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SUPPLEMENT TO TSCA 8(E) SUBSTANTIAL RISK NOTICE ON: N-Ethyl Perfluorooctyl Sulfonamido Ethanol and Perfluorooctane Sulfonate Docket Numbers 8EHQ -0998-373, 374, 394

Dear Sir:

Enclosed are the final reports for the two-generation rat reproduction studies reported under TSCA 8(e) by 3M September 14, 1998, for

N-Ethyl Perfluorooctyl Sulfonamido Ethanol (N-EtFOSE)
Perfluorooctane Sulfonate (PFOS)

Please contact Dr. John Butenhoff, 3M Toxicology 651-733-1962, for further information.

Sincerely,

Georjean L. Adams

8EHQ-80-373

Georjean L. Adams
Manager, Corporate Product Responsibility

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FINAL REPORT

PROTOCOL 418-009

COMBINED ORAL (GAVAGE) FERTILITY, DEVELOPMENTAL AND PERINATAL/POSTNATAL REPRODUCTION TOXICITY STUDY OF N-EtFOSE IN RATS.

SPONSOR'S STUDY NUMBER: 6316.5

FINAL REPORT DATE: 30 JUNE 1999

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PROTOCOL 418-009

COMBINED ORAL (GAVAGE) FERTILITY, DEVELOPMENTAL AND PERINATAL/POSTNATAL REPRODUCTION TOXICITY STUDY OF N-EtFOSE IN RATS

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TITLE: COMBINED ORAL (GAVAGE) FERTILITY, DEVELOPMENTAL AND PERINATAL/POSTNATAL REPRODUCTION TOXICITY STUDY OF N-EtFOSE IN RATS.

ARGUS RESEARCH LABORATORIES, INC.
PROTOCOL NUMBER: 418-009
SPONSOR'S STUDY NUMBER: 6316.5

I. SUMMARY AND CONCLUSION

A. Methods[a]

Text Figure 1 provides a schematic of the study design.

A.1. Fo Generation Rats/F1 Generation Litters

One hundred seventy-five male and female Crl:CD®BR VAF/Plus® (Sprague-Dawley) rats were assigned to five dosage groups (Groups I through V), 35 rats per sex per dosage group. The rats were administered the test article, N-EtFOSE[b], or the vehicle, 2.0% Tween® 80, orally (via gavage). The male rats were dosed once daily beginning 28 days before cohabitation and continuing through the day before sacrifice. The female rats were dosed once daily beginning 28 days before cohabitation and continuing through DG[c] 9 (rats assigned to Caesarean-sectioning), DG 24 (rats assigned to natural delivery that did not deliver a litter), or DL[d] 20 (rats that delivered a litter). The dosage volume was 5 mL/kg.

All Fo generation rats were observed for viability at least twice daily during the study and for effects of the test article twice daily during the dosage period before and approximately one hour postdosage) and on the day sacrificed. Body weights and feed consumption values for male rats were recorded weekly during the dosage period and at sacrifice. Body weights for the female rats were recorded weekly to cohabitation, daily during the gestation period, on DLs 1, 4, 7, 10 and 14 (rats assigned to natural delivery) and at sacrifice. Feed consumption values were recorded weekly to cohabitation, daily during the gestation period and on DLs 1, 4, 7, 10 and 14 (rats assigned to natural delivery).

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a. Detailed descriptions of all procedures used in the conduct of this study are provided in the appropriate sections of this report and in APPENDIX F (PROTOCOL AND AMENDMENTS).
b. N-EtFOSE is a metabolite of PFOS, the test article administered in Argus Research Laboratories, Inc., Protocol 418-008.
c. DG is used as an abbreviation for day of (presumed) gestation).
d. DL is used as an abbreviation for day of lactation or day postpartum.

The first ten female rats per dosage group with a confirmed date of mating were assigned to Caesarean-sectioning on DG 10. The remaining female rats were permitted to naturally deliver litters. These rats were evaluated for clinical observations during parturition, duration of gestation, litter size and pup viability at birth. Maternal behavior of the dams was evaluated daily when the pups were examined during the 21-day postpartum period.

Each litter was evaluated for viability at least twice each day during the 21-day postpartum period. Pups in each litter were counted once daily. Physical signs in the pups were recorded once daily for 21 days postpartum. Pup body weights and observed nursing behavior were recorded on DLs 1 (birth), 4, 7, 14 and 21. Surface righting reflex, pinna unfolding, eye opening, acoustic startle response and air r[illegible]ting reflex were monitored during the 21-day postpartum period until all pups in the litter reached the criterion for the specific test. Pupil constriction was evaluated once on DL 21. On DL 4, a table of random units was used to cull litters to four male and four female pups, where possible. On DL 21, a table of random units was used to select 25 male and 25 female pups in Groups I, II and III for continued evaluation.

Fo generation male rats were sacrificed after completion of the cohabitation period and necropsied; gross lesions were retained. The testes, epididymides, prostate and seminal vesicles (with and without fluid) were excised, individually weighed and retained.

Fo generation female rats assigned to Caesarean-sectioning were sacrificed on DG 10 and necropsied; pregnancy status was confirmed. Ovaries and gross lesions were retained. The rats were examined for the number and distribution of corpora lutea in each ovary and implantation sites, and viable and nonviable embryos. Embryos were discarded after examination.

Fo generation female rats assigned to natural delivery were sacrificed on DL 21 and necropsied. Ovaries and gross lesions were retained. The number and distribution of implantation sites were recorded.

At scheduled sacrifice after completion of the cohabitation period (males rats that sired litters of dams allowed to naturally deliver a litter) and on DL 21 (female rats allowed to naturally deliver a litter) blood samples (approximately 4 mL per rat) were collected from the inferior vena cava from five rats per sex per dosage group and shipped to the Sponsor for pharmacokinetic analysis. The liver was excised, weighed, and a sample section (lateral lobe) was frozen and shipped to the Sponsor for analysis.

Pups not selected for continued evaluation on DL 4 were sacrificed and necropsied. The stomach contents (milk curd) were collected from all culled pups from five of the largest litters from Groups I, II and III, frozen and shipped to the Sponsor for analysis.

Pups not selected for continued evaluation were sacrificed on DL 21.

The livers of the pups from the litters of the five dams in Groups I through IV selected for pharmacokinetic sample collection were excised, pooled per litter, frozen and shipped to the Sponsor for analysis. Dams in the 15 mg/kg/day dosage group (Group V) did not have surviving pups on DL 21.

A.2. F1 Generation Rats/F2 Generation Litters

Only the 0 (Vehicle), 1 and 5 mg/kg/day dosage groups were continued into the second generation because of severe pup mortality during lactation. There were 150 male and female F1 generation rats in the three dosage groups (Groups I through III), 25 rats per sex per dosage group.

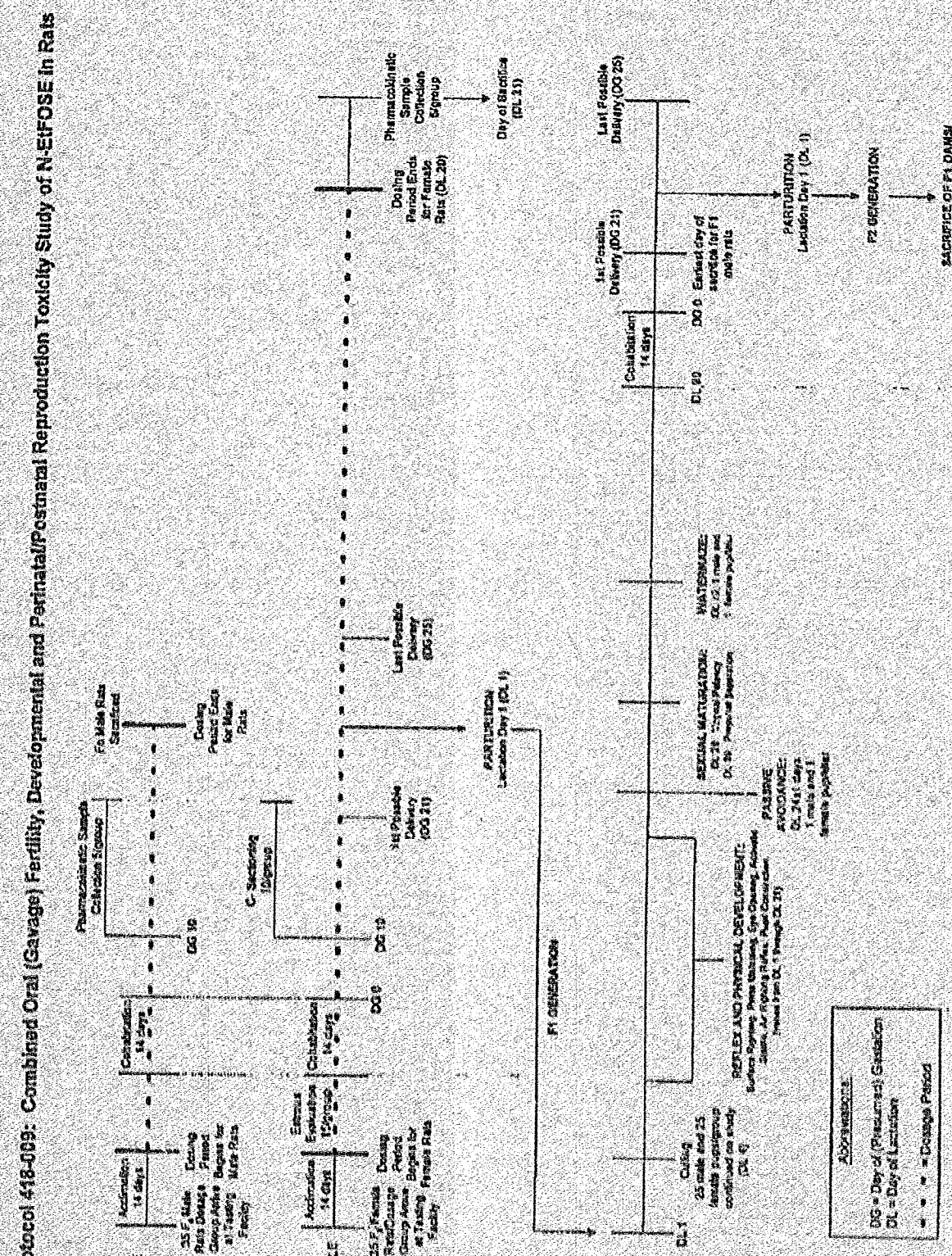
F1 generation male and female rats were given appropriate dosages of the test article orally (gavage) beginning on DL 22 and continuing through the day before sacrifice.

Beginning at 24 days of age, one male rat and one female rat from each litter in each dosage group were tested in a passive avoidance paradigm. Female rats were evaluated for the age of vaginal patency beginning on DL 28. Male rats were evaluated for the age of preputial separation beginning on DL 34. On postpartum day 70, one male rat and one female rat from each litter were evaluated in a water-filled M-maze. On approximately DL 90, the rats within each dosage group were assigned to cohabitation.

F1 generation male rats were sacrificed after completion of the cohabitation period and necropsied, as previously described for the Fo generation male rats. All F1 generation female rats were permitted to naturally deliver litters. All dams that delivered litters were sacrificed on DL 21 as previously described for Fo generation female rats.

On DL 21, all F2 generation pups were sacrificed and examined for gross lesions.

Text Figure 1



B. Results

B.1. Fo Generation Male Rats

No Fo generation male rats died during this study. The only clinical observation attributable to the test article was impaired righting reflex in the 15 mg/kg/day dosage group rats.

Groups administered 5 mg/kg/day and higher dosages of the test article had reduced body weight gains for the entire dosage period. The 10 and 15 mg/kg/day dosage groups had significantly reduced absolute feed consumption values for the entire treatment period.

Dosages of the test article as high as 15 mg/kg/day did not affect any mating and fertility parameters evaluated.

Necropsy observations considered related to the test article included a significantly increased number of male rats in the 15 mg/kg/day dosage group with small seminal vesicles and one 15 mg/kg/day dosage group male rat with a small prostate.

The 5, 10 and 15 mg/kg/day dosage groups had significantly reduced terminal body weights. The absolute weights of the left epididymis, seminal vesicles with fluid, and prostate were significantly reduced in the 15 mg/kg/day dosage group. The ratios of the weight of the left and right testes to terminal body weights were significantly increased in the 5 (left only), 10 and 15 mg/kg/day dosage groups.

B.2. Fo Generation Female Rats/F1 Generation Litters

No Fo generation female rats died during this study as a result of treatment with N-EtFOSE.

Observations of localized alopecia were significantly increased in the 5, 10 and 15 mg/kg/day dosage groups during the gestation and lactation periods.

Groups administered 5 mg/kg/day and higher dosages of the test article had significantly reduced body weight gains for the entire precohabitation period (DSs[a] 1 to 29). Gestation body weight gains were significantly reduced in the 10 and 15 mg/kg/day dosage groups on DGs 0 to 7 and in the 15 mg/kg/day dosage group on DGs 18 to 20. As a result, body weight gains were significantly reduced in the 15 mg/kg/day dosage group for the entire gestation period (DGs 0 to 20). Absolute body weights were significantly reduced in the 5 mg/kg/day dosage group on DGs 0 through 17, and in the 10 and 15 mg/kg/day dosage groups on all days of the gestation period. The 10 and 15 mg/kg/day dosage groups had significantly reduced body weights on DL 1. Lactation body weights continued to be significantly reduced in the 10 mg/kg/day dosage group on

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a. DS is used as an abbreviation for day of study.

DLs 4, 7, 10 and 14. Body weight loss occurred in the 10 and 15 mg/kg/day dosage groups on DLs 1 to 4. The 15 mg/kg/day dosage group was precluded from further evaluation because all pups died before DL 5. A significant increase in body weight gain on DLs 14 to 21 in the 10 mg/kg/day dosage group was possibly associated with reduced litter size and reduced milk demand and production.

Absolute and relative feed consumption values were significantly reduced in the 10 and 15 mg/kg/day dosage groups for the entire precohabitation period. The 10 and 15 mg/kg/day dosage groups continued to have significantly reduced absolute and relative feed consumption values early in the gestation period. Absolute feed consumption values were also significantly reduced in the 5 mg/kg/day dosage group on DGs 0 to 7. Absolute feed consumption values continued to be significantly reduced in the 10 mg/kg/day dosage group on DGs 10 to 12 and 12 to 15, and in the 15 mg/kg/day dosage group throughout the remainder of the gestation period (DGs 10 to 12, 12 to 15, 15 to 18 and 18 to 20). Absolute and relative feed consumption values were significantly reduced for the entire gestation period (DGs 0 to 20) in the 5 (absolute only), 10 and 15 mg/kg/day dosage groups.

Absolute and relative feed consumption values were significantly reduced in the 5 (absolute only) and 10 mg/kg/day dosage groups for the entire lactation period. Significant reductions in absolute and relative feed consumption values occurred on DLs 7 to 10 in the 5 mg/kg/day dosage group, and for all tabulated intervals in the 10 mg/kg/day dosage group. Absolute and relative feed consumption values were reduced in the one litter was available for evaluation in the 15 mg/kg/day dosage group on DLs 1 to 4. The 15 mg/kg/day dosage group was precluded from further evaluation because all pups died before DL 5.

Dosages of the test article as high as 15 mg/kg/day did not affect estrous cycling in the 15 rats per dosage group that were evaluated.

All necropsy observations were considered unrelated to treatment with the test article.

