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CENTRAL RESEARCH AND DEVELOPMENT DEPARTMENT
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NEWARK, DELAWARE 19711

C-8[1] GAVAGE: EMBRYO-FETAL TOXICITY AND TERATOGENICITY STUDY IN THE RAT

[redacted]

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[1] Octanoic acid, pentadecafluoro-, ammonium salt (ammonium perfluorooctanoate); [redacted]

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QUALITY ASSURANCE DOCUMENTATION

STUDY: [redacted] HLR 1-82 C-8 Gavage: Embryo-Fetal Toxicity and Teratogenicity Study in the Rat

QUALITY ASSURANCE AUDITS

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C-8 GAVAGE:
EMBRYO-FETAL TOXICITY AND TERATOGENICITY STUDY IN THE RAT

[redacted]

HASKELL LABORATORY REPORT NUMBER 1-82

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[1] individual animal data available upon request

* [redacted]

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C-8 GAVAGE:
EMBRYO-FETAL TOXICITY AND TERATOGENICITY STUDY IN THE RAT

[redacted]

HASKELL LABORATORY REPORT NUMBER 1-82

I. SUMMARY

C-8 [redacted] was administered to rats by gavage at 100 mg/kg body weight/day from Days 6 through 15 of gestation. In the group given C-8, maternal deaths occurred, and, during the dosing period, the surviving dams in the group gained about one-third less body weight than the control dams. A teratogenic response was not demonstrated among the fetuses from the C-8 group after sacrifice on Day 21 of gestation. External, visceral, and skeletal alterations were sought, and the eyes of several fetuses of each litter in both groups were examined stereoscopically and histologically for alterations. The only finding noted that could possibly be C-8 related was an increased incidence of fetuses in the C-8 group with ossification sites on the first lumbar vertebrae versus the incidence in the control group. This difference in incidence was statistically significant only if analyzed by a one-tailed test. Its presence was probably a response to generalized stress evoked by the toxic state of the dams. The postpartum viability, growth rate, and development of the offspring from additional dams given C-8 were not demonstrated to be adversely affected by the C-8 administered. Criteria of development included examination of the pups for external alterations and ophthalmoscopic examination of the eyes of each.

Hence, in this study, C-8 was not demonstrated to represent a unique hazard to the conceptus.

II. INTRODUCTION

A. Background

Du Pont obtains ammonium perfluorooctanoate (C-8) from 3M Company for use in the manufacture of a variety of fluoropolymer dispersions, including some of Du Pont's Teflon® products.

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A. Background (cont)

A study of the embryotoxicity and teratogenic potential of C-8 was requested by [redacted] Polymer Products Department, and by [redacted] Chemicals and Pigments Department, at a meeting held at Haskell Laboratory on June 11, 1981. This request was initiated in response to TSCA, Section 8(e)'s filed by 3M between the last part of 1980 and March 20, 1981 on this and several related chemicals.[1] The possible teratogenic activity relayed to us by 3M included lens changes in the eyes of the near-term offspring of rats exposed to the test chemical by gavage from Days 6 through 15 of gestation. It was not determined whether the lens changes persisted after birth of the offspring.

Inhalation of C-8 was not demonstrated to be teratogenic in the rat after exposure from Days 6 through 15 of gestation (1) even though the concentrations tested included those that were overtly toxic to the dam. No additional adverse effects were noted among similarly exposed dams or their offspring when maintained through weaning.

B. Protocol (Appendix A)

An MR request was sent on July 17, 1981; it was authorized on July 28, 1981. The protocol was issued on July 23, 1981, and it was amended on December 3, 1981.

[redacted]

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C. Purpose

This study was designed to test the teratogenic potential of C-8 in the rat after administration by gavage to determine whether the preliminary findings reported by 3M could be repeated, and, if so, to determine whether the changes seen persist after birth.

III. MATERIALS AND METHODS

The study consisted of two experiments. Experiment I was a teratogenicity study by gavage with the dams sacrificed the day before expected delivery. The dams for Experiment II were dosed as in the first experiment, but were allowed to give birth, and the offspring were maintained till 35 days postpartum.

A. Test Material

1. Physical characteristics - C-8 is a white powder which sublimes at 110°C. Its molecular weight is 431, and its structural formula is [redacted] The purity of the sample used was [redacted] and contaminants present were [redacted]

2. Source - The C-8 sample (CAS Registry Number 3825-26-1) was supplied by the Polymer Products Department. It was assigned Haskell Number 14,045.

B. Animals

The rat was chosen for this test because previous toxicity testing on C-8 was conducted in this species. The Crl:CD®(SD)BR strain was selected because the preliminary teratogenicity test on C-8 conducted by 3M used the Sprague-Dawley (SD) derived rat

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B. Animals (cont)

obtained from the same supplier, and because extensive background information from previous teratogenicity testing at Haskell Laboratory exists on this rat strain.

