | 185.8±26.5 |
| 100 | 113.9± 8.8 | 152.2±11.2 | 205.0±16.8 | 258.6±23.4 | 296.2±28.0 | 182.2±22.8 |
| 400 | 114.2± 8.9 | 151.3±11.1 | 201.0±20.8 | 249.6±29.2 | 288.2±33.2 | 174.0±29.6 |
| | | | $F_1$ Males | | | |
| 0 | 56.2± 5.1† | 98.1± 7.7† | 217.8±10.9† | 372.3±28.3† | 512.7±35.2 | 456.5±35.1 |
| 25 | 52.7± 8.7 | 92.8±10.6 | 210.5±20.6 | 375.5±41.0 | 519.6±51.5 | 466.9±46.8 |
| 100 | 54.6± 4.3 | 94.4± 6.9 | 215.0±13.6 | 382.8±22.4 | 530.1±29.3 | 475.5±29.1 |
| 400 | 45.7± 6.3* | 82.0±11.0* | 197.4±20.6* | 350.1±28.0* | 489.5±46.4 | 443.9±42.8 |
| | | | $F_1$ Females | | | |
| 0 | 54.2± 5.1 | 91.2± 8.0† | 169.4±17.8† | 237.2±31.3 | 299.5±33.5 | 245.3±31.6 |
| 25 | 51.2± 7.6 | 87.3± 9.9 | 163.9±12.4 | 228.5±21.0 | 296.6±31.1 | 245.4±30.2 |
| 100 | 52.5± 5.7 | 88.3± 9.2 | 164.1±14.2 | 232.8±24.6 | 300.8±32.8 | 248.3±31.5 |
| 400 | 44.2± 4.3* | 76.7± 7.2* | 157.1±10.8* | 224.3±19.8 | 292.6±32.6 | 248.3±32.6 |

†Significant trend across dose groups ($p \leq 0.05$).
*Significantly different from control value ($p \leq 0.05$).

TABLE 2. Mean Gestation and Lactation Body Weights and Body Weight Changes (g±SD) of Females Fed INY-6202 for Two Generations

| Dose Level (ppm) | Gestation | | | | Lactation | | | |
|---|---|---|---|---|---|---|---|---|
| | Day 0 | Day 14 | Day 21 | Wt. Change Days 0-21 | Day 0 | Day 14 | Day 21 | Wt. Change Days 0-21 |
| | | | | $F_0$-$F_{1a}$ Litter Interval | | | | |
| 0 | 283.0±20.2 | 337.0±22.2 | 413.1±22.7 | 130.1±16.1 | 317.4±21.0 | 334.2±25.0 | 317.7±19.5† | 1.4±13.0 |
| 25 | 284.9±19.1 | 340.7±19.4 | 416.2±22.7 | 131.3±19.3 | 320.9±17.7 | 339.8±21.2 | 319.4±31.4 | 0.4±31.6 |
| 100 | 291.7±29.6 | 346.0±32.0 | 418.1±53.3 | 126.4±39.0 | 324.6±34.8 | 342.3±28.0 | 329.6±30.0 | 4.4±18.7 |
| 400 | 287.5±31.6 | 341.2±35.7 | 411.5±46.6 | 121.7±36.0 | 326.4±31.7 | 353.8±32.1 | 339.0±29.4 | 12.6±14.5 |
| | | | | $F_0$-$F_{1b}$ Litter Interval | | | | |
| 0 | 300.4±23.3† | 365.3±21.9 | 445.9±28.1† | 148.9±28.4 | 347.6±21.7† | 357.9±32.0† | 328.7±38.2† | -18.9±36.1† |
| 25 | 314.2±21.0 | 380.8±30.5 | 460.2±34.2 | 139.7±33.0 | 361.7±31.9 | 370.5±27.9 | 349.2±23.9 | -12.5±18.6 |
| 100 | 322.3±26.0 | 387.5±31.9 | 470.8±40.4 | 149.3±22.6 | 365.7±32.5 | 389.7±28.8* | 359.6±25.0* | -6.1±18.3 |
| 400 | 314.4±30.6 | 379.9±34.2 | 475.6±40.5 | 161.2±16.2 | 368.5±35.1 | 387.7±36.2* | 368.4±37.0* | -0.1±13.8 |
| | | | | $F_1$-$F_{2a}$ Litter Interval | | | | |
| 0 | 298.6±37.4 | 355.5±38.2 | 430.3±45.8 | 132.5±27.8 | 334.4±39.4 | 357.4±34.2 | 333.5±29.2 | -1.0±17.7 |
| 25 | 295.2±28.0 | 352.2±26.2 | 425.4±34.7 | 130.2±25.3 | 322.1±25.4 | 347.2±20.4 | 327.0±19.6 | 4.9±20.9 |
| 100 | 292.5±30.8 | 353.4±30.4 | 430.4±37.8 | 137.9±29.2 | 332.4±32.8 | 353.0±28.2 | 331.4±24.5 | -1.1±17.9 |
| 400 | 288.0±32.6 | 347.9±36.8 | 419.4±38.3 | 136.7±24.0 | 327.4±39.2 | 350.7±31.5 | 331.5±34.1 | 4.1±21.4 |
| | | | | $F_1$-$F_{2b}$ Litter Interval | | | | |
| 0 | 328.8±31.9 | 388.4±34.0 | 475.4±49.7 | 143.7±23.9 | 369.5±38.2 | 376.5±37.1 | 350.7±31.0 | -18.8±21.7† |
| 25 | 314.5±25.4 | 374.9±26.9 | 449.4±28.8 | 141.8±15.8 | 357.4±26.3 | 371.6±18.7 | 351.8±22.0 | -5.6±19.6 |
| 100 | 327.7±36.0 | 390.6±31.5 | 476.7±41.3 | 146.2±36.3 | 375.6±38.4 | 379.5±36.8 | 362.4±35.3 | -13.2±24.9 |
| 400 | 305.4±25.6 | 367.2±32.3 | 437.7±42.1* | 134.9±43.6 | 353.8±33.4 | 374.9±30.5 | 363.5±26.9 | 9.7±20.0* |