Statistically significant reductions in the averages for implantations and viable embryos occurred in the 15 mg/kg/day dosage group at Caesarean-sectioning on DG 10. There were no biologically important or statistically significant differences in the litter averages for corpora lutea or nonviable embryos at Caesarean-sectioning on DG 10.

The duration of gestation was significantly reduced in the 5, 10 and 15 mg/kg/day dosage groups, an observation associated with preimplantation loss in the 15 mg/kg/day dosage group (the average number of implantation sites per dam was significantly reduced, resulting in a significantly reduced litter size).

Pup viability was significantly affected by the 10 and 15 mg/kg/day dosages of the test article. Reflecting these effects, the viability and lactation indices were significantly reduced in the 10 and 15 mg/kg/day dosage groups.

A dosage-dependent pattern of reduced pup body weights was evident in each group administered the test article. The 1 mg/kg/day dosage group tended to have reduced pup body weights on DL 4 (pre-and postculling). The 5, 10 and 15 mg/kg/day dosage groups had significantly reduced pup body weights on all weighing days (no pups survived after DL 4 in the 15 mg/kg/day dosage group).

Clinical and necropsy observations associated with reduced pup viability and potential reduction in maternal care occurred in the 5, 10 and 15 mg/kg/day dosage groups. Clinical observations included 1, 7 and 3 litters with pups that were not nursing in the 5, 10 and 15 mg/kg/day dosage groups, respectively; the incidence was significant in the 10 mg/kg/day dosage group. The number of litters with cold to touch pups was also significantly increased in the 10 mg/kg/day dosage group. Necropsy observations in pups that were found dead included significant increases in the number of pups with no milk in stomach in the 10 and 15 mg/kg/day dosage group litters.

Reversible delays in reflex and physical development that are highly correlated with body weight occurred in the 5 and 10 mg/kg/day dosage groups. Surface righting was delayed in the 5, 10 and 15 mg/kg/day dosage groups. The time of development for pinna unfolding was delayed in the 5, 10 and 15 mg/kg/day dosage groups. Eye opening was delayed in the 5 and 10 mg/kg/day dosage groups (no 15 mg/kg/day dosage group pups were evaluated for this parameter). The time of development of the acoustic startle reflex was delayed in the 5 and 10 mg/kg/day dosage groups (no 15 mg/kg/day dosage group pups survived to be tested for this reflex). The ability to air right was delayed in the 5 and 10 mg/kg/day dosage groups (no 15 mg/kg/day dosage group pups survived to be tested for this reflex).

B.3. F1 Generation Male Rats

No deaths occurred in the F1 generation male rats. All clinical observations were considered unrelated to the test article.

The 1 and 5 mg/kg/day dosage groups weighed less than the control group on day 1 postweaning (significant in the 5 mg/kg/day dosage group) and body weight gains were reduced in these groups throughout the postweaning period. Weight gains in the 1 and 5 mg/kg/day dosage groups were significantly reduced for the precohabitation period and for day 1 postweaning to termination (significant at 5 mg/kg/day only). Absolute body weights were significantly reduced in the 5 mg/kg/day dosage group throughout the postweaning dosage period.

Absolute and relative feed consumption values in the F1 generation male rats were significantly reduced in the 5 mg/kg/day dosage group on days 8 to 15

postweaning. The absolute feed consumption value was significantly reduced in this dosage group on days 1 to 8 postweaning. Relative feed consumption values were also significantly reduced on days 15 to 22, 22 to 29, 29 to 36 and 36 to 43 in the 5 mg/kg/day dosage group.

The average day of preputial separation was significantly delayed for the F1 generation male rats in the 5 mg/kg/day dosage group value, but the increase was approximately one day and not considered toxicologically important.

There were no biologically important differences in the values for learning, short-term retention, long-term retention or response inhibition in the F1 generation male rats, as evaluated by performance in a passive avoidance or watermaze performance paradigm.

Dosages of the test article as high as 5 mg/kg/day did not affect any mating and fertility parameters evaluated in the F1 generation male rats.

All necropsy observations in the F1 generation male rats were considered unrelated to the test article. Terminal body weights were significantly reduced in the 5 mg/kg/day dosage group. The ratios of the left and right testis weights to the terminal body weight in the 5 mg/kg/day dosage group were significantly increased. No statistically significant differences occurred in the absolute weights of the epididymides, testes, seminal vesicles (with and without fluid) and prostate, and the ratios of the epididymides, seminal vesicles (with and without fluid) and prostate weights to the terminal body weight of the F1 generation male rats.

B.4. <u>F1 Generation Female Rats/F2 Generation Litters</u>

All F1 generation female rats survived until scheduled sacrifice. All clinical observations during the precohabitation, gestation and lactation periods were considered unrelated to the test article.

The 1 and 5 mg/kg/day dosage groups weighed less than the control group on day 1 postweaning (the reduction was significant in the 5 mg/kg/day dosage group) and body weight gains were generally reduced in these groups throughout the precohabitation period. Body weight gains were significantly reduced in both test article-treated groups for the entire precohabitation period [day 1 postweaning to precohabitation and day 1 to 57 postweaning (significant at 5 mg/kg/day only)]. Absolute body weights in the 5 mg/kg/day dosage group were significantly reduced throughout the precohabitation dosage period.

Maternal body weight gains during the gestation period were significantly reduced in the 1 and 5 mg/kg/day dosage groups on days 0 to 7 of gestation. Body weight gains in the 5 mg/kg/day dosage group were significantly increased on DGs 14 to 17. Absolute body weights were significantly reduced on DGs 0 through 18 in the 1 and 5 mg/kg/day dosage groups. Maternal body weights

remained significantly reduced in the 5 mg/kg/day dosage group on DLs 1, 4, 7, 10, 14 and 21 and in the 1 mg/kg/day dosage group on DLs 1, 7 and 14.

Feed consumption values were significantly reduced for the entire precohabitation period (days 1 to 57 postweaning) in both groups administered the test article. Relative feed consumption values in the 5 mg/kg/day dosage group were significantly increased on days 8 to 15, 15 to 22 and 22 to 29 postweaning. Absolute feed consumption values in the 1 and 5 mg/kg/day dosage groups were significantly reduced during the first week of the gestation period. Absolute feed consumption values for the 5 mg/kg/day dosage group were also significantly reduced on DGs 7 to 10. The relative feed consumption value on DGs 14 to 17 was significantly reduced in the 5 mg/kg/day dosage group. The absolute maternal feed consumption value was significantly reduced for the entire lactation in the 5 mg/kg/day dosage group. The relative feed consumption value was significantly reduced on DLs 4 to 7 and 7 to 10 in the 5 mg/kg/day dosage group, as compared to the control group values.

Dosages of the test article as high as 5 mg/kg/day did not affect the average day of vaginal patency in the F1 generation female rats. There were no biologically important differences in the values for learning, long-term retention or response inhibition in the F1 generation female rats, as evaluated by performance in a passive avoidance or watermaze performance paradigm.

Dosages of the test article as high as 5 mg/kg/day did affect any mating and fertility parameters evaluated in the F1 generation female rats.

All necropsy observations in the F1 generation female rats were considered unrelated to the test article.

As occurred in the previous generation, there was a tendency for reduced pup viability in the 5 mg/kg/day dosage group. The number of dams with stillborn pups was significantly increased, and the number of pup deaths was significantly increased in this dosage group. As also occurred in the previous generation, pup body weights were significantly reduced in the 5 mg/day dosage group on days 1, 4 (pre and postculling), 7, 14 and 21.

No clinical or necropsy observations in the F2 generation were attributable to dosages of the test article as high as 5 mg/kg/day.

C. Conclusion

On the basis of these data, the Fo generation maternal and paternal no-observable-effect-level (NOEL) of N-EtFOSE is 1 mg/kg/day (5 mg/kg/day and higher dosages caused reductions in body weight gain, the 10 and 15 mg/kg/day dosages also caused reduced feed consumption values).

The Fo generation reproductive NOEL is greater than 15 mg/kg/day; no effects on mating, fertility or estrous cycling occurred. The NOEL for viability and growth in the F1 generation offspring is 1 mg/kg/day (the 5 mg/kg/day dosage caused reductions in pup body weight gains, the 10 and 15 mg/kg/day dosages caused preimplantation loss and reductions in litter size, pup viability, growth and survival).

The F1 generation maternal and paternal NOEL of N-EtFOSE is less than 1 mg/kg/day (the 1 and 5 mg/kg/day dosages caused reductions in body weight gain and feed consumption).

The F1 generation reproductive NOEL is a dosage of 5 mg/kg/day; no effects on mating or fertility occurred. The NOEL for viability and growth in the F2 generation offspring is 1 mg/kg/day (the 5 mg/kg/day dosage caused stillbirths and reductions in litter size, pup viability, growth and survival).

George E. Dearlove 30-JUN-99
for Mildred S. Christian, Ph.D., Fellow, ATS Date
Executive Director of Research

George E. Dearlove 30-JUN-99
for Alan M. Hoberman, Ph.D., DABT Date
Director of Research

Raymond G. York 30-JUN-99
Raymond G. York, Ph.D., DABT Date
Associate Director of Research and Study Director

II. DESCRIPTION OF TEST PROCEDURES

A. Conduct of Study:

A.1. Sponsor:

3M Corporate Toxicology, 3M Center, Building 220-2E-02, St. Paul, Minnesota 55144-1000

A.2. Testing Facility:

Argus Research Laboratories, Inc., 905 Sheehy Drive, Building A, Horsham, Pennsylvania 19044-1297

A.3. Study Number:

418-009

A.4. Sponsor's Study Number:

6316.5

A.5. Purpose of the Study:

The purpose of this study was to test for toxic effects/disturbances resulting from N-EtFOSE treatment of Crl:CD®BR VAF/Plus® male and female rats before cohabitation and continuing through mating, gestation and lactation. This study was designed to evaluate ICH Harmonised Tripartite Guideline stages A through F of the reproductive process and should detect effects on the estrous cycle, tubal transport, implantation, gestation, parturition, lactation and maternal behavior in female rats, on the development of the offspring of the treated male and female rats, and permit detection of functional effects (e.g., effects on libido or epididymal sperm maturation) that may not be detected by histological examinations of male rat reproductive organs. Because manifestations of effects induced during this period may be delayed in the offspring, observations were continued through production of F2 litters.

A.6. Study Design:

A modification of the requirements of the U.S. Food and Drug Administration (FDA)[1] was used as the basis for study design.

A.7. Regulatory Compliance:

The study was conducted in compliance with the Good Laboratory Practice (GLP) regulations of the U.S. Food and Drug Administration (FDA)[2], the Japanese Ministry of Health and Welfare (MHW)[3] and the European Economic Community (EEC)[4]. There were no deviations from the GLP regulations that affected the quality or integrity of the study. Quality Assurance Unit findings derived from the inspections during the conduct of this study are documented and have been provided to the Study Director and the Testing Facility Management.

A.8. Ownership of the Study:

The Sponsor owns the study. All raw data, analyses, reports and preserved tissues are the property of the Sponsor.

A.9. Study Monitor:

Marvin T. Case, D.V.M., Ph.D.

A.10. Alternate Study Monitor:

Andrew M. Seacat, Ph.D.

A.11. Study Director:

Raymond G. York, Ph.D., DABT (Associate Director of Research)

A.12. Technical Performance:

John F. Barnett, B.S. (Director of Laboratory Operations)
Aaron J. Weilerstein, B.S. (Research Assistant)
Karen D. Klein, B.S. (Laboratory Scheduler)

A.13. Report Preparation:

Raymond G. York, Ph.D., DABT
Jo Ann Frazee, M.S. (Study Coordinator)
Erin Hagan, B.A. (Data Management Specialist)
Karen G. Parker, A.A. (Report Administrator)

A.14. Report Review:

Alan M. Hoberman, Ph.D., DABT (Director of Research)
Mildred S. Christian, Ph.D., Fellow, ATS (Executive Director of Research)

A.15. Date Protocol Signed:

28 May 1998

A.16. Dates of Technical Performance:

A.16.a. Fo Generation Male Rats:

Rat Arrival Date	02 JUN 98
Dosage Period (28 days before cohabitation, and continuing through a 14-day cohabitation period and until day before sacrifice)	08 JUN 98 - 29 JUL 98
Scheduled Sacrifice	30 JUL 98

A.16.b. Fo Generation Female Rats:

Rat Arrival Date	02 JUN 98
Dosage Period - Female Rats Assigned to Caesarean-Sectioning (28 days before cohabitation and continuing through DG[a] 9)	08 JUN 98 - 29 JUL 98
Dosage Period - Female Rats Assigned to Natural Delivery [28 days before cohabitation and continuing through DG 24 (rats that did not deliver a litter) or DL[b] 20 (rats that delivered a litter)]	08 JUN 98 - 30 AUG 98
Dosage Period Estrous Cycle Evaluation	23 JUN 98 - 06 JUL 98
Cohabitation Period	
Male 1	06 JUL 98 PM - 13 JUL 98 AM
Male 2	13 JUL 98 PM - 20 JUL 98 AM
DG 10 Caesarean-Sectioning	17 JUL 98 - 21 AUG 98
Natural Delivery Period (DL 1)	28 JUL 98 - 11 AUG 98
DG 25 Sacrifice (rats that did not deliver a litter)	01 AUG 98 - 03 AUG 98
DL 21 Sacrifice (dams and pups not selected for continued study)	17 AUG 98 - 31 AUG 98

a. DG is used as an abbreviation for day of (presumed) gestation.
b. DL is used as an abbreviation for day of lactation or day postpartum.

A.16.c. **F1 Generation Rats:**

Dosage Period (Male Rats)	18 AUG 98 - 17 NOV 98
Dosage Period (Female Rats)	18 AUG 98 - 28 DEC 98
Passive Avoidance Testing	20 AUG 98 - 11 SEP 98
Watermaze Testing	07 OCT 98 - 26 OCT 98
Cohabitation Period	
Male 1	02 NOV 98 PM - 09 NOV 98 AM
Male 2	09 NOV 98 PM - 16 NOV 98 AM
Male Rats Sacrificed	18 NOV 98
Natural Delivery Period	24 NOV 98 - 09 DEC 98
DL 21 Sacrifice	14 DEC 98 - 29 DEC 98

A.17. **Records Maintained:**

The original report, raw data and reserve samples of the bulk test article and vehicle components are retained in the archives of Argus Research Laboratories, Inc. Any preserved tissues are retained in the archives of the Testing Facility for one year after the mailing of the draft final report, after which time the Sponsor will decide their final disposition. All unused prepared formulations were discarded at the Testing Facility. Unused bulk test article was returned to the Study Monitor.

B. **Test Article Information:**

B.1. **Description:**

N-EtFOSE - a waxy solid

B.2. **Lot Number:**

FM-3929 [30035, 30037, 30039 (Expiration date: May 2000)]

B.3. **Date Received and Storage Conditions:**

The test article was received on 20 May 1998, and stored at room temperature. Prepared suspensions were stored frozen (-20°C).

B.4. Special Handling Instructions:

Standard safety precautions (use of protective clothing, gloves, dust-mist respirator and safety goggles) were taken when handling the bulk test article and prepared suspensions.

B.5. Analysis of Purity:

Information regarding the identity, composition, strength and purity of the test article is on file with the Sponsor.

C. Vehicle Information:

C.1. Description:

2.0% Tween® 80 in reverse osmosis membrane processed deionized water (R.O. deionized water)

C.2. Lot Number:

M03H05

C.3. Dates Received and Storage Conditions:

Shipments of Tween® 80 were received from J.T. Baker, Phillipsburg, New Jersey, on 22 May, 1 July and 8 July 1998, and stored at room temperature. The R.O. deionized water is available from a continuous source at the Testing Facility and is maintained at room temperature.