Female rats about 56 days of age (nulliparous) were received from Charles River Breeding Laboratories, Inc., North Wilmington, Massachusetts. They arrived on July 2, 1981, and weighed 170.0±0.7 g (S.E.M.). Individual weights ranged from 153.7 to 198.5 g. Male rats of the same strain and from the same supplier were used for cohabitation with the females. They ranged in age from one to two weeks older than the females.

Upon arrival at Haskell Laboratory, each female rat was identified by a combination of toe clips and ear slashes and by a cage card bearing its assigned number. The male rats were identified by ear slashes and cage cards. The rats were housed two per cage in suspended, wire-mesh, stainless steel cages. Purina Certified Rodent Chow® 5002, Checkers and water from the Wilmington Suburban Water Corporation (WSWC) were supplied ad libitum. The water was provided by an automatic watering device. A lighting cycle of 12 hr light: 12 hr dark (dark period was from 6:00 P. M. to 6:00 A. M.) was maintained throughout the study. Before mating, the temperature of the animal rooms was maintained at 74±2°F. After mating, the animal room temperature was maintained between 75 and 78°F, and relative humidity was maintained between 40 and 80%.

Since the historical incidence of cataracts or opacities in adult CD rats is about 3% (personal communication with James M. Clinton, V.M.D.; consultant ophthalmologist), all prospective parental rats were examined for these alterations before breeding. The eyes of each rat were dilated with 1% atropine ophthalmic solution and examined in semidarkness by the consultant ophthalmologist using focal illumination, indirect ophthalmoscopy, and, when indicated, slit lamp microscopy. Affected rats were eliminated from the colony before the breeding began.

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C. Experimental Design

An outline of the experimental design is presented below:

Group	Dose	Period of Administration	No. Mated Females/Group (Experiment) I	No. Mated Females/Group (Experiment) II	Day Offspring Sacrificed (Experiment) I	Day Offspring Sacrificed (Experiment) II
Control	5 mL corn oil/kg/day	Days 6-15 G[a]	25	12	Day 21 G	Day 35 PP[b]
C-8	100 mg/kg/day in 5 mL corn oil/kg/day	Days 6-15 G	25	12	Day 21 G	Day 35 PP

Breeding Lots	No. Mated Females	Day 1 G	Day 21 G	Expected Day 1 PP	Expected Day 22 PP	Expected Day 35 PP
A	10	7/14/81	8/3/81	8/4/81	8/25/81	9/7/81
B	22	7/15/81	8/4/81	8/5/81	8/26/81	9/8/81
C	21	7/16/81	8/5/81	8/6/81	8/27/81	9/9/81
D	21	7/17/81	8/6/81	8/7/81	8/28/81	9/10/81

a - G = gestation day(s); Day 1 G = day sperm detected in vaginal lavage

b - PP = postpartum day(s); Day 1 PP = day delivery detected (9:00 A. M. - 9:00 A. M.)

C. Experimental Design (cont)

The female rats were quarantined for 11 days after arrival at Haskell Laboratory and then they were mated on an as-needed basis.

After the necessary number of females were bred, the mated females were ranked by body weight and assigned to groups by rotation in order of rank. The exposure group that the first animal was assigned to was selected randomly.

In a preliminary study conducted by 3M, the C-8 was given by gavage with corn oil as the vehicle. Hence, for the current study, the same route and vehicle was used. Stripped corn oil[1] was purchased in 400-g cans from Eastman Kodak Company, Rochester, New York. Most of the tocopherols present in the refined corn oil had been removed by stripping off the most volatile fraction by molecular distillation.

The amount of C-8 required was removed from a sealed plastic bag, which was contained in a Fiber-Pak® carton. The C-8 removed was ground manually with a mortar and pestle, and was placed in a sealed glass jar, which was retained at room temperature. C-8 is known to be stable under these conditions. During the dosing period (Days 6-15 G), suspensions of C-8 in corn oil were prepared daily such that 100 mg C-8/kg body weight could be delivered by gavage in 5 mL of suspension/kg body weight. The body weight most recently recorded was used to calculate the dose to be given to each dam. The dams were dosed between 1:30 and 3:30 P. M. daily. A sample of the suspension remaining after completion of each day's dosing was stored at about 4°C for possible analysis of concentration and of uniformity of mixture. The control group in each experiment received 5 mL stripped corn oil/kg body weight for the same period of gestation. To minimize

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[1] CAS Registry Number 8001-30-7; Item 13266, Lot No. D 4-45

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C. Experimental Design (cont)

oxidation during the dosing period, the corn oil from opened cans was stored at about 4°C in red bottles with ground glass stoppers. This practice previously was shown to limit peroxide concentration to <25 ppm after storage for one year(2). Aflatoxin concentration contained in stripped corn oil received previously from the same source (Lot D 4-25) was determined to be <2 ppb(2).