†Significant trend across dose groups ($p \leq 0.05$).
*Significantly different from control value ($p \leq 0.05$).

Premating food consumption data were comparable for all male groups in the $F_0$ generation (Table 3). In the second generation, significant trends of decreased food consumption were reported for the dose groups when compared to controls during the first 2 weeks of feeding. During week 1, the high-dose males consumed significantly less than controls, and during week 2, all dosed males consumed significantly less than controls.

For $F_0$ females, a significant dose-related trend of decreased premating food consumption was reported for the first week of feeding. The study authors also noted a nonsignificant dose-related trend toward (slightly) decreased food consumption over the 70-day premating period. In the second generation, a significant dose-related decrease in food consumption for week 1 was reported, with high-dose $F_1$ females consuming significantly less than controls.

During gestation, maternal food consumption was significantly affected on days 0-7 of the $F_{1a}$ interval only; a significant dose-related decrease in food intake occurred and low- and high-dose females consumed significantly less than controls (Table 4). Food consumption during the $F_{1b}$, $F_{2a}$, and $F_{2b}$ litter intervals was generally comparable for all groups.

Fertility rates were comparable for all groups of both generations except for low-dose $F_0$ males and females, which had significantly reduced fertility rates when compared to controls at the first mating (Table 5). All pregnant females successfully delivered live pups and maintained their litters until weaning. Mating data were not reported.

Gross necropsy findings did not indicate any compound-related effects. Histopathological examination showed foci of nodular hyperplasia of interstitial cells in the testes of one high-dose $F_1$ male; the study author stated that this lesion is not usually spontaneous in rats under 1 year of age. However, abnormal testicular pathology was not noted in any other parental males examined (10/group) nor were fertility or testicular weights decreased at the high-dose level. In addition, no compound-related testicular effects were reported in a 2-year study using the same dietary concentrations. Thus, the study author concluded that a compound-related testicular effect was not indicated.

Mean absolute and relative testicular weights were comparable for all groups of $F_0$ and $F_1$ parental males. No other organs from parental animals were weighed.