C.4. Special Handling Instructions:

Standard safety precautions (use of protective clothing, gloves, dust-mist respirator, safety goggles or safety glasses and a face-shield) were taken when handling the vehicle.

C.5. Analysis of Purity:

Neither the Sponsor nor the Study Director was aware of any potential contaminants likely to be present in the vehicle that would interfere with the results of this study.

D. Test Article Preparation:

Suspensions of N-EtFOSE were prepared daily at concentrations of 0, 0.2, 1, 2 and 3 mg/mL. The test article was considered 100% pure for the purpose of dosage calculations.

D.1 Sample Information:

Sample Type	Size	Date Retained	Storage/Shipping Conditions	Shipped To	Date Shipped
Homogeneity	5 mL[a]	08 JUN 98	Frozen (-20°C)	Sponsor	09 JUN 98
Concentration	5 mL[b]	08 JUN 98[c] 15 JUL 98[c] 22 DEC 98[d] 27 DEC 98[e] 28 DEC 98[f]	Frozen (-20°C)	Sponsor	09 JUN 98 15 JUL 98 04 JAN 99 04 JAN 99 04 JAN 99
Vehicle Component Reserve Tween® 80 R.O. Deionized Water	 5 mL 5 mL	 10 JUN 98 10 JUN 98	Room temperature	Testing Facility Archives	 20 JUL 98 20 JUL 98

a. A syringe was used to withdraw samples from the top, middle and bottom of the highest concentration on the first day prepared. Each sample was divided into two aliquots (2 mL and 3 mL, respectively). One aliquot (2 mL) was shipped for analysis. The other aliquot (3 mL) was retained at the Testing Facility as a backup.
b. A syringe was used to withdraw samples during the first and sixth weeks of dosage administration for the Fo generation and during the first and last weeks of dosage administration for the F1 generation. Each sample was divided into two aliquots (2 mL and 3 mL, respectively). One aliquot (2 mL) was shipped for analysis. The other aliquot (3 mL) was retained at the Testing Facility as a backup.
c. 0, 0.2 and 1 mg/mL concentrations.
d. 0 mg/mL concentration.
e. 0.2 mg/mL concentration.
f. 1 mg/mL concentration.

D.2. Analytical Results:

Information on the stability of the bulk test article is on file with the Sponsor. Stability data for prepared formulations bracketing the range of concentrations and conditions of this study are on file with the Sponsor. Results of the concentration and homogeneity analyses were not available at the time of the writing of this report.

E. Test System:

E.1. Species:

Rat

E.2. Strain:

Crl:CD®BR VAF/Plus® (Sprague-Dawley)

E.3. Supplier (Source):

Charles River Laboratories, Inc., Raleigh, North Carolina

E.4. Sex:

Male and female

E.5. Rationale for Test System:

The Crl:CD®BR VAF/Plus® (Sprague-Dawley) rat was selected as the Test System because: 1) this strain of rat has been demonstrated to be sensitive to reproductive and developmental toxins and has been widely used throughout industry for reproductive and developmental toxicity evaluations; 2) historical data and experience exist at the Testing Facility[5-7]; and 3) the test article is pharmacologically active in the species and strain.

E.6. Test System Data:

	Male Rats		Female Rats
	Shipment 1	Shipment 2	
Number of Rats	100	95	205
Approximate Dates of Birth	28 MAR 98	06 APR 98	30 MAR 98
Approximate Age at Arrival	67 days	58 days	65 days
Weight (g) on the Day after Arrival	223 - 331		179 - 229
Weight (g) at Study Assignment	223 - 336		193 - 216

E.7. Method of Randomization:

E.7.a. Fo Generation Rats:

Upon arrival, Fo generation rats were assigned to individual housing on the basis of computer-generated random units. After acclimation, male and female rats were selected for study on the basis of physical appearance and body weights recorded during acclimation. Separate shipments of male rats were randomized as one group to ensure an equivalent distribution of body weights. Rats were assigned to five dosage groups (Groups I through V), 35 rats per sex per dosage group, using a computer-generated (weight-ordered) randomization procedure.

The first ten female rats per dosage group with a confirmed date of mating were assigned to Caesarean-sectioning on DG 10. The remaining female rats were permitted to naturally deliver litters.

A table of random units was used to select five male rats per dosage group from among those that successfully mated a female rat assigned to natural delivery for scheduled sacrifice after completion of the cohabitation period. The same method was used to select five female rats per group from among those that delivered a litter for scheduled for sacrifice on DL 21.

E.7.b. F1 Generation Pups:

On DL 4, a table of random units was used to select pups to be culled, and litters were reduced to eight pups each. Whenever possible, the same number of male and female pups per litter were continued on study.

At weaning of the F1 generation pups on DL 21, a table of random units was used to select 25 male and 25 female pups in each of Groups I, II and III, resulting in a total of 150 F1 generation rats (75 per sex) chosen for continued postnatal evaluation. At least one male pup and one female pup per litter, when possible, was selected. Group IV was not continued on the study due to severe pup toxicity (mortality) during lactation. This decision was made in consulation with the study veterinarian and the Sponsor. There were no surviving pups in Group V after DL 5.

E.8. System of Identification:

E.8.a. Fo Generation Rats:

Male and female rats were assigned temporary numbers at receipt and given unique permanent identification numbers when assigned to the study before administration of the first dosage of the test article. Rats were permanently identified using Monel® self-piercing ear tags (Gey Band and Tag Co., Inc., No. MSPT 20101).

E.8.b. F1/F2 Generation Pups and Rats:

Pups were not individually identified during lactation; all parameters were evaluated in terms of the litter. At weaning, each F1 generation rat selected for continued observation was identified with a Monel® self-piercing ear tag.

F. Husbandry:

F.1. Research Facility Registration:

USDA Registration No. 23-R-099 under the Animal Welfare Act, 7 U.S.C. 2131 *et seq.*

F.2. Study Rooms:

The study rooms were maintained under conditions of positive airflow relative to a hallway and independently supplied with a minimum of ten changes per hour of 100% fresh air that had been passed through 99.97% HEPA filters. Room temperature and humidity were monitored constantly throughout the study. Room temperature was targeted at 64°F to 79°F(18°C to 26°C); relative humidity was targeted at 30% to 70%. See APPENDIX H (TEMPERATURE AND RELATIVE HUMIDITY REPORTS).

F.3. Housing:

All cage sizes and housing conditions were in compliance with the *Guide for the Care and Use of Laboratory Animals*[8].

F.3.a. Fo Generation Rats/F1 Generation Litters:

Fo generation rats were individually housed in stainless steel wire-bottomed cages except during the cohabitation and postpartum periods. During cohabitation, each pair of rats was housed in the male rat's cage. Beginning no later than DG 20, Fo generation female rats assigned to natural delivery were individually housed in nesting boxes. Each dam and delivered litter were housed in a common nesting box during the postpartum period.

F.3.b. F1 Generation Rats/F2 Generation Litters:

After weaning, the F1 generation rats were individually housed before cohabitation, housed in pairs (one male rat per female rat) during cohabitation, and individually housed after cohabitation. The same type of caging was used as described for the Fo generation rats. Beginning no later than DG 20, F1 generation female rats were individually housed in nesting boxes. Each dam and delivered litter were housed in a common nesting box during the postpartum period.

F.4. Lighting:

An automatically-controlled fluorescent light cycle was maintained at 12-hours light:12-hours dark, with each dark period beginning at 1900 hours EST.

F.5. Sanitization:

Cage pan liners were changed approximately three times each week. Cages were changed approximately every other week. Bedding was changed as often as necessary to keep the rats dry and clean.

F.6. Feed:

Rats were given *ad libitum* access to Certified Rodent Diet® #5002 (PMI Nutrition International, St. Louis, Missouri) in individual feeders.

F.7. Feed Analysis:

Analyses were routinely performed by the feed supplier. No contaminants at levels exceeding the maximum concentration for certified feed or deviations from expected nutritional requirements were detected by these analyses. Copies of the results of the feed analyses are available in the raw data.

Neither the Study Director nor the Sponsor was aware of any agent present in the feed that was known to interfere with the results of this study.

F.8. Water:

Local water that had been processed by passage through a reverse osmosis membrane (R.O. water) was available to the rats *ad libitum* from an automatic watering access system and/or individual water bottles. Chlorine was added to the processed water as a bacteriostat.

F.9. Water Analysis:

The processed water is analyzed twice annually for possible chemical contamination (Lancaster Laboratories, Lancaster, Pennsylvania) and monthly for possible bacterial contamination (Analytical Laboratories, Inc., Chalfont, Pennsylvania). Copies of the results of the water analyses are available in the raw data.

Neither the Study Director nor the Sponsor was aware of any agent present in the water that was known to interfere with the results of this study.

F.10. Nesting Material:

Bed-o'cobs® was used as nesting material (The Andersons Industrial Products Group, Maumee, Ohio).

F.11. Bedding Analysis:

Neither the Sponsor nor the Study Director was aware of any potential contaminants likely to be present in the bedding that would interfere with the results of this study. Analyses for possible contamination are conducted annually. Copies of the results of the bedding analyses are available in the raw data.

G. Methods:

G.1. Dosage Administration[a]:

Dosage Group	Dosage (mg/kg/day)	Concentration (mg/mL)	Dosage Volume (mL/kg)	Number of Fo Generation Rats Per Sex	Number of F1 Generation Rats Per Sex
I	0 (Vehicle)	0	5	35	25
II	1	0.2	5	35	25
III	5	1	5	35	25
IV	10	2	5	35	a
V	15	3	5	35	b

a. Group IV F1 generation pups were sacrificed at weaning on DL 21 because of severe pup mortality during lactation.
b. There were no surviving Group V F1 generation pups after DL 5.

The test article was considered 100% pure for the purpose of dosage calculations.

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a. See APPENDIX G (DEVIATIONS FROM THE PROTOCOL AND STANDARD OPERATING PROCEDURES OF THE TESTING FACILITY), items 1 and 2.

G.2. **Assigned Rat Numbers:**

Dosage Group	Assigned Fo Generation Rat Numbers		Assigned F1 Generation Rat Numbers	
	Male	Female	Male	Female
I	9001 - 9935	10076 - 10110	12076 – 12100	12202 - 12225
II	9936 - 9970	10111 - 10124, 16400[a], 10126 - 10145	12101 – 12125	12226 - 12260
III	9971 - 10005	10146 - 10180	12126 - 12150	12251 - 12275
IV	10006 - 10040	10181 - 10215	b	b
V	10041 - 10075	10216 - 10250	c	c

a. Female rat 10125 escaped and was missing overnight; this rat was excluded and replaced with femal rat 16400 on day 1 of study, at the request of the Study Director.
b. Group IV F1 generation pups were sacrificed at weaning on DL 21 because of severe pup mortality during lactation.
c. There were no surviving Group V F1 generation pups after DL 5.

G.3. **Rationale for Dosage Selection:**

Dosages were selected by the Sponsor on the basis of previous studies conducted with the test article.

G.4. **Route of Administration:**

Oral (gavage)

G.5. **Rationale for Route of Administration:**

The oral (gavage) route was selected for use because: 1) in comparison with th dietary route, the exact dosage can be accurately administered; and 2) it is one of the possible routes of human exposure.

G.6. **Frequency of Administration:**

G.6.a. **Fo Generation Rats:**

The Fo generation male rats were given appropriate dosages of the test article o vehicle once daily[a] beginning 28 days before cohabitation (which continued for a maximum of 14 days) and continuing through the day before sacrifice after the completion of the cohabitation period. All male rats received a total of 52 consecutive daily dosages.

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a. See APPENDIX G, item 3.

The Fo generation female rats were given the appropriate dosages of the test article or vehicle once daily beginning 28 days before cohabitation (which continued for a maximum of 14 days) and continuing through DG 9 (rats assigned to Caesarean-sectioning), DG 24 (rats assigned to natural delivery that did not deliver a litter), or DL 20 (rats that delivered a litter). The dosage volume (5 mL/kg) was adjusted daily on the basis of the most recently recorded body weight and given at approximately the same time each day.

Dams in the process of delivering pups were not dosed until completion of parturition, in order to preclude possible disruption of maternal behavior and/or cannibalization of the pups. Consequently, some dams were not administered one daily dosage during the delivery period. No dam missed more than one daily dosage.

G.6.b. F1 Generation Rats:

F1 generation male and female rats were given appropriate dosages of the test article[a] or vehicle once daily beginning on day 1 postweaning (DL 21) and continuing through the day before sacrifice. F2 generation pups were not directly given the test article, but may have been possibly exposed to the test article during the maternal gestation (*in utero* exposure) or via maternal milk during the lactation period.

G.7. Length of Study:

Approximately seven months

G.8. Method of Study Performance:

G.8.a. Fo Generation Rats:

All Fo generation rats were observed for viability at least twice daily during all periods of the study. Rats were also observed for general appearance at least once during the acclimation period, and examined for clinical observations of effects of the test article, abortions, premature deliveries and deaths prior to and approximately one hour after dosage and on the day sacrificed.

Body weights for Fo generation male rats were recorded at least once during the acclimation period, weekly during the dosage period and at sacrifice. Feed consumption values for male rats were recorded weekly during the dosage period.

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a. See APPENDIX G, Items 4 and 5.

Body weights for Fo generation female rats were recorded at least once during the acclimation period, weekly to cohabitation, daily during the gestation period, on DLs 1, 4, 7, 10 and 14 (rats assigned to natural delivery) and at sacrifice. Feed consumption values were recorded weekly to cohabitation, daily during the gestation period and on DLs 1, 4, 7, 10 and 14 (rats assigned to natural delivery). Feed consumption values were not recorded after DL 14, when it was expected that the pups would begin to consume maternal feed.

A table of random units was used to select 15 female rats per dosage group for evaluation of estrous cycling by examination of vaginal cytology for 14 days before the start of the cohabitation period and continuing until mating.

Within each dosage group, consecutive order was used to assign rats to cohabitation, one male rat per female rat. The cohabitation period consisted of a maximum of 14 days. During cohabitation, all female rats were evaluated daily until spermatozoa were observed in a smear of the vaginal contents and/or a copulatory plug was observed *in situ* (DG 0) and were assigned to individual housing. Female rats not mated within the first seven days of cohabitation were assigned alternate male rats that had mated (within the same dosage group) and remained in cohabitation for a maximum of seven additional days.

Rats allowed to naturally deliver litters were evaluated for clinical observations during parturition, duration of gestation (DG 0 to the day the first pup was observed), litter size (all pups delivered) and pup viability at birth. Pups that either appeared stillborn or that died before initial examination of the litters for viability were examined for vital status at birth. The lungs were removed and immersed in water. Pups with lungs that sank were considered stillborn; pups with lungs that floated were considered liveborn and to have died shortly after birth. Each litter was subsequently examined daily for pup viability. Maternal behavior of the dams was evaluated daily when the pups were examined during the 21-day postpartum period. Maternal behavior was recorded on DLs 1, 4, 7, 14 and 21. Variations from expected maternal behavior were recorded, if present, on all other days of the postpartum period.

Fertility parameters were assessed for all dams assigned to natural delivery. These parameters included a fertility index (percentage of matings that resulted in pregnancies), gestation index (percentage of pregnancies that resulted in the birth of live litters), number of offspring per litter (live and dead pups), number of implantation sites, general condition of the dam and litter during the postpartum period, viability indices (percentage of pups born that survived 4 and 7 days), and lactation index (percentage of pups born that survived 21 days).

G.8.b. F1/F2 Generation Pups – Preweaning Observations:

Day 1 of lactation (postpartum) was defined as the day of birth and was also the first day in which all pups in a litter were individually weighed (pup body weights were recorded after all pups in a litter were delivered and groomed by the dam).

Vital status at birth was determined for pups that either appeared stillborn or that died before initial examination of the litter for viability. Pups that either appeared stillborn or that died before initial examination of the litter for viability were examined for vital status at birth, as previously described.

Each litter was evaluated for viability at least twice each day during the 21-day postpartum period. Pups in each litter were counted once daily. Physical signs (including variations from expected nursing behavior and gross external physical anomalies) in the pups were recorded once daily for 21 days postpartum. Dead pups observed at these times were removed from the nesting box. When not precluded by autolysis or cannibalization by the dam, any pup found dead was necropsied and examined for the cause of death. Pup body weights were recorded on DLs 1 (birth), 4, 7, 14 and 21.

Reflex and physical development parameters in the F1 generation pups only were monitored during the 21-day postpartum period. Surface righting reflex [ability to right in 5 seconds (from DL 1)], pinna unfolding (from DL 2), eye opening (from DL 12), acoustic startle response (from DL 13) and air righting reflex (from DL 14) were monitored daily until all pups (100%) in the litter reached the criterion for the specific test. Pupil constriction was evaluated once on DL 21; the number of pups per litter with this reflex present was recorded.

G.8.c. F1 Generation Rats - Postweaning Observations:

"Postweaning day" observations were recorded beginning on DL 22. All F1 generation rats were observed for viability at least twice daily during all periods of the study. Rats were also observed for clinical observations of effects of the test article, abortions and premature deliveries prior to and approximately one hour after dosage and at sacrifice. Body weights of F1 generation male rats were recorded weekly during the dosage period and at sacrifice. Body weights for F1 generation female rats were recorded weekly to cohabitation, daily during the gestation period, on DLs 1, 4, 7, and 14 postpartum (rats assigned to natural delivery) and at sacrifice. Feed consumption values were recorded weekly except during cohabitation and on DGs 0, 7, 10, 14 and 20 and DLs 1, 4, 7, 10 and 14 (female rats only).

Beginning at 23 to 25 days of age, one male rat and one female rat from each litter, where possible, were evaluated in a passive avoidance test for learning,

short-term retention and long-term retention. Each rat was tested on two days separated by a one-week interval, and the criterion for learning was the same for both days of testing. The passive avoidance apparatus consisted of a two-compartment chamber with hinged Plexiglas® lids. One compartment was fitted with a bright light and Plexiglas® floor. The other compartment was fitted with a grid floor to which a brief (1 second) pulse of mild electric current (1 mA) could be delivered. The two compartments were separated by a sliding door.

During each trial, the rat was placed into the "bright" compartment, the sliding door was opened and the light was turned on. The rat was allowed to explore the apparatus until it entered the "dark" compartment. The sliding door was then immediately closed, the light was turned off and the brief pulse of current was delivered to the grid floor. The rat was then removed from the apparatus and placed into a holding cage for 30 seconds before the start of the next trial. Trials were repeated until the rat remained in the "bright" compartment for 60 seconds on two consecutive trials (the criterion for learning) or until 15 trials were completed. The latency to enter the dark compartment or the maximum 60-second interval was recorded for each trial.

Dosage groups were compared for the following dependent measures: the number of trials to the criterion in the first session (overall learning performance); the latency (in seconds) to enter the "dark" compartment from the "bright" compartment on trial 1 in the first test session (activity level and exploratory tendency in a novel environment); the latency (in seconds) to enter the "dark" compartment from the "bright" compartment on trial 2 in the first test session (short-term retention); the number of trials to the criterion in the second test session (long-term retention); and the latency (in seconds) to enter the "dark" compartment from the "bright" compartment on trial 1 in the second session (long-term retention).

Beginning at approximately 70 days postpartum, one male rat and one female rat from each litter were evaluated in a water-filled M-maze for overt coordination, swimming ability, learning and memory. Each rat was tested in a watertight 16-gauge stainless steel modified M-maze. The maze was filled with water to a depth of approximately nine inches, and the water was monitored for temperature (range of 21°C ± 1°C). On each test trial, the rat was placed into the starting position (base of the M-maze stem farthest from the two arms) and required to swim to one of the two goals of the M-maze, in order to be removed from the water. On the first trial, the rat was required to enter both arms of the maze before being removed from the water. The initial arm chosen on trial 1 was designated the incorrect goal during the remaining trials. Rats that failed to make a correct goal choice within 60 seconds in any given trial were guided to the correct goal and were then removed from the water. A 15-second intertrial interval separated each trial. Each rat was required to reach a criterion of five

consecutive errorless trials to terminate the test session. The maximum number of trials in any test session was 15. Latency (measured in seconds) to choose the correct goal or the maximum 60-second interval was recorded for each trial, as is the number of errors (incorrect turns in the maze) during each trial.

Each rat was tested twice. The test sessions were separated by a one-week interval, and the correct goal and the criterion were the same for both test sessions. Dosage groups were compared for the following dependent measures: the number of trials to criterion on the first day of testing (overall learning); the average number of errors (incorrect turns in the maze) for each trial on the first day of testing (overall learning); the latency (in seconds) to reach the correct goal on trial 2 of the first day of testing (short-term retention); the number of trials to criterion on the second day of testing (long-term retention); the average number of errors for each trial on the second day of testing (long-term retention); and the latency (in seconds) to reach the correct goal on trial 1 of day 2 of testing (long-term retention).

Female rats were evaluated for the age of vaginal patency beginning on day 28 postpartum[a]. Male rats were evaluated for the age of preputial separation beginning on day 39 postpartum.

On DLs 84 to 97, the F1 generation rats within each dosage group were assigned to cohabitation, one male rat per female rat, based on computer-generated random units, with the exclusion of sibling matings. The cohabitation period consisted of a maximum of 14 days. Female rats with spermatozoa observed in a smear of the vaginal contents and/or a copulatory plug observed *in situ* were considered to be at DG 0 and assigned to individual housing. Female rats that did not mate within the first seven days of cohabitation were assigned alternate male rats from the same dosage group that had mated. Female rats were allowed to naturally deliver and maintain litters through a 21-day postpartum period.

These rats were evaluated for clinical observations during parturition, duration of gestation (DG 0 to the day the first pup was observed), litter size (all pups delivered) and pup viability at birth. Pups that either appeared stillborn or that died before initial examination of the litters for viability were examined for vital status at birth. The lungs were removed and immersed in water. Pups with lungs that sank were considered stillborn; pups with lungs that floated were considered liveborn and to have died shortly after birth. Each litter was subsequently examined daily for pup viability. Maternal behavior of the dams was evaluated daily when the pups were examined during the 21-day postpartum period. Maternal behavior was recorded on DLs 1, 4, 7, 14 and 21.

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a. See APPENDIX G, Item 6.

Variations from expected maternal behavior were recorded, if present, on all other days of the postpartum period.

Fertility parameters were assessed for all dams assigned to natural delivery. These parameters included a fertility index (percentage of matings that resulted in pregnancies), gestation index (percentage of pregnancies that resulted in the birth of live litters), number of offspring per litter (live and dead pups), number of implantation sites, general condition of the dam and litter during the postpartum period, viability indices (percentage of pups born that survived 4 and 7 days), and lactation index (percentage of pups born that survived 21 days).

G.9. **Gross Necropsy**[a]:

G.9.a. **Fo Generation Male and Female Rats Assigned to Pharmacokinetic Sample Collection:**

At scheduled sacrifice after completion of the cohabitation period (male rats that sired litters of dams allowed to naturally deliver a litter) and on DL 21 (female rats allowed to naturally deliver a litter) blood samples (approximately 4 mL per rat) were collected from five rats per sex per dosage group from the inferior vena cava into serum separator tubes and centrifuged. The resulting serum was immediately frozen on dry ice and maintained frozen (-70°C) until shipment to the Sponsor for analysis. The liver was excised, weighed, and a sample section (lateral lobe) was frozen and retained at -70°C until shipment to the Sponsor for analysis.

The livers of the pups from the litters of the five dams in Groups I to IV selected for pharmacokinetic sample collection were excised, pooled per litter, frozen and retained at -70°C until shipment to the Sponsor for analysis. The dams in the 15 mg/kg/day dosage group (Group V) did not have surviving pups on DL 21.

G.9.b. **Fo and F1 Generation Male Rats:**

Male rats were sacrificed by carbon dioxide asphyxiation after completion of the cohabitation period, and a gross necropsy of the thoracic, abdominal and pelvic viscera was performed. Gross lesions were retained in neutral buffered 10% formalin for possible future evaluation. Representative photographs of gross lesions are available in the raw data. The following organs were excised, individually weighed and retained for possible histologic evaluation: testes,

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a. A table of random units was used to select one control group Fo and F1 generation rat of each sex from which all tissues examined at necropsy were retained, in order to provide control tissues for any possible histopathological evaluations of gross lesions.

epididymides, prostate and seminal vesicles (with and without fluid). The testes were fixed in Bouin's solution for 48 to 96 hours and then retained in neutral buffered 10% formalin. The remaining organs were retained in neutral buffered 10% formalin.

G.9.c. Fo Generation Female Rats Assigned to Caesarean-Sectioning:

Female rats assigned to Caesarean-sectioning were sacrificed by carbon dioxide asphyxiation on DG 10, and a gross necropsy of the thoracic, abdominal and pelvic viscera was performed. Uteri of apparently nonpregnant rats were stained with 10% ammonium sulfide to confirm the absence of implantation sites[9]. All ovaries and gross lesions were retained in neutral buffered 10% formalin for possible future evaluation. Representative photographs of gross lesions are available in the raw data. The rats were examined for placentae that appeared abnormal (size, color or shape) and the number of corpora lutea in each ovary, implantation sites and viable and nonviable embryos. A viable embryo is oval or crescent shaped, pink and enclosed in an amniotic sac filled with clear fluid. A nonviable embryo is amorphous, small, pale pink to tan or deep red to black, soft and enclosed in an amniotic sac filled with clear, cloudy or opaque fluid. Embryos were discarded after examination.

G.9.d. Fo Generation Female Rats Assigned Natural Delivery and F1 Generation Female Rats:

After completion of the 21-day postpartum period, all dams that delivered litters were sacrificed, and gross necropsy of the thoracic, abdominal and pelvic viscera was performed. The number and distribution of implantation sites was recorded. Female rats assigned to natural delivery that did not deliver a litter were sacrificed on DG 25 and examined for gross lesions. To confirm the pregnancy status, uteri from rats that appeared nonpregnant were stained with 10% ammonium sulfide[9]. Dams with no surviving pups were sacrificed after the last pup was found dead, missing or presumed cannibalized. A gross necropsy of the thoracic, abdominal and pelvic viscera was performed. All ovaries were retained in neutral buffered 10% formalin for possible future evaluation.

The Fo generation female rat in the 5 mg/kg/day dosage group selected for natural delivery that died was examined for the cause of death on the day the observation was made. The rat was examined for gross lesions. Pregnancy status and uterine contents were recorded. Delivered pups were examined to the extent possible. Ovaries were retained in neutral buffered 10% formalin.

G.9.e. F1/F2 Generation Pups:

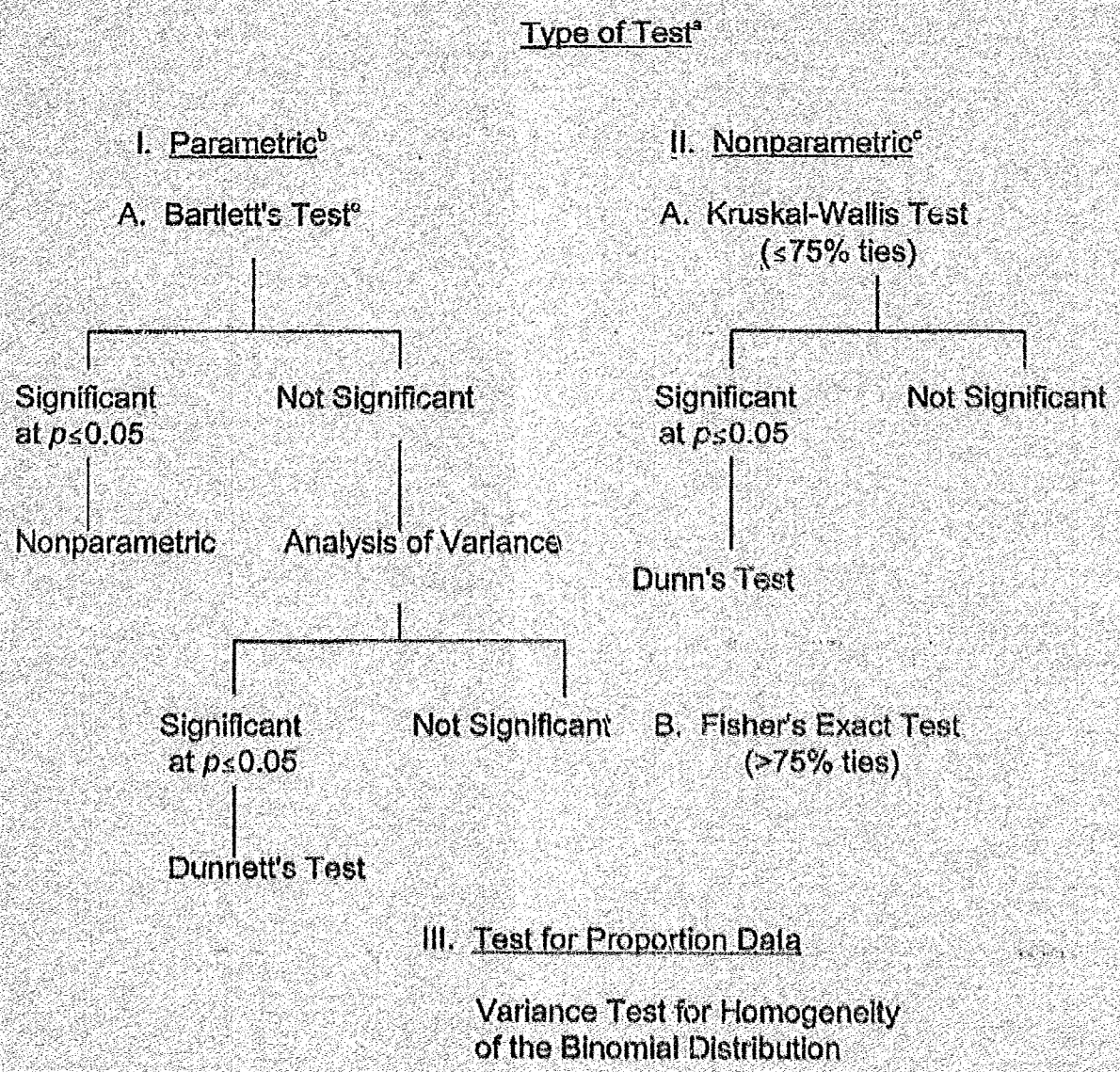
Pups that died before examination of the litter for pup viability were evaluated for vital status at birth, as described previously. Pups found dead were examined for gross lesions and for the cause of death. Pups with gross lesions found on DLs 1 to 4 were preserved in Bouin's solution. Gross lesions of pups found on DLs 5 to 21 were preserved in neutral buffered 10% formalin. Representative photographs of pup gross lesions are available in the raw data.