TABLE 3. Mean Premating Food Consumption (g/rat/day ± SD) for Rats Fed INY-6202 for Two Generations

| Dose Level (ppm) | Days on Study | | | | Total Premating Interval |
|---|---|---|---|---|---|
| | 0-7 | 7-14 | 42-49 | 63-70 | |
| | | | $F_0$ Males | | |
| 0 | 23.1±1.5 | 25.8±1.4 | 30.3±1.8 | 31.3±2.0 | 29.0±1.3 |
| 25 | 23.3±1.4 | 25.9±1.6 | 30.3±2.0 | 31.1±3.0 | 28.9±1.7 |
| 100 | 23.0±1.8 | 25.5±1.9 | 30.4±2.6 | 31.8±3.2 | 28.7±2.1 |
| 400 | 23.0±2.1 | 25.3±2.2 | 30.7±4.3 | 30.9±3.4 | 28.6±2.5 |
| | | | $F_0$ Females | | |
| 0 | 20.6±4.2† | 20.4±2.1 | 22.4±2.4 | 22.7±2.7 | 21.9±2.3 |
| 25 | 19.5±1.8 | 20.2±2.3 | 22.7±3.0 | 22.6±2.4 | 21.7±2.2 |
| 100 | 19.3±1.7 | 19.6±3.8 | 21.6±2.2 | 21.9±2.2 | 21.1±2.0 |
| 400 | 19.1±1.9 | 19.4±2.6 | 21.8±3.1 | 22.1±2.7 | 20.9±2.6 |
| | | | $F_1$ Males | | |
| 0 | 15.4±3.2† | 22.8±2.3† | 34.0±3.9 | 31.5±2.7 | 29.1±1.8 |
| 25 | 14.5±1.1 | 21.0±2.1* | 32.7±3.6 | 31.0±2.9 | 28.3±2.7 |
| 100 | 15.1±1.1 | 21.5±1.2* | 34.7±3.8 | 31.9±2.7 | 29.5±1.8 |
| 400 | 13.6±2.2* | 20.3±2.5* | 33.3±9.0 | 32.4±2.7 | 28.8±2.0 |
| | | | $F_1$ Females | | |
| 0 | 15.7±2.6† | 20.6±2.5 | 26.7±3.5 | 23.4±3.1 | 23.0±2.4 |
| 25 | 14.4±1.9 | 19.8±2.1 | 24.5±3.4 | 21.5±2.2* | 21.7±1.6 |
| 100 | 14.8±3.5 | 19.8±2.5 | 25.4±6.2 | 20.2±2.8* | 22.1±2.1 |
| 400 | 12.5±2.9* | 19.5±2.7 | 25.1±4.6 | 23.7±2.5 | 23.0±2.4 |

†Significant trend across dose groups ($p \leq 0.05$).
*Significantly different from control value ($p \leq 0.05$).

TABLE 4. Mean Maternal Food Consumption During Gestation (g/rat/day ± SD) of Rats Fed INY-6202 for Two Generations

| Dose Level (ppm) | Gestation Days 0-7 | 7-14 | 0-14 |
|---|---|---|---|
| | $F_0$-$F_{1a}$ Litter Interval | | |
| 0 | 23.4† | 24.9 | 24.2 |
| 25 | 21.8* | 24.5 | 23.1 |
| 100 | 22.5 | 24.5 | 23.5 |
| 400 | 21.4* | 23.5 | 22.4 |
| | $F_0$-$F_{1b}$ Litter Interval | | |
| 0 | 28.4 | 29.5 | 29.1 |
| 25 | 28.8 | 29.1 | 28.9 |
| 100 | 28.3 | 29.8 | 28.9 |
| 400 | 28.2 | 29.0 | 28.9 |
| | $F_1$-$F_{2a}$ Litter Interval | | |
| 0 | 24.4 | 26.6 | 25.6 |
| 25 | 24.3 | 25.4 | 24.8 |
| 100 | 25.7 | 27.1 | 26.4 |
| 400 | 24.8 | 26.6 | 25.7 |
| | $F_1$-$F_{2b}$ Litter Interval | | |
| 0 | 28.2 | 27.9 | 28.1 |
| 25 | 28.4 | 26.8 | 27.6 |
| 100 | 28.3 | 27.9 | 28.1 |
| 400 | 28.3 | 27.7 | 28.0 |

†Significant trend across dose groups ($p \leq 0.05$).
*Significantly different from control value ($p \leq 0.05$).