Pups not selected for continued evaluation on DL 4 were sacrificed by carbon dioxide asphyxiation and examined for gross lesions; pups with gross lesions were preserved in Bouin's solution. Necropsy included a single cross-section of the head at the level of the frontal-parietal suture and examination of the cross-sectioned brain for apparent hydrocephaly. The stomach contents (milk curd) were collected from culled pups from Groups I, II and III. Samples were collected from all pups from five of the largest litters in these three dosage groups. Individual pup samples were combined by litter into polypropylene tubes and frozen at -20°C. After completion of sample collection, samples were shipped (frozen on dry ice) to the Sponsor for analysis.

On DL 21, F1 generation pups not continued on study and all F2 generation pups were sacrificed and examined for gross lesions; gross lesions were preserved in neutral buffered 10% formalin. The 10 mg/kg/day F1 generation (Group IV) litters were sacrificed on DL 21 because of the severe pup toxicity during lactation (mortality and reduced body weights and delayed development). Necropsy procedures were the same as those used for pups culled on DL 4.

G.10. Statistical Analyses:

The following schematic represents the statistical analyses of the data:



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a. Statistically significant probabilities are reported as either $p \leq 0.05$ or $p \leq 0.01$.
b. Used only to analyze data with homogeneity of variance.
c. Proportion data are not included in this category.
d. Test for homogeneity of variance.

Proportion data were analyzed using the Variance Test for Homogeneity of the Binomial Distribution[10].

Continuous data (e.g., body weights, body weight changes, feed consumption data and organ weights) were analyzed using Bartlett's Test of Homogeneity of Variances[11] and the Analysis of Variance[12], when appropriate [i.e., Bartlett's Test was not significant ($p>0.05$)]. If the Analysis of Variance was significant ($p\leq0.05$), Dunnett's Test[13] was used to identify the statistical significance of the individual groups. If the Analysis of Variance was not appropriate [i.e., Bartlett's Test was significant ($p\leq0.05$)], the Kruskal-Wallis Test[14] was used (≤75% ties). In cases where the Kruskal-Wallis Test was statistically significant ($p\leq0.05$), Dunn's Method of Multiple Comparisons[15] was used to identify the statistical significance of the individual groups. If there were greater than 75% ties, Fisher's Exact Test[16] was used.

Data obtained at Caesarean-sectioning, natural delivery, preweaning reflex/physical developmental data involving discrete data (e.g., number of corpora lutea, number of pups per litter trials to a criterion), were evaluated by the Kruskal-Wallis Test[14], as described above.

One F1 generation dam (12228) in the 1 mg/kg/day dosage group had a litter consisting of only two pups. Because such occurrences can abnormally skew the distribution of the data[17], statistical analyses of gestation body weights, feed consumption values and natural delivery and litter data were made without the values for this dam and litter.

III. RESULTS - Fo GENERATION MALE RATS

A. Mortality and Clinical Observations (Summary - Table B1; Individual Data - Table B10)

No Fo generation male rats died during this study.

The only clinical observation attributable to the test article was impaired righting reflex. This observation occurred in significant numbers ($p \leq 0.01$) of 15 mg/kg/day dosage group rats, as compared with the control group values. All other adverse clinical observations were considered unrelated to the test article because the incidences were not dosage-dependent. These observations included localized alopecia on the limbs, dental problems (missing, broken or misaligned incisors), chromodacryorrhea, red peripenal substance and chromorhinorrhea.

B. Body Weights and Body Weight Changes (Figure 1; Summaries - Tables B2 and B3; Individual Data - Table B11)

Groups administered 5 mg/kg/day and higher dosages of the test article had reduced body weight gains. The values were significantly reduced ($p \leq 0.05$ or $p \leq 0.01$) in the 5 mg/kg/day dosage group on DSs 15 to 22, 22 to 29 and 29 to 36; in the 10 mg/kg/day dosage group on DSs 8 to 15, 15 to 22, 22 to 29, 29 to 36, 36 to 43 and 50 to 53; and at all tabulated intervals in the 15 mg/kg/day dosage group. Reflecting these effects of the test article, body weight gains were significantly reduced ($p \leq 0.01$) for the entire dosage period (DSs 1 to 53) in groups administered 5 mg/kg/day and higher of the test article. Absolute body weights were significantly reduced ($p \leq 0.05$ or $p \leq 0.01$) in the 5 mg/kg/day dosage group on DSs 29, 36, 43, 50 and 53, and in the 10 and 15 mg/kg/day dosage groups on DSs 15, 22, 29, 36, 43, 50 and 53.

Body weights and body weight gains were unaffected by the 1 mg/kg/day dosage of the test article.

C. Absolute (g/day) and Relative (g/kg/day) Feed Consumption Values (Summaries - Tables B4 and B5; Individual Data - Table B12)

Absolute (g/day) feed consumption values were significantly reduced ($p \leq 0.05$ or $p \leq 0.01$) in the 10 and 15 mg/kg/day dosage groups after the first week of treatment (DSs 8 to 15, 15 to 22, 22 to 29, 43 to 50 and 50 to 53). Reflecting these effects of the test article, the 10 and 15 mg/kg/day dosage groups had significantly reduced ($p \leq 0.01$) absolute feed consumption values for the entire treatment period (DSs 1 to 53).

Absolute and relative (g/kg/day) feed consumption values were significantly reduced ($p \leq 0.05$ or $p \leq 0.01$) in the 15 mg/kg/day dosage group on DSs 15 to 22, 22 to 29, 43 to 50 and 50 to 53, as compared with the control group values.

Feed consumption values were unaffected by the 5 mg/kg/day dosage of the test article.

D. **Mating and Fertility (Summary - Table B5; Individual Data - Table B13)**

Dosages of the test article as high as 15 mg/kg/day did not affect any mating and fertility parameters evaluated in the male rats. Values for the fertility and pregnancy indices (number of pregnancies per number of rats in cohabitation and rats that mated, respectively), the number of days to inseminate, the number of rats that mated and the number of rats with confirmed mating dates during the first week of cohabitation were comparable among the five dosage groups.

E. **Necropsy Observations (Summary - Table B7; Individual Data - Table B14)**

A significantly increased ($p \leq 0.01$) number of male rats in the 15 mg/kg/day dosage group had small seminal vesicles. One of these rats mated but did not impregnate a female rat; the other two rats impregnated female rats. One 15 mg/kg/day dosage group male rat (10051) had a small prostate; this male rat was cohabited with and impregnated two female rats. Both of these gross lesions were considered related to the test article because: 1) the incidences were dosage-dependent; and 2) the absolute weights of the seminal vesicles with fluid and prostate were significantly reduced ($p \leq 0.01$) in the 15 mg/kg/day dosage group (see section III.F).

All other necropsy observations were considered unrelated to the test article because: 1) the observation occurred in a control group rat; or 2) the observation was considered a congenital malformation. One control group rat had a missing portion of a hindpaw digit. One 5 mg/kg/day dosage group male rat did not have a right testis and epididymis; this rat did not mate.

F. **Terminal Body Weights and Organ Weights and Ratios (%) of Organ Weight to Terminal Body Weight (Summaries - Tables B8 and B9; Individual Data - Table B15)**

The 5, 10 and 15 mg/kg/day dosage groups had significantly reduced ($p \leq 0.01$) terminal body weights. The absolute weights of the left epididymis, seminal vesicles with fluid, and prostate were significantly reduced ($p \leq 0.05$ or $p \leq 0.01$) in the 15 mg/kg/day dosage group. The absolute weights of the seminal vesicles

without fluid and the right epididymis were also reduced in the 15 mg/kg/day dosage group, however not significantly ($p>0.05$). Testes weights were unaffected by dosages of the test article as high as 15 mg/kg/day.

The ratios of the weight of the left and right testes to terminal body weights were significantly increased ($p\leq0.05$ or $p\leq0.01$) in the 5 (left only), 10 and 15 mg/kg/day dosage groups, as compared to the control group values. These observations were associated with the significantly reduced ($p\leq0.01$) terminal body weights in these dosage groups. The ratios of the weights of the epididymides, seminal vesicles and prostate were generally comparable among the five dosage groups.

IV. RESULTS - Fo GENERATION FEMALE RATS/F1 GENERATION LITTERS

A. Mortality and Clinical Observations (Summaries - Table C1 and C15; Individual Data - Tables C22 and C30)

A.1. Mortality

No deaths were caused by dosages of EtFOSE as high as 15 mg/kg/day. One dam (10170) in the 5 mg/kg/day dosage group died during delivery on gestation day (DG) 22. The event was considered unrelated to the test article because the incidence was not dosage-dependent. No other adverse clinical observations occurred in this dam, and its body weight and feed consumption values were unremarkable throughout the gestation period. Necropsy of the dam revealed external observations of red and wet perioral and perivaginal substance presumed to be evidence of bleeding during parturition. There were 15 implantation sites; eight normal pups were delivered and six dead fetuses were *in utero*. One conceptus was presumed cannibalized, because pup tissues were found in the stomach of the dam. All other dams survived to scheduled sacrifice.

A.2. Clinical Observations

Relatively low incidences of localized alopecia occurred during the precohabitation, gestation and lactation periods only in groups treated with EtFOSE. These incidences of localized alopecia (total of all areas and limbs) were significant ($p \leq 0.05$ or $p \leq 0.01$) in the 5, 10 and 15 mg/kg/day dosage groups during the gestation and lactation periods.

All other clinical observations during the precohabitation, gestation and lactation periods were considered unrelated to the test article because: 1) the incidences were not dosage-dependent; and/or 2) the observation occurred in only one rat. These observations included dehydration, cold to touch, portion of tail black or missing, chromodacryorrhea, dental problems and chromorhinorrhea.

B. Body Weights (Figure 2; Summaries - Tables C2 through C7; Individual Data - Tables C23 through C25)

B.1. Precohabitation

Groups administered 5 mg/kg/day and higher dosages of the test article had significantly reduced ($p \leq 0.01$) body weight gains for the entire precohabitation period (DSs 1 to 29). During this period, values were significantly reduced ($p \leq 0.05$ or $p \leq 0.01$) in the 5 mg/kg/day dosage group on DSs 8 to 16 and 23 to

29; and in the 10 and 15 mg/kg/day dosage groups on DSs 1 to 8, 8 to 16, 16 to 23 and 23 to 29, as compared with the control group values.
Body weights were significantly reduced ($p \leq 0.05$ or $p \leq 0.01$) in the 5 mg/kg/day dosage group on DSs 16 and 29, and in the 10 and 15 mg/kg/day dosage groups on DSs 16, 23 and 29, as compared with the control group values.

Body weights and body weight gains during the precohabitation period were unaffected by the 1 mg/kg/day dosage of the test article.

B.2. Gestation

Gestation body weight gains tended to be reduced in the 1 and 5 mg/kg/day dosage groups and were significantly reduced ($p \leq 0.01$) in the 10 and 15 mg/kg/day dosage groups on DGs 0 to 7. Weight gains generally continued to be reduced in these groups until DGs 10 to 12 (1 and 5 mg/kg/day dosage groups) or 12 to 15 (10 and 15 mg/kg/day dosage groups). Body weight gains were again significantly reduced ($p \leq 0.01$) in the 15 mg/kg/day dosage group on DGs 18 to 20. Reflecting these effects of the test article, body weight gains tended to be reduced in the 5 and 10 mg/kg/day dosage groups and were significantly reduced ($p \leq 0.01$) in the 15 mg/kg/day dosage group for the entire gestation period (DGs 0 to 20).

Body weights continued to be significantly reduced ($p \leq 0.05$ or $p \leq 0.01$) in the 5 mg/kg/day dosage group on DGs 0 through 17, and in the 10 and 15 mg/kg/day dosage groups on all days of the gestation period (DGs 0 through 20).

Body weights and body weight gains during gestation were unaffected by the 1 mg/kg/day dosage of the test article.

B.3. Lactation

The 10 and 15 mg/kg/day dosage groups had significantly reduced ($p \leq 0.01$) body weights on DL 1. Lactation body weights continued to be significantly reduced ($p \leq 0.01$) in the 10 mg/kg/day dosage group on DLs 4, 7, 10 and 14.

Body weight loss occurred in the 10 and 15 mg/kg/day dosage groups on DLs 1 to 4. The 15 mg/kg/day dosage group was precluded from further evaluation because all pups died before DL 5. A significant increase ($p \leq 0.05$) in body weight gain on DLs 14 to 21 in the 10 mg/kg/day dosage group was possibly associated with reduced litter size, milk demand and production.

Body weights and body weight gains during lactation were unaffected by the 1 and 5 mg/kg/day dosage of the test article.

C. Absolute (g/day) and Relative (g/kg/day) Feed Consumption Values (Summaries - Tables C8 through C13; Individual Data - Tables C26 through C28)

C.1. Precohabitation

Absolute (g/day) and relative (g/kg/day) feed consumption values were significantly reduced ($p \leq 0.05$ or $p \leq 0.01$) during the precohabitation period in the 10 and 15 mg/kg/day dosage groups on DSs 8 to 16, 16 to 23 and 23 to 29. Reflecting these effects of the test article, absolute and relative feed consumption values were significantly reduced ($p \leq 0.05$ or $p \leq 0.01$) in the 10 and 15 mg/kg/day dosage group for the entire precohabitation period (DSs 1 to 29).

Feed consumption values during the precohabitation period were unaffected by the 5 mg/kg/day dosage of the test article.

C.2. Gestation

The 10 and 15 mg/kg/day dosage groups continued to have significantly reduced ($p \leq 0.01$) absolute and relative feed consumption values early in the gestation period (DGs 0 to 7 and 7 to 10). Absolute feed consumption values were also significantly reduced ($p \leq 0.05$) in the 5 mg/kg/day dosage group on DGs 0 to 7. Absolute feed consumption values continued to be significantly reduced ($p \leq 0.05$ or $p \leq 0.01$) in the 10 mg/kg/day dosage group on DGs 10 to 12 and 12 to 15, and in the 15 mg/kg/day dosage group throughout the remainder of the gestation period (DGs 10 to 12, 12 to 15, 15 to 18 and 18 to 20). As a result of these changes, absolute and relative feed consumption values were significantly reduced ($p \leq 0.05$ or $p \leq 0.01$) for the entire gestation period (DGs 0 to 20) in the 5 (absolute only), 10 and 15 mg/kg/day dosage groups.

Feed consumption values during gestation were unaffected by the 1 mg/kg/day dosage of the test article.

C.3. Lactation

Absolute and relative feed consumption values were significantly reduced ($p \leq 0.05$ or $p \leq 0.01$) in the 5 (absolute only) and 10 mg/kg/day dosage groups for the entire lactation period (DLs 1 to 14). Significant reductions ($p \leq 0.05$ or $p \leq 0.01$) in absolute and relative feed consumption values occurred on DLs 7 to 10 in the 5 mg/kg/day dosage group, and for all tabulated intervals in the 10 mg/kg/day dosage group. Absolute and relative feed consumption values were reduced in the 15 mg/kg/day dosage group on DLs 1 to 4, however the reductions were not statistically significant because only one litter was available for evaluation in this dosage group. The 15 mg/kg/day dosage group was precluded from further evaluation because all pups died before DL 5.

Feed consumption values during lactation were unaffected by the 1 mg/kg/day dosage of the test article.

D. **Estrous Cycling, Mating and Fertility (Summary - Table C14; Individual Data - Table C29)**

Dosages of the test article as high as 15 mg/kg/day did not affect estrous cycling (number of estrus stages per 14 days) in the 15 rats per dosage group that were evaluated. A total of 34 or 35 female rats mated in each dosage group. The number of days in cohabitation, the number of rats that mated, the fertility and pregnancy indices (number of pregnancies per number of rats that mated and rats in cohabitation, respectively), and the number of rats with confirmed mating dates during the first and second week of cohabitation were comparable in the five dosage groups. Pregnancy occurred in 35, 33, 32, 32 and 34 female rats in Groups I through V, respectively.

E. **Necropsy Observations (Summary - Table C15; Individual Data - Table C30)**

All necropsy observations were considered unrelated to treatment with the test article. One, one and two rats in the 1, 10 and 15 mg/kg/day dosage groups, respectively, had moderate or marked dilation of the pelvis of one or both kidneys, observations that are common in this species and strain. Red and wet perioral and perivaginal substance occurred in the 5 mg/kg/day dosage group rat that died during delivery and was previously discussed. One rat in the 5 mg/kg/day dosage group had localized alopecia at necropsy.

F. **Caesarean-Sectioning and Litter Observations (Summary - Table C16; Individual Data - Tables C31 through C33)**

Caesarean-sectioning observations on DG 10 were based on 10 pregnant dams in each of the five dosage groups.

Statistically significant reductions ($p \leq 0.01$) in the averages for implantations and viable embryos occurred in the 15 mg/kg/day dosage group, as compared to the control group.

There were no biologically important or other statistically significant differences in the litter averages for corpora lutea or nonviable embryos. There were no dams with all nonviable embryos.

G. **Natural Delivery and Litter Observations (Summaries - Table C17 and C18; Individual Data – Tables C34 through C37)**

There were 25 presumed pregnant rats assigned to natural delivery in each of the five dosage groups and 22 to 25 pregnant dams in each dosage group. One pregnant dam in the 5 mg/kg/day dosage group died during delivery on DG 22, as previously described. One dam in the 10 mg/kg/day dosage group was not observed to have delivered a litter but had one implantation site *in utero* at necropsy on DG 25, an event that may indicate resorption of the litter, as such generally occurs for litters of one conceptus. All other pregnant rats delivered a litter.

The duration of gestation was significantly reduced ($p \leq 0.05$ or $p \leq 0.01$) in the 5, 10 and 15 mg/kg/day dosage groups, an observation associated with preimplantation loss in the 15 mg/kg/day dosage group [the average number of implantation sites per dam was significantly reduced ($p \leq 0.01$), resulting in a significantly reduced ($p \leq 0.01$) litter size] and an effect of the test article.

Pup viability was significantly affected ($p \leq 0.05$ or $p \leq 0.01$) by the 10 and 15 mg/kg/day dosages of the test article, as described in the following information. The 10 mg/kg/day dosage group had a significantly increased ($p \leq 0.01$) number of dams with stillborn pups and the numbers of pups that died or were presumed cannibalized were significantly increased ($p \leq 0.01$) on DLs, 1, 2 to 4, 5 to 7 and 8 to 14. The 15 mg/kg/day dosage group had postimplantation loss evident as reduced pup viability at birth [the gestation index (number of dams with liveborn pups per pregnant rats) was significantly reduced ($p \leq 0.01$), four** dams in this dosage group had no liveborn pups, the number of dams with stillborn pups was significantly increased ($p \leq 0.01$), the litter average and number of stillborn pups was significantly increased ($p \leq 0.01$) and the litter average and number of liveborn pups was significantly reduced ($p \leq 0.01$)], as well as peripartum deaths [significant ($p \leq 0.01$) numbers of dead and presumed cannibalized pups on DLs 1, 2 to 4 and 5 to 7 (all liveborn pups died by DL 5) and significant ($p \leq 0.01$) numbers of dams had all pups dead or presumed cannibalized on DLs 1 to 4]. Reflecting these effects, the viability and lactation indices were significantly reduced ($p \leq 0.01$) in the 10 and 15 mg/kg/day dosage groups, as also were the averages for surviving pups ($p \leq 0.01$) in the 10 mg/kg/day dosage group on DLs 4 (pre- and postculling) and 7, and in the 15 mg/kg/day dosage group on DLs 1 and 4 (preculling). The live litter size at weighing was significantly reduced ($p \leq 0.05$ or $p \leq 0.01$) on DL 1 in the 10 and 15 mg/kg/day dosage groups and on DLs 1, 4 (postculling), 7, 14 and 21 in the 10 mg/kg/day dosage group.

** Significantly different from the vehicle control group value ($p \leq 0.01$).

A dosage-dependent pattern of reduced pup body weights was evident in each group administered the test article. The 1 mg/kg/day dosage group tended to have reduced pup body weights on DL 4 (pre-and postculling). The 5, 10 and 15 mg/kg/day dosage groups had significantly reduced ($p \leq 0.01$) pup body weights on all weighing days (no pups survived after DL 4 in the 15 mg/kg/day dosage group).

The percentage of male pups was comparable across all five dosage groups.

H. Pup Clinical and Necropsy Observations (Summaries – Tables C19 and C21; Individual Data – Tables C38 and C45)

Adverse clinical and necropsy observations associated with reduced pup viability and potential reduction in maternal care occurred in the 5, 10 and 15 mg/kg/day dosage groups. Adverse clinical observations included 1, 7 and 3 litters with pups that were not nursing in the 5, 10 and 15 mg/kg/day dosage groups, respectively; the incidence was significant ($p \leq 0.01$) in the 10 mg/kg/day dosage group. The number of litters with cold to touch pups was also significantly increased ($p \leq 0.01$) in the 10 mg/kg/day dosage group. Two litters in the 10 mg/kg/day dosage group had pups that were not nesting and one 10 mg/kg/day dosage group litter had dehydrated pups. The placentae and umbilical cords were not removed from pups in one 5 mg/kg/day dosage group litter.

Necropsy observations in pups that were found dead included increases in the number of pups with no milk in the stomach in the 5, 10 and 15 mg/kg/day dosage group litters; the incidence was significant ($p \leq 0.01$) in the 10 and 15 mg/kg/day dosage groups.

All other clinical and necropsy observations in the F1 generation pups were considered unrelated to the test article because: 1) the incidences were not dosage-dependent; or 2) the observation occurred in only one or two pups. Clinical observations included a black area on the right hindpaw, swollen left hindlimb, mass(es) in mouth, lesion on the neck and portion of tail missing or black. Necropsy revealed one 15 mg/kg/day dosage group stillborn pup with a short jaw and one 5 mg/kg/day pup with moderate dilation of the pelvis of the right kidney at necropsy on DL 21.

I. Reflex and Physical Development (Summary – Table C20; Individual Data – Tables C39 through C44)

Reversible delays in reflex and physical development that are highly correlated with body weight[1,18] occurred in the 5 and 10 mg/kg/day dosage groups, as described below.

I.1. Surface Righting

Surface righting was delayed in the 5, 10 and 15 mg/kg/day dosage groups, as described below. The percentage of pups that could surface right was significantly reduced ($p \leq 0.05$ or $p \leq 0.01$) in the 5 mg/kg/day dosage group on DLs 2, 3, 6, 9 and 10, in the 10 mg/kg/day dosage group on DLs 2 through 18, and in the 15 mg/kg/day dosage group on DL 1. After DL 1, no pups in the 15 mg/kg/day dosage group surface righted; all pups were dead after DL 4. The average day that at least 50% of the pups in a dosage group had the ability to surface right was significantly increased ($p \leq 0.01$) in the 10 mg/kg/day dosage group. All surviving pups ultimately attained the ability to surface right.

The ability to surface right was unaffected by the 1 mg/kg/day dosage of the test article.

I.2. Pinna Unfolding

Pinna unfolding was delayed in the 1, 5, 10 and 15 mg/kg/day dosage groups. The effect was transient (evident only on one day) in the 1 mg/kg/day dosage group, and considered of no toxicological importance [the percentage of pups per litter with pinna unfolding was significantly reduced ($p \leq 0.05$) on DL 3 only]. The percentage of pups with an unfolded pinna was significantly reduced ($p \leq 0.05$ or $p \leq 0.01$) on DLs 3 and 4 in the 5 mg/kg/day dosage group, on DLs 3, 4, 5 and 6 in the 10 mg/kg/day dosage group and on DLs 3 and 4 in the 15 mg/kg/day dosage group. No pups in the 15 mg/kg/day dosage group had an unfolded pinna on DLs 2, 3 or 4; all pups in this group were dead after DL 4. The average day that at least 50% of the pups in a dosage group had an unfolded pinna was significantly increased ($p \leq 0.01$) in the 5 and 10 mg/kg/day dosage groups. All surviving pups ultimately had unfolded pinnae.

I.3. Eye Opening

Eye opening was delayed in the 5 and 10 mg/kg/day dosage groups (no 15 mg/kg/day dosage group pups were evaluated for this parameter). The percentage of pups with at least one open eye was significantly reduced ($p \leq 0.05$ or $p \leq 0.01$) in the 5 and 10 mg/kg/day dosage groups on DLs 14, 15 and 16. The day that at least 50% of the pups had at least one open eye was increased in the 5 and 10 mg/kg/day dosage groups; the value was significant ($p \leq 0.01$) in the 5 mg/kg/day dosage group. All pups had open eyelids by DL 21, when pupil constriction was tested.

The time to eye opening was unaffected by the 1 mg/kg/day dosage of the test article.

I.4. Acoustic Startle

The time of development of the acoustic startle reflex was delayed in the 1, 5 and 10 mg/kg/day dosage groups; no 15 mg/kg/day dosage group pups survived to be tested for this reflex. The effect was transient (evident only on two days) in the 1 mg/kg/day dosage group, and considered of no toxicological importance [the percentage of pups per litter with this reflex was significantly reduced ($p \leq 0.05$ or $p \leq 0.01$) on DLs 14 and 15]. The percentage of pups in the 10 and 15 mg/kg/day dosage groups with this reflex was significantly reduced ($p \leq 0.05$ or $p \leq 0.01$) on DLs 13, 14 and 15. The day that at least 50% of the pups had the acoustic startle reflex was significantly increased ($p \leq 0.01$) in the 5 and 10 mg/kg/day dosage groups.

I.5. Air Righting

The ability to air right was delayed in the 5 and 10 mg/kg/day dosage groups; no 15 mg/kg/day dosage group pups survived to be tested for this reflex. The percentage of pups that air righted was significantly reduced ($p \leq 0.05$ or $p \leq 0.01$) in the 5 mg/kg/day dosage group on DLs 14 through 18 and in the 10 mg/kg/day dosage group on DLs 14 through 20. The day that at least 50% of the pups could air right was significantly increased ($p \leq 0.05$ or $p \leq 0.01$) in the 5 and 10 mg/kg/day dosage groups.

The ability to air right was unaffected by the 1 mg/kg/day dosage of the test article.

I.6. Pupil Constriction

All live pups in the 0 (Vehicle), 1, 5 and 10 mg/kg/day dosage groups had the pupil constriction response present on DL 21.

V. RESULTS– F1 GENERATION MALE AND FEMALE RATS

A. F1 Generation Male Rats

A.1. Mortality and Clinical Observations (Summary - Table D1; Individual Data - Table D13)

All F1 generation male rats survived until scheduled sacrifice.

All adverse clinical observations were considered unrelated to the test article because 1) the incidences were not dosage-dependent; and/or 2) the observation occurred in only one or two rats. These observations included dental problems (missing, broken and/or misaligned incisors), chromodacryorrhea, chromorhinorrhea and swollen and purple ears. Observations of emaciation, dehydration, brown perinasal discharge, labored breathing, brown or red perioral substance, excess salivation, gasping and rales occurred in one rat (12123) in the 1 mg/kg/day dosage group on days 70 to 85 postweaning and were considered related to a possible intubation injury on day 70 postweaning. This rat lost 101 g from days 71 to 78 postweaning but regained 88 g by day 85 postweaning.

A.2. Body Weights and Body Weight Changes (Figure 3; Summaries - Tables D2 and D3; Individual Data - Table D14)

The 1 and 5 mg/kg/day dosage groups weighed less than the control group on day 1 postweaning [the reduction was significant ($p \leq 0.01$) in the 5 mg/kg/day dosage group] and body weight gains were reduced in these groups throughout the postweaning period. Body weight gains were significantly reduced ($p \leq 0.01$ and $p \leq 0.05$) in the 1 and 5 mg/kg/day dosage groups on days 22 to 29 and 43 to 50 postweaning, and additionally in the 5 mg/kg/day dosage group on days 1 to 8, 8 to 15 and 15 to 22 postweaning. Reflecting this pattern of weight gain, weight gains in the 1 and 5 mg/kg/day dosage groups were significantly reduced ($p \leq 0.05$ and $p \leq 0.01$) for the precohabitation period (day 1 postweaning to precohabitation) and for day 1 postweaning to termination (significant at 5 mg/kg/day only). Absolute body weights were significantly reduced ($p \leq 0.05$) in the 1 mg/kg/day dosage group on days 36, 50 and 57 postweaning and significantly reduced ($p \leq 0.01$) in the 5 mg/kg/day dosage group throughout the postweaning dosage period.

A.3. Absolute (g/day) and Relative (g/kg/day) Feed Consumption Values (Summaries - Tables D4 and D5; Individual Data - Table D15)

Absolute (g/day) and relative (g/kg/day) feed consumption values in the F1 generation male rats were significantly reduced ($p \leq 0.01$) in the 5 mg/kg/day dosage group on days 1 to 8 and 8 to 15 postweaning. Relative feed

consumption values were also significantly reduced ($p \leq 0.01$) on days 15 to 22, 22 to 29, 29 to 36, 36 to 43 and 1 to 57 in the 5 mg/kg/day dosage group.

Feed consumption values were unaffected by the 1 mg/kg/day dosage of the test article.

A.4. Sexual Maturation (Summary - Table D6; Individual Data - Table D16)

The average day of preputial separation was unaffected by the 1 mg/kg/day dosage of the test article. The average day of preputial separation was significantly delayed ($p \leq 0.01$) for the F1 generation male rats in the 5 mg/kg/day dosage group, as compared to the control group value, but the increase was approximately one day and not considered toxicologically important.

A.5. Passive Avoidance Performance (Summary - Table D7; Individual Data - Table D17)

There were no biologically important differences in the values for learning, short-term retention, long-term retention or response inhibition in the F1 generation male rats, as evaluated by performance in a passive avoidance paradigm. No statistically significant differences occurred in the number of trials to criterion, trial latencies or numbers of rats that failed to learn.

A.6. Watermaze Performance (Summary - Table D8; Individual Data - Table D18)

No biologically important dosage-dependent differences occurred in watermaze performance of the F1 generation male rats regarding learning, short-term retention, long-term retention or response inhibition. No statistically significant differences occurred in the number of trials to criterion, trial latencies or numbers of rats that failed to learn.

A.7. Mating and Fertility (Summary - Table D9; Individual Data - Table D19)

Dosages of the test article as high as 5 mg/kg/day did not affect any mating and fertility parameters evaluated. Values for the fertility and pregnancy indices (number of pregnancies per number of rats that mated and rats in cohabitation, respectively), the number of days to inseminate, the number of rats that mated and the number of rats with confirmed mating dates during the first week of cohabitation were comparable among the three dosage groups. Of the male rats assigned to cohabitation, 80.0%, 88.0% and 92.0% impregnated the cohort female rat.

A.8. Necropsy Observations (Summary - Table D10; Individual Data - Table D20)

All necropsy observations in the F1 generation male rats were considered unrelated to the test article because: 1) the incidences were not dosage-dependent; and/or 2) the observation occurred in only one rat in a group.