TABLE 5. Fertility Indices of Rats Fed INY-6202 for Two Generations

| Dose Level (ppm) | No. Females Cohabited | Females with Litters | | Proven Sires | |
|---|---|---|---|---|---|
| | | No. | % | No. | % |
| | | $F_0$-$F_{1a}$ Litters | | $F_0$-$F_{1a}$ Litters | |
| 0 | 23 | 22 | 95.7 | 22 | 95.7 |
| 25 | 22 | 15 | 68.2* | 15 | 68.2* |
| 100 | 23 | 20 | 87.0 | 19 | 82.6 |
| 400 | 23 | 20 | 87.0 | 19 | 82 6 |
| | | $F_0$-$F_{1b}$ Litters | | | |
| 0 | 22 | 21 | 95.4[a] | | |
| 25 | 22 | 17 | 77.2[b] | | |
| 100 | 23 | 19 | 82.6 | | |
| 400 | 23 | 19 | 82.6 | | |
| | | $F_1$-$F_{2a}$ Litters | | $F_1$-$F_{2a}$ and $F_{2b}$ Litters Combined | |
| 0 | 23 | 20 | 87.0 | 21 | 91.5[c] |
| 25 | 23 | 21 | 91.3 | 22 | 95.7 |
| 100 | 23 | 19 | 82.6 | 22 | 95.7 |
| 400 | 23 | 21 | 91.3 | 23 | 100.0 |
| | | $F_1$-$F_{2b}$ Litters | | | |
| 0 | 23 | 18 | 78.3 | | |
| 25 | 23 | 22 | 95.7 | | |
| 100 | 23 | 22 | 95.7 | | |
| 400 | 23 | 19 | 82.6 | | |

NOTE: Male fertility for $F_{1b}$ litters was not reported because in the first generation nonfertile males were excluded from second matings.

[a]Reviewers' calculations indicate 95.5.
[b]Reviewers' calculations indicate 77.3.
[c]Reviewers' calculations indicate 91.3.

*Significantly different from control value ($p \leq 0.05$).

C. Litter Data and Offspring Effects: A significant trend of lower percentages of liveborn pups was reported for the $F_{1a}$ litters; in the high-dose group the percentage of liveborn pups was significantly lower than controls. At the $F_{2a}$ litter interval, a similar trend was reported for the number of liveborn pups, and the number of high-dose pups alive at birth and day 4 was significantly lower than control values. No other significant differences in offspring survival were observed in any generation (Table 6). The study author stated that the number of $F_{2a}$ males born and alive at day 4 was lower in the high-dose group than in controls; however, no data for the sex of offspring were presented.

High-dose pup body weights were significantly lower than controls in all generations; $F_{1a}$ and $F_{1b}$ pup weights were significantly decreased from birth throughout lactation, while $F_{2a}$ and $F_{2b}$ pup weights were significantly reduced starting at day 7 and day 4 (postculling), respectively (Table 7). In addition, low-dose pup weights were significantly reduced in $F_{1b}$ litters on day 7 of lactation and in $F_{2b}$ litters on days 7-21.

Clinical observations of the offspring showed a significantly higher incidence of pups with hematomas at birth in all compound-treated $F_{1b}$ groups and mid- and high-dose $F_{2a}$ groups when compared to controls (Table 8). The author did not consider the hematomas compound related because the incidence reported for $F_{1b}$ and $F_{2a}$ pups was similar to the incidence for $F_{1a}$ control pups.

Gross necropsy findings for $F_{2b}$ weanlings did not reveal any distinct compound-related effect. At sacrifice, body weights of the low- and high-dose $F_{2b}$ weanlings were significantly lower than controls (Table 9). Significant increases in absolute and relative liver weights of mid- and high-dose weanlings and significantly decreased spleen weights at the high-dose level were attributed to compound administration; dose-related trends were also reported for these parameters (Table 9). Other significant differences in organ weight data were not considered to be direct compound-related effects; most were associated with decreased body weights and the relative organ weights did not show corresponding changes.

Histopathologic examination of tissues from $F_{2b}$ weanlings revealed compound-related eosinophilic changes of the liver at the mid- and high-dose levels (Table 10). These changes were indicative of proliferation of the smooth endoplasmic reticulum and were interpreted as a physiologic response to the compound or metabolites of the compound passing through the dam's milk.

13. STUDY AUTHOR'S CONCLUSIONS/QUALITY ASSURANCE MEASURES:

A. The study author concluded that the NOEL for this study was 25 ppm based on increased liver weights and pathological changes of the liver observed in $F_{2b}$ weanlings at 100 and 400 ppm. Other effects noted in this study that the author considered

TABLE 6. Mean Litter Size and Pup Survival per Litter (± SD) of Rats Fed IHY-6202 for Two Generations

| Dose Level (ppm) | No. Pups Born | Live Pups: At Birth No. | At Birth % | At Day 4 (Preculling) No. | At Day 4 (Preculling) % of Pups Born Alive | At Day 21 No. | At Day 21 % of Pups After Culling |
|---|---|---|---|---|---|---|---|
| | | | | $F_{1a}$ Litters | | | |
| 0 | 13.2±2.9 | 13.2±2.9 | 100† | 12.5±3.6[a] | 97.9±4.6[b] | 7.5±1.1 | 100 ±0 |
| 25 | 12.8±2.8 | 12.6±2.7 | 99.0±3.6 | 12.5±2.6 | 99.4±2.1 | 7.6±0.9 | 100 ±0 |
| 100 | 14.2±2.7 | 14.0±2.6 | 99.1±3.0 | 13.8±2.5 | 98.3±3.0 | 7 8±0.7 | 100 ±0 |
| 400 | 13.0±3.3[c] | 12.4±3.2[d] | 95.7±7.3* | 11.8±3.2[e] | 95.5±9.4 | 7.2±1.2[f] | 98.8±3.8 |
| | | | | $F_{1b}$ Litters | | | |
| 0 | 14.0±3.6 | 13.9±3.5 | 99.0±2.4 | 13.7±3.3 | 98.4±2.8 | 7.7±1.3 | 100 ±0 |
| 25 | 15.1±2.2 | 15.1±2.2 | 100 ±0 | 14.6±2.1 | 97.0±4.6 | 8.0±0 | 100 ±0 |
| 100 | 15.1±2.0 | 15.0±2.1 | 99.3±2.2 | 14.4±2.2 | 96.5±9.5 | 8.0±0 | 100 ±0 |
| 400 | 14.9±2.2 | 14.5±3.4 | 96.2±16.7 | 13.8±4.0 | 92.5±16.6 | 7.6±1.6 | 99.3±2.9 |
| | | | | $F_{2a}$ Litters | | | |
| 0 | 14.5±1.6 | 14.2±1.9† | 97.9±7.0 | 13.7±1.7 | 96.5±5.3 | 8.0±0 | 100 ±0 |
| 25 | 13.4±3.7 | 13.1±3.4 | 97.9±4.5 | 12.6±3.3 | 96.9±7.8 | 7.6±1.0 | 98.8±3.8 |
| 100 | 14.0±2.0 | 13.7±2.1 | 97.8±5.2 | 13.5±2.0 | 98.9±2.5 | 7.7±1.1 | 96.7±14.3 |
| 400 | 12.7±3.9 | 12.0±3.4* | 95.8±6.7 | 11.8±3.3* | 98.3±3.8 | 7.6±1.2 | 99.4±2.7 |
| | | | | $F_{2b}$ Litters | | | |
| 0 | 13.7±3.1 | 13.4±3.2 | 97.9±6.1 | 13.4±3.2 | 100 ±0 | 7.7±1.2 | 100 ±0 |
| 25 | 13.3±3.5 | 13.0±3.4 | 97.7±5.4 | 12.9±3.4 | 99.4±1.9 | 7.8±0.9 | 100 ±0 |
| 100 | 13.8±3.2 | 13.6±3.2 | 98.7±3.8 | 13.5±3.2 | 99.3±2.4 | 8.0±0.2 | 100 ±0 |
| 400 | 12.9±4.4 | 12.5±4.4 | 97.3±7.2 | 12.4±4.4 | 98.9±2.6 | 7.3±1.8 | 98.7±3.9 |

†Significant trend across dose groups ($p \leq 0.05$).
*Significantly different from control value ($p \leq 0.05$).

[a]Reviewers' calculations indicate mean to be 12.6.
[b]Reviewers' calculations indicate mean to be 98.0.
[c]Reviewers' calculations indicate mean to be 13.1.
[d]Reviewers' calculations indicate mean to be 12.5.
[e]Reviewers' calculations indicate mean to be 11.9.
[f]Reviewers' calculations indicate mean to be 7.3.