These observations included one control rat (12080) with small and flaccid left testis and epididymis and one 1 mg/kg/day dosage group male rat (12123) with a small left epididymis. Control group rat 12080 sired a litter. Rat 12123 in the 1 mg/kg/day dosage group did not mate, however this rat had clinical observations that suggested an intubation injury occurred two days before cohabitation, as previously described.

A.9. Terminal Body Weights and Organ Weights and Ratios (%) of Organ Weight to Terminal Body Weight (Summaries - Tables D11 and D12; Individual Data - Table D21)

Terminal body weights were significantly reduced ($p \leq 0.01$) in the 5 mg/kg/day dosage group, as compared to the control group value.

The ratios of the left and right testis weights to the terminal body weight in the 5 mg/kg/day dosage group were significantly increased ($p \leq 0.01$), as compared to the control group values. This significant increase in the relative testis weights reflects the significant reduction in terminal body weights for this dosage group.

The absolute weights of the epididymides, testes, seminal vesicles (with and without fluid) and prostate, and the ratios of the epididymides, seminal vesicles (with and without fluid) and prostate weights to the terminal body weight of the F1 generation male rats were comparable among the three dosage groups.

The mean absolute weight of the left testis in the 1 mg/kg/day dosage group was significantly reduced ($p \leq 0.01$), as compared to the control group value. This significant reduction was not considered an effect of the test article because: 1) the value was not dosage-dependent; and 2) a similar reduction did not occur for the weight of the right testis.

B. F1 Generation Female Rats

B.1. Mortality and Clinical Observations (Summary - Table E1; Individual Data - Table E23)

All F1 generation female rats survived until scheduled sacrifice.

All clinical observations during the precohabitation, gestation and lactation periods were considered unrelated to the test article because: 1) the incidences were not dosage-dependent; and/or 2) the observation occurred in only one to two rats. These clinical observations included portion of tail missing, dental problems (misaligned, missing and/or broken incisors), chromodacryorrhea, swollen and purple ears, localized alopecia on the back, head and/or limbs, ptosis, urine-stained abdominal fur and scab or lesion on the head.

In contrast to the slightly increased incidences of localized alopecia in the EtFOSE treated Fo generation female rats, the total incidence of localized alopecia was significantly reduced ($p \leq 0.01$) during the gestation period in the 1 and 5 mg/kg/day dosage groups, as compared to the control group. This significant reduction was not considered treatment-related because: 1) it was not dosage-dependent; and 2) the incidence of an adverse clinical observation is expected to increase, not decrease, in a toxicology study.

B.2. Maternal Body Weights and Body Weight Changes (Figure 4; Summaries - Tables E2 through E7; Individual Data - Tables E24 through E26)

B.2.a. Precohabitation

The 1 and 5 mg/kg/day dosage groups weighed less than the control group on day 1 postweaning [the reduction was significant ($p \leq 0.01$) in the 5 mg/kg/day dosage group] and body weight gains were generally reduced in these groups throughout the precohabitation period. Body weight gains were reduced in the 1 mg/kg/day dosage group to 86% to 95% of the control group values on days 22 to 29, 36 to 43 and 43 to 50 postweaning and body weight gains were significantly reduced ($p \leq 0.01$) in the 5 mg/kg/day dosage group on days 1 to 8 and 8 to15 postweaning. Reflecting these effects of the test article, body weight gains were significantly reduced ($p \leq 0.05$ and $p \leq 0.01$) in both test article-treated groups for the entire precohabitation period [day 1 postweaning to precohabitation and days 1 to 57 postweaning (significant at 5 mg/kg/day only)].

Absolute body weights were slightly reduced (97% to 98% of the control group value) in the 1 mg/kg/day dosage group throughout the precohabitation period and were significantly reduced ($p \leq 0.05$) on the first day of the cohabitation period. Absolute body weights in the 5 mg/kg/day dosage group were significantly reduced ($p \leq 0.01$) throughout the precohabitation dosage period.

B.2.b. Gestation

Maternal body weight gains during the gestation period were significantly reduced ($p \leq 0.05$ or $p \leq 0.01$) in the 1 and 5 mg/kg/day dosage groups on days 0 to 7 of gestation (DGs 0 to 7), as compared to the control group values. Body

weight gains in the 5 mg/kg/day dosage group were significantly increased ($p \leq 0.01$) on DGs 14 to 17.

Body weights were significantly reduced ($p \leq 0.05$ or $p \leq 0.01$) on DGs 0 through 18 in the 1 and 5 mg/kg/day dosage groups and in the 5 mg/kg/day dosage group only on DGs 19 and 20.

B.2.c. Lactation

Maternal body weights remained significantly reduced ($p \leq 0.05$ or $p \leq 0.01$) in the 5 mg/kg/day dosage group on DLs 1, 4, 7, 10, 14 and 21 and in the 1 mg/kg/day dosage group on DLs 1 and 7.

Maternal body weight gains during the lactation period were significantly reduced ($p \leq 0.05$) in the 1 and 5 mg/kg/day dosage groups on DLs 1 to 21.

B.3. Maternal Absolute (g/day) and Relative (g/kg/day) Feed Consumption Values (Summaries - Tables E8 through E13; Individual Data - Tables E27 through E29)

B.3.a. Precohabitation

The 1 mg/kg/day dosage of the test article was associated with a significant reduction ($p \leq 0.05$) in absolute (g/day) feed consumption values on days 43 to 50 and 50 to 57 of the precohabitation period. Absolute feed consumption values for the 5 mg/kg/day dosage group were significantly reduced ($p \leq 0.01$) on days 1 to 8, 36 to 43, 43 to 50 and 50 to 57 postweaning, as compared to the control group values. Reflecting these effects of the test article, feed consumption values were significantly reduced ($p \leq 0.05$ and $p \leq 0.01$, respectively) for the entire precohabitation period (days 1 to 57 postweaning) in both groups administered the test article.

Relative (g/kg/day) feed consumption values in the 5 mg/kg/day dosage group were significantly increased ($p \leq 0.05$ or $p \leq 0.01$) on days 8 to 15, 15 to 22 and 22 to 29 postweaning, compared to the control group.

B.3.b. Gestation

Absolute feed consumption values in the 1 and 5 mg/kg/day dosage groups were significantly reduced ($p \leq 0.05$) during the first week of the gestation period (DGs 0 to 7), as compared to the control group. Absolute feed consumption values for the 5 mg/kg/day dosage group were also significantly reduced ($p \leq 0.01$) on DGs 7 to 10, as compared to the control group.

The relative feed consumption value on DGs 14 to 17 was significantly reduced ($p \leq 0.05$) in the 5 mg/kg/day dosage group, as compared to the control group value.

B.3.c. Lactation

The absolute maternal feed consumption value was significantly reduced ($p \leq 0.05$ to $p \leq 0.01$) for the entire lactation period (DLs 1 to 14) and at all tabulated intervals during the lactation period in the 5 mg/kg/day dosage group.

The relative feed consumption value was significantly reduced ($p \leq 0.01$) on DLs 4 to 7 and 7 to 10 in the 5 mg/kg/day dosage group, as compared to the control group values.

Absolute and relative feed consumption values during the lactation period were unaffected by the 1 mg/kg/day dosage of the test article.

B.4. Sexual Maturation (Summary - Table E14; Individual Data - Table E30)

The average day of vaginal patency was unaffected by the 1 and 5 mg/kg/day dosages of the test article. The average day of vaginal patency was significantly delayed ($p \leq 0.01$) for the F1 generation female rats in the 1 mg/kg/day dosage group, as compared to the control group value, but the delay was not dosage-dependent and not considered toxicologically important.

B.5. Passive Avoidance Performance (Summary - Table E15; Individual Data - Table E31)

There were no biologically important differences in the values for learning, long-term retention or response inhibition in the F1 generation female rats, as evaluated by performance in a passive avoidance paradigm. No statistically significant differences occurred in the number of trials to criterion or numbers of rats that failed to learn.

The latency of the second trial in the first testing session was significantly increased ($p \leq 0.05$) in the 5 mg/kg/day dosage group, as compared to the control group. This observation was not considered an effect of the test article because a decrease in short-term memory, rather than an increase, would be expected as an indication of toxicity.

B.6. **Watermaze Performance (Summary - Table E16; Individual Data - Table E32)**

No biologically important dosage-dependent differences occurred in watermaze performance of the F1 generation female rats regarding learning, short-term retention, long-term retention or response inhibition. No statistically significant differences occurred in the number of trials to criterion, trial latencies or numbers of rats that failed to learn.

B.7. **Mating and Fertility (Summary - Table E17; Individual Data - Table E33)**

Dosages of the test article as high as 5 mg/kg/day did not affect any mating and fertility parameters evaluated. Values for the number of days in cohabitation, the number of rats that mated, the fertility and pregnancy indices (number of pregnancies per number of rats that mated and rats in cohabitation, respectively), and the number of rats with confirmed mating dates during the first and second week of cohabitation were comparable among the three dosage groups.

B.8. **Necropsy Observations (Summary - Table E18; Individual Data - Table E34)**

The only necropsy observation was moderate dilation of the pelvis of the right kidney in one 1 mg/kg/day dosage group female rat (12239). This observation was considered unrelated to the test article because it was a single occurrence of an observation that commonly occurs in this strain.

B.9. **Natural Delivery and Litter Observations (Summaries - Tables E19 and E20; Individual Data - Tables E35 through E38)**

Pregnancy occurred in 23 (92.0%), 25 (100.0%) and 24 (96.0%) of the 25 female rats in each dosage group assigned to cohabitation in the 0 (Vehicle), 1 and 5 mg/kg/day dosage groups, respectively. All pregnant dams delivered litters. The gestation index (the percentage of pregnant rats with live offspring) was comparable across the three dosage groups.

As occurred in the previous generation, there was a tendency for reduced pup viability in the 5 mg/kg/day dosage group. The number of dams with stillborn pups was significantly increased ($p \leq 0.01$) in this dosage group. The number of pups found dead or presumed cannibalized was significantly increased ($p \leq 0.01$) on DLs 1, 2 to 4 and 8 to 14 and one 5 mg/kg/day dosage group dam (12255) had all pups die by DL 4. As a result, the viability and lactation indices and the average number of surviving pups per litter on days 4 (preculling), 14 and 21 postpartum were significantly reduced ($p \leq 0.05$) in this dosage group.

As also occurred in the previous generation, pup body weights were significantly reduced ($p \leq 0.01$) in the 5 mg/kg/day dosage group on days 1, 4 (pre and postculling), 7, 14 and 21, as compared to the control group values. There was not effect on pup viability or pup body weights in the 1 mg/kg/day dosage group.

Administration of the test article at dosages as high as 5 mg/kg/day did not adversely affect any other parameter evaluated at natural delivery or during the 21-day lactation period (averages for implantations and live litter sizes and pup sex ratios). The duration of gestation was significantly reduced ($p \leq 0.05$) in the 5 mg/kg/day dosage group. This slight reduction was considered unrelated to the test article because the magnitude of the change was small [0.3 day, or 99% of the 0 (Vehicle) and 1 mg/kg/day dosage group values].

B.10. <u>Pup Clinical and Necropsy Observations (Summaries - Tables E21 and E22; Individual Data - Tables E39 and E40)</u>

All clinical and necropsy observations were unrelated to dosages of the test article as high as 5 mg/kg/day because: 1) the incidences were not dosage-dependent; and 2) the observation occurred in only one or two litters. These clinical observations included one pup in the 1 mg/kg/day dosage group and three pups in the 5 mg/kg/day dosage group that were not nursing; nine littermates in the 5 mg/kg/day dosage group that were cold to the touch, one control group pup with exophthalmos and a traumatized cornea; and one control group pup with a portion of the tail black.

No milk in the stomach occurred in 2 (100%), 2 (50.0%) and 13 (52.0%) of the pups that were found dead in the three respective dosage groups. The only necropsy observation at scheduled sacrifice was an absent left eye in one control group pup.

REFERENCES

1. Study Design as Modification of: U.S. Food and Drug Administration (1994). International Conference on Harmonisation; Guideline on detection of toxicity to reproduction for medicinal products. Federal Register, September 22, 1994, Vol. 59, No. 183.

2. U.S. Food and Drug Administration. Good Laboratory Practice Regulations; Final Rule. 21 CFR Part 58.

3. Japanese Ministry of Health and Welfare (1997). Good Laboratory Practice Standard for Safety Studies on Drugs, MHW Ordinance Number 21, March 26, 1997.

4. European Economic Community (1989). Council decision on 28 July 1989 on the acceptance by the European Economic Community of an OECD decision/recommendation on compliance with principles of good laboratory practice. Official Journal of the European Communities: Legislation. 32 (No. L 315; 28 October): 1-17.

5. Christian, M.S. and Voytek, P.E. (1982). In Vivo Reproductive and Mutagenicity Tests. Environmental Protection Agency, Washington, D.C. National Technical Information Service, U.S. Department of Commerce, Springfield, VA 22161.

6. Christian, M.S. (1984). Reproductive toxicity and teratology evaluations of naltrexone (Proceedings of Naltrexone Symposium, New York Academy of Sciences, November 7, 1983), J. Clin. Psychiat. 45(9):7-10.

7. Lang, P.L. (1988). Embryo and Fetal Developmental Toxicity (Teratology) Control Data in the Charles River Crl:CD7BR Rat. Charles River Laboratories, Inc., Wilmington, MA 01887-0630. (Data base provided by Argus Research Laboratories, Inc.)

8. Institute of Laboratory Animal Resources (1996). Guide for the Care and Use of Laboratory Animals. National Academy Press, Washington, D.C.

9. Salewski, E. (1964). Färbemethode zum makroskopischen Nachweis von Implantationsstellen am Uterus der Ratte. Arch. Pathol. Exp. Pharmakol. 247:367.

10. Snedecor, G.W. and Cochran, W.G. (1967). Variance test for homogeneity of the binomial distribution. Statistical Methods, 6th Edition, Iowa State University Press, Ames, pp. 240-241.

11. Sokal, R.R. and Rohlf, F.J. (1969). Bartlett's test of homogeneity of variances. Biometry, W.H. Freeman and Co., San Francisco, pp. 370-371.

12. Snedecor, G.W. and Cochran, W.G. (1967). Analysis of Variance. Statistical Methods, 6th Edition, Iowa State University Press, Ames, pp. 258-275.

13. Dunnett, C.W. (1955). A multiple comparison procedure for comparing several treatments with a control. J. Amer. Stat. Assoc. 50:1096-1121.

14. Sokal, R.R. and Rohlf, F.J. (1969). Kruskal-Wallis Test. Biometry, W.H. Freeman and Co., San Francisco, pp. 388-389.

15. Dunn, O.J. (1964). Multiple comparisons using rank sums. Technometrics 6(3):241-252.

16. Siegel, S. (1956). Nonparametric Statistics for the Behavioral Sciences, McGraw-Hill, New York, pp. 96-104.

17. Zambrama, M.A. and Greenwald, G.S. (1971). Effects of fetal ovarian and placental weight of various number of fetuses in the rat. Biol. of Reprod. 4:216-223.

18. Lochry, E.A., Hoberman, A.M. and Christian, M.S. (1984). Positive correlation of pup body weight with other commonly used developmental landmarks. Teratology 29(2):44A.