TABLE 7. Mean Pup Body Weights per Litter (g±SD) for Rats Fed INY-6202 for Two Generations

| Dose Level (ppm) | Lactation Day | | | |
|---|---|---|---|---|
| | Day 0 | Day 4 (Preculling) | Day 14 | Day 21 |
| | | | $F_{1a}$ Litters | |
| 0 | 6.1±0.5† | 10.2±1.2† | 32.5±2.3† | 54.6±4.2† |
| 25 | 6.1±0.4 | 9.9±1.1 | 32.8±2.2 | 52.6±6.7 |
| 100 | 6.1±0.5 | 9.7±0.9 | 32.4±1.9 | 53.0±3.5 |
| 400 | 5.7±0.5* | 9.3±1.1* | 29.4±2.3* | 45.4±3.3* |
| | | | $F_{1b}$ Litters | |
| 0 | 6.2±0.8† | 10.0±1.9 | 33.5±3.6† | 54.6±9.5† |
| 25 | 5.8±0.5 | 9.1±1.2 | 31.5±4.1 | 52.6±5.3 |
| 100 | 5.8±0.5 | 9.3±0.9 | 32.6±3.0 | 53.4±5.5 |
| 400 | 5.6±0.4* | 8.8±1.0* | 29.6±3.5* | 47.3±4.5* |
| | | | $F_{2a}$ Litters | |
| 0 | 6.1±0.6 | 9.6±1.0 | 31.9±2.3† | 51.4±4.3† |
| 25 | 6.2±0.7 | 9.8±1.7 | 30.8±3.5 | 49.8±5.9 |
| 100 | 6.1±0.6 | 9.4±1.6 | 30.6±5.1 | 48.7±7.3 |
| 400 | 6.0±0.9 | 9.3±1.8 | 28.3±2.8* | 42.0±3.6* |
| | | | $F_{2b}$ Litters | |
| 0 | 6.2±0.7 | 10.2±1.2 | 33.6±3.0† | 55.4±4.1[a] † |
| 25 | 6.0±0.5 | 9.6±1.1 | 30.9±4.3* | 50.5±6.3* |
| 100 | 6.3±0.6 | 10.0±1.4 | 32.6±3.8 | 53.3±5.1 |
| 400 | 6.0±1.0 | 9.5±2.0 | 28.8±3.0* | 44.6±4.8* |

†Significant trend across dose groups ($p \leq 0.05$).
*Significantly different from control value ($p \leq 0.05$).

[a]Reviewers' calculations indicate an SD of 4.3.

TABLE 8. Incidence of Clinically Observed Hematomas in Offspring of Rats Fed INY-6202 for Two Generations

| Litter Interval | Dose Level (ppm) 0 | 25 | 100 | 400 |
|---|---|---|---|---|
| $F_{1a}$ No. pups observed | 277 | 188 | 280 | 249 |
| No. pups with hematomas | 27 (9.7%) | 21 (11.2%) | 37 (13.2%) | 30 (12.0%) |
| $F_{1b}$ No. pups observed | 291 | 257 | 285 | 275 |
| No. pups with hematomas | 7†(2.4%) | 33*(12.8%) | 25* (8.8%) | 32*(11.6%) |
| $F_{2a}$ No. pups observed | 284 | 275 | 260 | 253 |
| No. pups with hematomas | 6 (2.1%) | 15 (5.5%) | 22* (8.5%) | 18* (7.1%) |
| $F_{2b}$ No. pups observed | 241 | 286 | 299 | 238 |
| No. pups with hematomas | 2 (0.8%) | 2 (0.7%) | 3 (1.0%) | 3 (1.3%) |

†Significant trend across dose groups ($p \leq 0.05$).
*Significantly different from control value ($p \leq 0.05$).