F 04

APPENDIX A

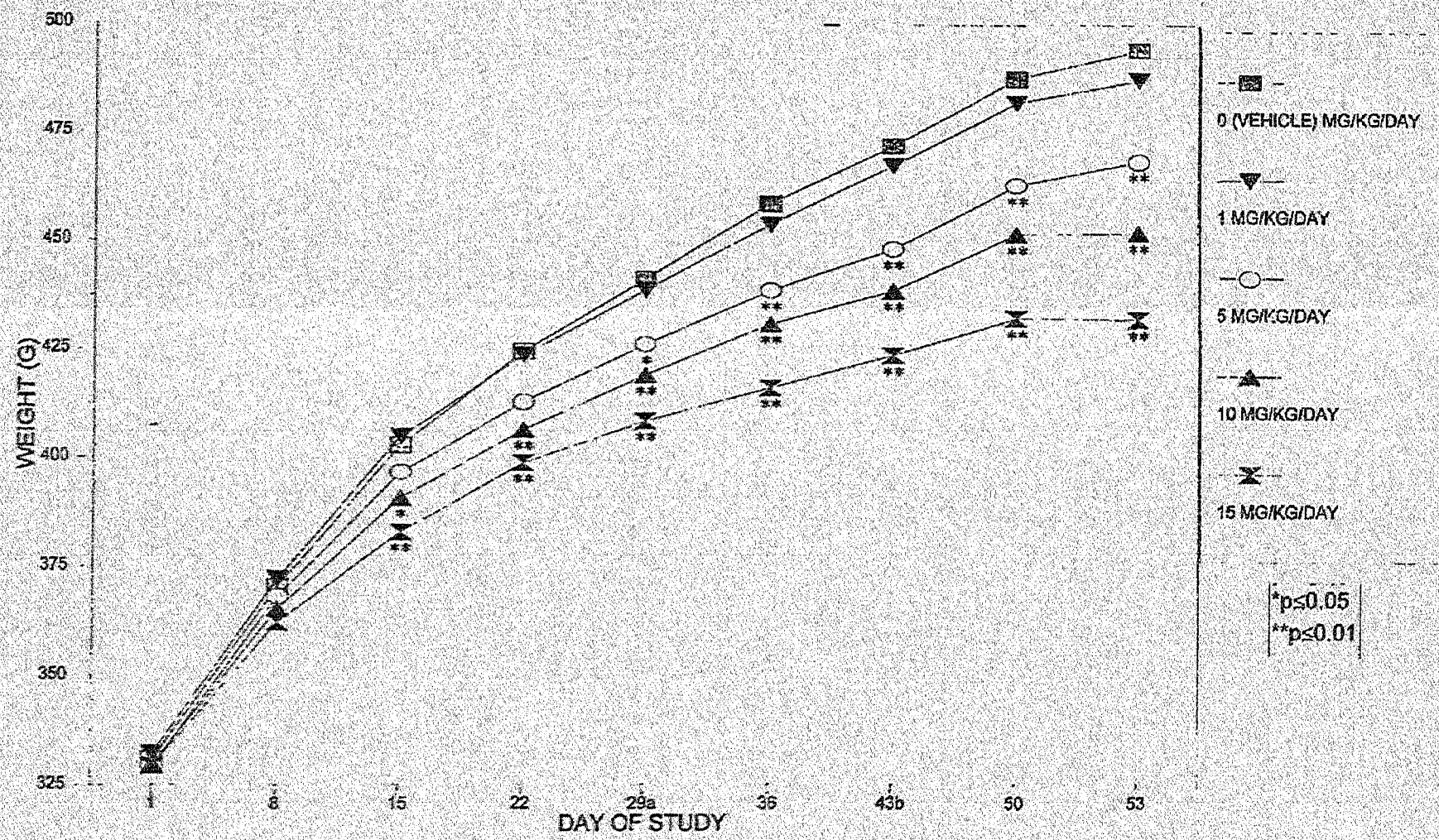
REPORT FIGURES

F 03

BODY WEIGHTS

Fo GENERATION MALE RATS

Figure 1

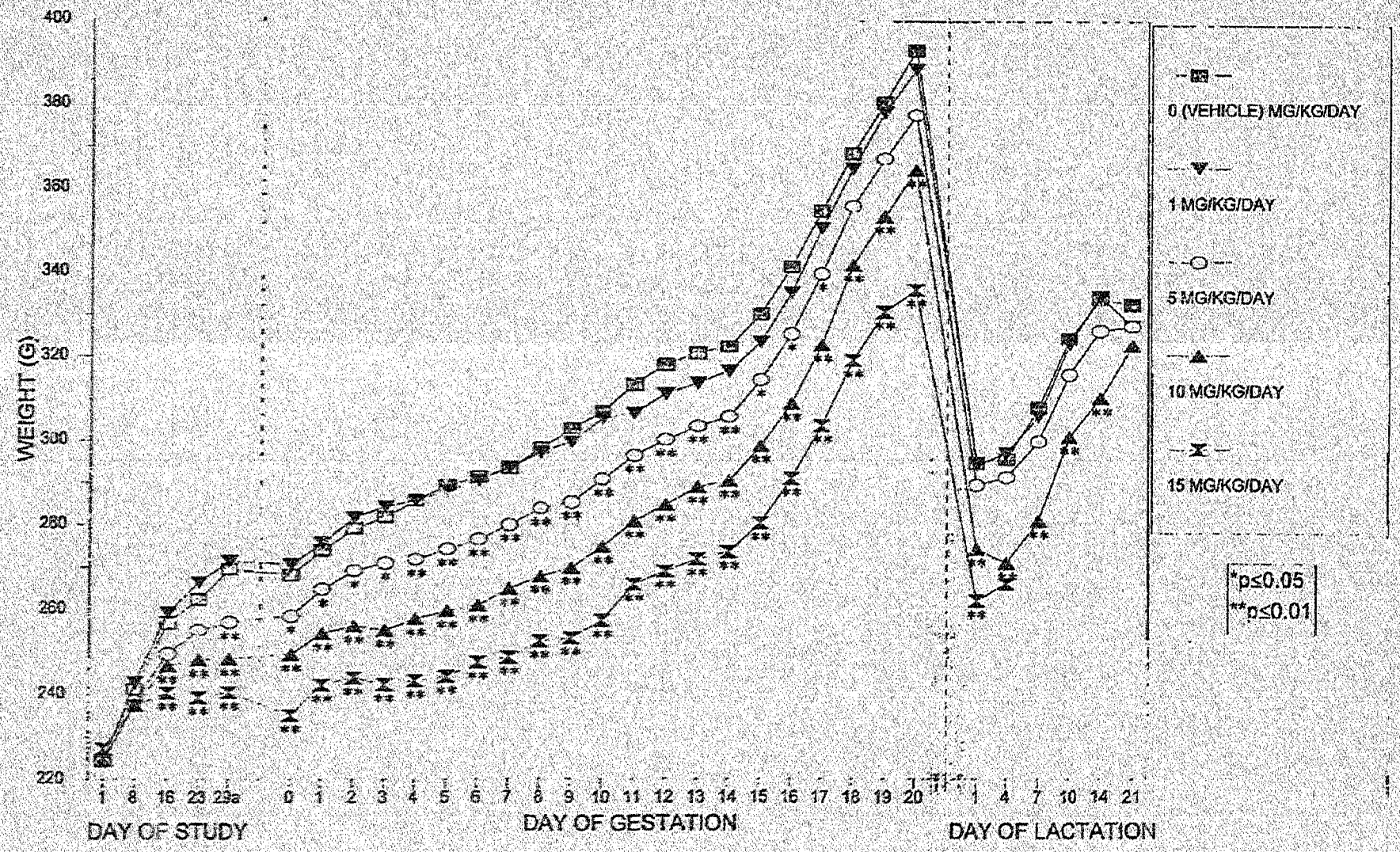


a. Last value recorded before cohabitation.
b. First value recorded after cohabitation.

BODY WEIGHTS

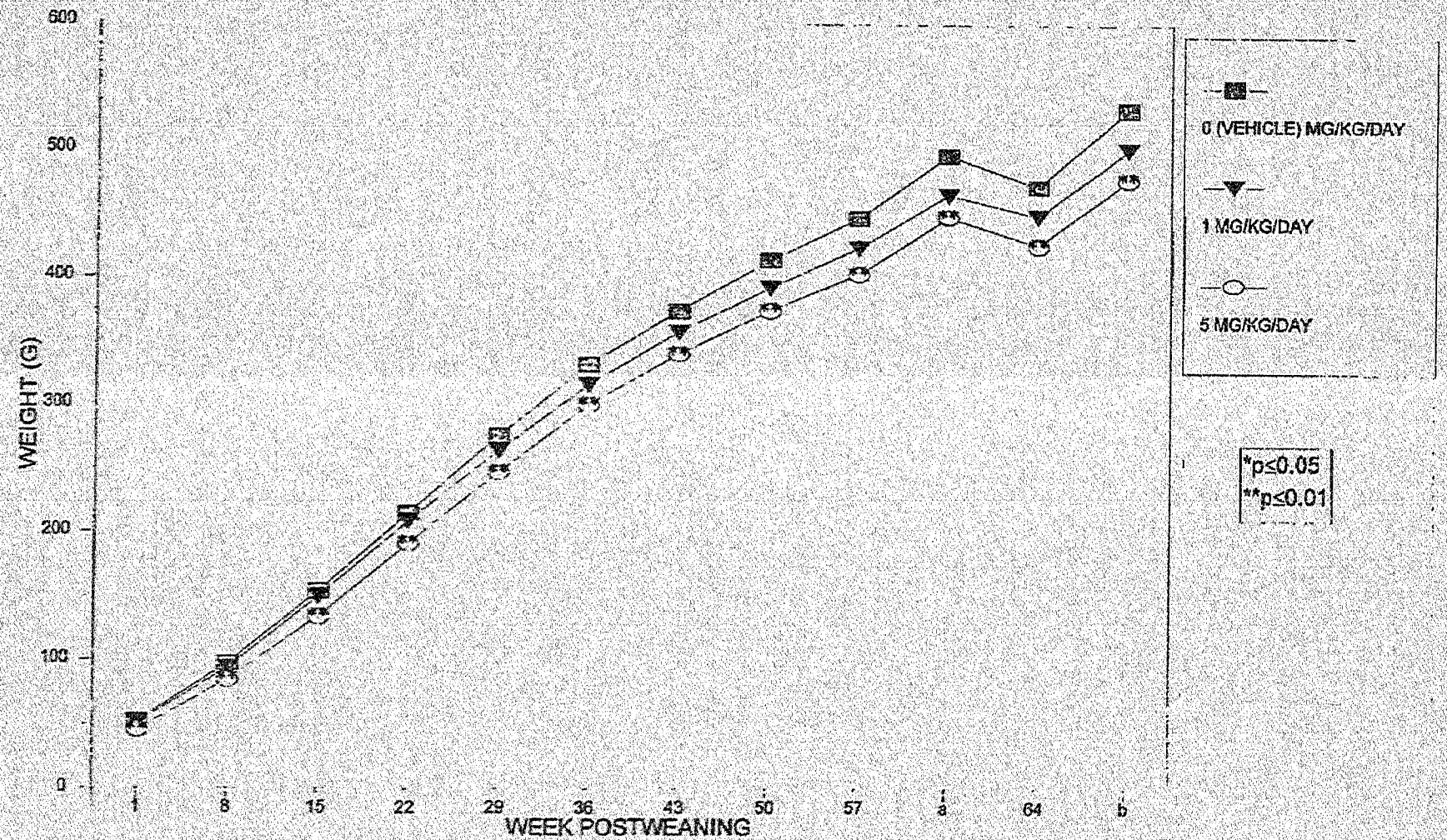
Fo GENERATION FEMALE RATS

Figure 2



a. Last value recorded before cohabitation.

BODY WEIGHTS
F1 GENERATION MALE RATS
Figure 3

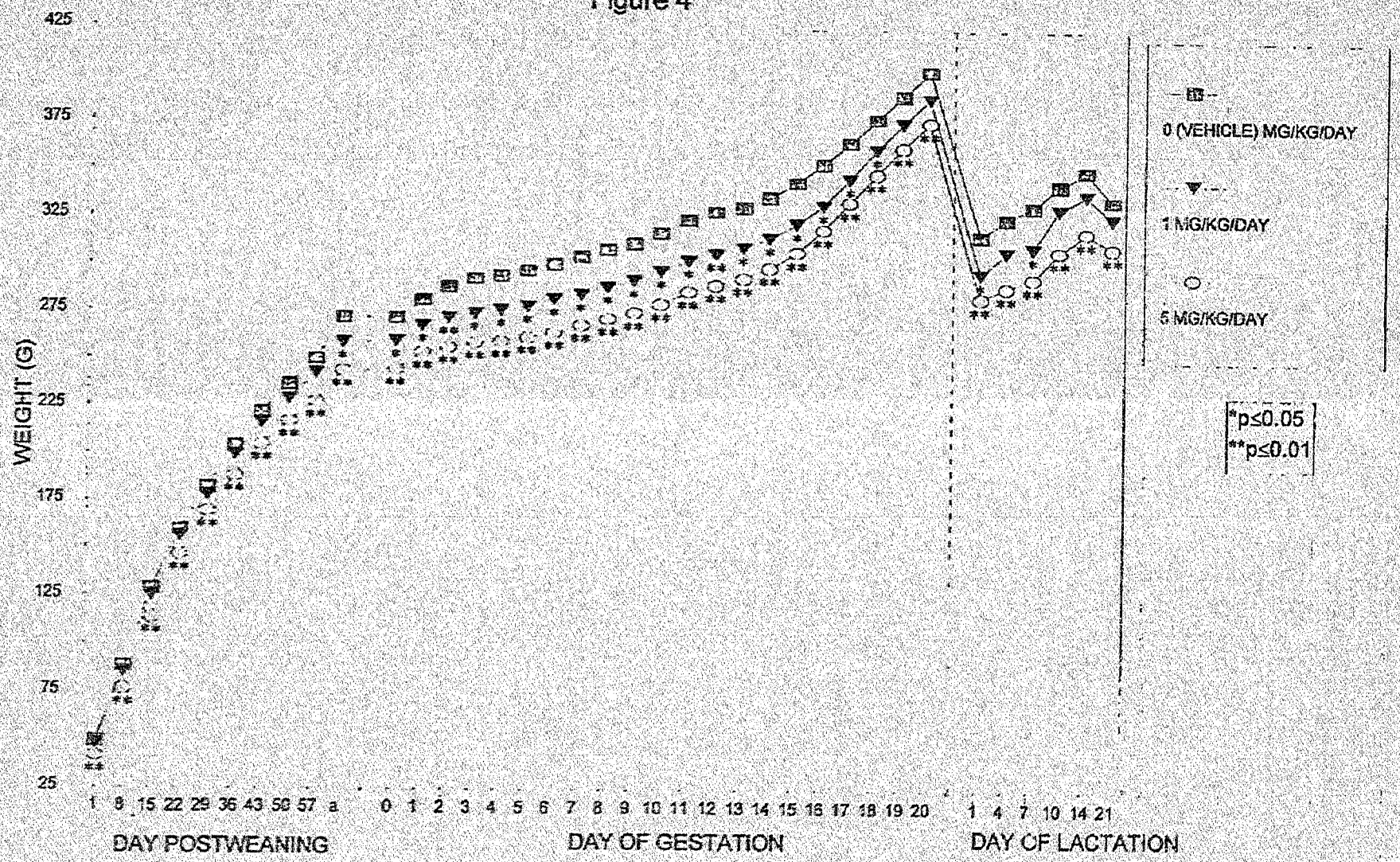


a. Precohabitation body weights.
b. Terminal body weights.

BODY WEIGHTS

F1 GENERATION FEMALE RATS

Figure 4



a. Precohabitation body weight.