TABLE 9. Mean Absolute and Relative Organ Weights for $F_{2b}$ Weanlings of Rats Fed INY-6202 for Two Generations

Dose Level (ppm) — MALES

Mean Absolute Organ Weight (g)

| | Body | Liver | Kidney | Lung | Heart | Spleen | Thymus | Testes |
|---|---|---|---|---|---|---|---|---|
| 0 | 58.4† | 2.311† | 0.726† | 0.511† | 0.331† | 0.260† | 0.294 | 0.258† |
| 25 | 52.4* | 2.131 | 0.675 | 0.507 | 0.316 | 0.237 | 0.250 | 0.245 |
| 100 | 55.3 | 2.695* | 0.716 | 0.512 | 0.308 | 0.244 | 0.287 | 0.249 |
| 400 | 43.2* | 2.534 | 0.603* | 0.426* | 0.282 | 0.157* | 0.284 | 0.199* |

Mean Relative Organ Weights (% Body Weight)

| | Liver | Kidney | Lung | Heart | Spleen | Thymus | Testes |
|---|---|---|---|---|---|---|---|
| 0 | 3.962† | 1.241† | 0.886† | 0.566† | 0.445† | 0.503 | 0.442 |
| 25 | 4.064 | 1.281 | 0.976 | 0.603 | 0.447 | 0.480 | 0.465 |
| 100 | 4.868* | 1.294 | 0.930 | 0.558 | 0.442 | 0.520 | 0.451 |
| 400 | 5.871* | 1.398* | 0.989 | 0.654* | 0.363* | 0.642 | 0.458 |

Dose Level (ppm) — FEMALES

Mean Absolute Organ Weight (g)

| | Body | Liver | Kidney | Lung | Heart | Spleen | Thymus |
|---|---|---|---|---|---|---|---|
| 0 | 52.7 | 2.077† | 0.673 | 0.496 | 0.335 | 0.252† | 0.264 |
| 25 | 47.4* | 1.964 | 0.627 | 0.443* | 0.285 | 0.198* | 0.245 |
| 100 | 53.6 | 2.596* | 0.709 | 0.508 | 0.332 | 0.239 | 0.306* |
| 400 | 47.1* | 2.731* | 0.644 | 0.459 | 0.303 | 0.157* | 0.243 |

Mean Relative Weight (% Body Weight)

| | Liver | Kidney | Lung | Heart | Spleen | Thymus |
|---|---|---|---|---|---|---|
| 0 | 3.938† | 1.277 | 0.940 | 0.633 | 0.477† | 0.499 |
| 25 | 4.137 | 1.321 | 0.954 | 0.602 | 0.415* | 0.520 |
| 100 | 4.824* | 1.320 | 0.954 | 0.620 | 0.446 | 0.572 |
| 400 | 5.799* | 1.367 | 0.974 | 0.644 | 0.331* | 0.516 |

18

†Significant trend across dose groups ($p \leq 0.05$).
*Significantly different from control value ($p \leq 0.05$).

TABLE 10. Incidence of Hepatic Eosinophilic Change in $F_{2b}$ Weanlings of Rats Fed INY-6202 for Two Generations

| | Dose Level (ppm) | | | |
|---|---|---|---|---|
| | 0 | 25 | 100 | 400 |
| | | MALES | | |
| No. livers examined | 10 | 10 | 10 | 10 |
| No. with eosinophilic change | 0 | 0 | 10 | 10 |
| | | FEMALES | | |
| No. livers examined | 10 | 10 | 10 | 10 |
| No. with eosinophilic change | 0 | 0 | 9 | 10 |

compound related included decreased premating body weights and/or weight gains of mid-dose $F_0$ males and high-dose $F_0$ males and $F_1$ males and females and reduced body weights of high-dose pups at all litter intervals. The author also stated that the decrease in the percentage of high-dose $F_{1a}$ pups and number of high-dose $F_{2a}$ pups born alive may be a "minimal" compound-related effect.

B. A signed quality assurance statement, listing study intervals and audit dates, was presented in the final report; the statement was not dated.

14. REVIEWERS' DISCUSSION AND INTERPRETATION OF STUDY RESULTS:

A. Review of clinical observations and survival of parental animals did not indicate adverse compound effects.

We attribute the decreased body weights and premating body weight gain reported for high-dose males of both generations to compound administration. $F_0$ females did not appear to be similarly affected. The significantly reduced weights reported for $F_1$ high-dose females for days 0-21 were associated with reduced weights of these animals prior to weaning. The total premating body weight gain of the high-dose females was comparable to controls.

No consistent dose-related or cross-generational trends were evident for female body weights and weight changes during gestation or lactation.

In general, food consumption of the dosed animals was not adversely affected by compound administration. Although a significant trend toward reduced food intake was reported for $F_0$ females during the first week of feeding, mean group values were not significantly different from controls. Significant trends toward reduced food intake and significant group differences reported for $F_1$ males and females during the first 1-2 weeks of feeding may be attributable to the reduced body weights of these animals at weaning. In both generations, food consumption values were comparable for all groups for the remainder of the premating interval, as were values for the total premating interval. No consistent dose-related or cross-generational trends were evident for female food consumption during gestation.

Review of mean testicular weights and gross and histopathologic findings for parental animals did not indicate any compound-related effects.

No clear pattern of compound effect was evident for male or female fertility indices. In the $F_0$ generation, fertility was reduced in all groups when compared to controls at both mating intervals; reductions were significant for low-dose males and females at the $F_{1a}$ interval. In contrast, fertility indices for $F_1$ animals were higher for the dosed animals than for controls at both mating intervals (except for mid-dose females at the $F_{2a}$ interval). The length of the mating interval and length of gestation were not reported and therefore could not be assessed for compound effects.

We assess that the mean number and/or percentage of liveborn pups per litter and offspring survival during lactation were adversely affected by compound administration at the high-dose level. Although differences from control values were generally small, they were noted consistently across all litter intervals. At the $F_{1a}$ interval, the percentage of liveborn pups per litter was significantly lower in the high-dose group when compared to controls. At the $F_{2a}$ interval, the numbers of high-dose pups born alive and surviving to day 4 of lactation were significantly reduced.

We consider the decreased body weights reported for high-dose progeny at all litter intervals to be compound related. The significant reductions in body weights of low-dose offspring at the $F_{1b}$ and $F_{2b}$ litter intervals are considered incidental.

The clinical observations reported for offspring do not clearly suggest any compound-related effects. The incidence of $F_{1b}$ pups with hematomas was significantly increased at all dose levels when compared with controls. Similar increases were noted in the $F_{2a}$ generation, except that the increases were significant only at 100 and 400 ppm. Although the incidences of hematomas in the dosed $F_{1a}$ and $F_{2b}$ pups were comparable to their corresponding controls, the possibility of a compound effect at all dose levels in the $F_{1b}$ and $F_{2a}$ generations cannot be ruled out. The possible associations of this vascular effect in pups with previously reported effects of the test material on hematologic parameters in adult rats (Chronic Oncogenic Toxicity Study in Rats, Accession No. 073531-5) should be investigated. The assessment of potential effects of INY-6202 on the litter incidence of hematomas was precluded by the absence of litter data for this finding.

Review of gross necropsy findings for the $F_{2b}$ weanlings did not indicate any compound-related effects.

Differences in organ weight data that we attribute to compound administration include significant increases in absolute and relative liver weights of mid- and high-dose $F_{2b}$ weanlings and significantly decreased spleen weights for high-dose weanlings.

We agree with the study author that the increased incidence of eosinophilic changes in the livers of mid- and high-dose $F_{2b}$ weanlings was a compound-related effect.

B. Our assessment of the study findings differs from the conclusions of the study author with regard to $F_1$ female body weights and weight gains, offspring survival, and the incidence of hematomas in the pups.

The study author attributed the significantly reduced body weights for the high-dose $F_1$ females on days 0-21 of the premating interval to compound administration. We agree that a compound effect was evident, but because the body weights of these females were already reduced during the postnatal period and because their body weight gain for the premating period was comparable to controls, we consider the early reductions evidence of offspring toxicity, rather than parental toxicity.

The study author concluded that offspring survival was not affected by compound administration. Our review of the data suggests that offspring survival during lactation was decreased at the high-dose level when compared to controls.

The study author did not consider the significantly higher incidence of hematomas reported for all compound-treated $F_{1b}$ pups and mid- and high-dose $F_{2a}$ pups to be compound related. However, although a consistent cross-generational trend toward increased incidence of hematomas was not evident, we could not rule out the possibility of a compound-related effect at all dose levels.

Item 15--see footnote 1.

16. CBI APPENDIX: Appendix A, Materials and Methods, CBI pp. 12-20; Appendix B, Protocol and Protocol Amendments, CBI pp. 75-108.

# APPENDIX A

## Materials and Methods

ASSURE 005547

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