In a pretest, two non-pregnant female rats were administered C-8 by gavage at 150 mg/kg/day which was the highest dose used in the preliminary study for 3M. The first rat, which weighed 278 g, showed severe clinical signs of toxicity by the fourth day and was found dead on the morning of the fifth day by which time it had lost about 40 g body weight. The second rat, which weighed 260 g, lost about 11 g by the third day. Two additional non-pregnant female rats were then given C-8 at 100 mg/kg/day, the second highest dose level used for the 3M study. After five days of dosing, adverse clinical signs were not noted in one rat that lost about 6 g and were minimal in the other which lost about 14 g. On this basis, the 100 mg/kg/day dosage level was judged to be the maximum that the dams could tolerate for the planned exposure period of ten days.

For Experiment I, the dams were weighed on the day of arrival, before breeding, and on the morning of Days 1, 6, 9, 13, 16, and 21 G. They were observed for clinical signs and changes in demeanor upon arrival at Haskell Laboratory, at breeding, and daily from Days 6-21 G. After mating, the rats were housed individually in suspended, wire-mesh, stainless steel cages. Feed consumption was measured during gestation.

To prevent bias in the examination of maternal and fetal specimens, the dams were coded from just before sacrifice till all maternal and fetal data

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C. Experimental Design (cont)

were collected, and till all structural alterations noted among the fetuses were classified.

After sacrifice of the dams by cervical dislocation on Day 21 G, gross pathologic changes were sought, liver weight was recorded, and reproductive status was determined. The number of corpora lutea and implantation sites were counted, and the number and position of all live, dead, and resorbed fetuses were recorded. The uterus of each apparently "non-pregnant" rat was stained with ammonium sulfide to detect very early resorptions; data collected were used only to determine the incidence of pregnancy. The weight of the intact and empty uterus for each dam was recorded to allow calculation of actual maternal gain in body weight.

All live and dead fetuses were weighed and sexed externally and internally, and the live fetuses were examined at a magnification of 2.5X (Ednalite®) for external alterations. The Ednalite® also was used to count the corpora lutea.

About one-half of the fetuses of each litter that were alive when removed from the dam were examined for visceral alterations(3); in addition, all stunted or malformed fetuses also were examined similarly. The heads of all fetuses examined for visceral alterations and sufficient of the remainder to total two-thirds of each litter were fixed in Bouin's solution. Two of the fetal heads of each litter that were fixed in Bouin's solution were sliced in vertical cross-section in front of the eyes, through the center of the eyes, and through the widest portion of the head(4). The remainder that were fixed in Bouin's solution were sectioned immediately in front of and behind the eyes (rather than through the eyes) and through the widest portion of the head. Three of the fetal heads of each litter that were fixed in Bouin's solution, but not cut through the eyes, were processed, and the eyes were examined histologically via light

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C. Experimental Design (cont)

microscopy by a pathologist. The histologic specimens were coded for this examination, and particular emphasis was placed upon the structural integrity of the lens.

All fetuses, except for the heads of those that were fixed in Bouin's solution, were fixed in 70% ethanol, eviscerated (if not done previously), macerated in 1% aqueous KOH solution, and stained with alizarin red S to permit examination for skeletal alterations. On an as-indicated basis at sacrifice, some tissues were fixed in Bouin's solution for storage or for histologic evaluation. The identity of each fetus was retained at least till the report was written.

For Experiment II, the procedures used till Day 21 G were the same as for Experiment I, except that the dams were weighed on Days 1, 6, and 21 G and twice between Days 9 and 16 G, feed consumption was not measured, and the identity of each offspring within litters was not retained. At least two days before expected parturition, each dam was housed in a 13" X 15" polycarbonate cage outfitted with a water bottle and a wire-mesh lid. The bedding (Bed-O-Cobs®; 1/4" size) was changed weekly following the seventh day postpartum (Day 7 PP). The date of parturition was noted, and it was termed Day 1 PP. Each parturition day was considered to begin at 9:00 A. M. The dams were weighed and examined for clinical signs on Days 1, 7, 14, and 22 PP. For each test group, a Fertility Index (% matings resulting in pregnancy) and a Gestation Index (% pregnant resulting in live births) were calculated, and for each litter, a Viability Index (% animals born that survived to Day 4 PP and a Lactation Index (% animals alive at four days that survived to Day 22 PP) were calculated. All dams were sacrificed on Day 23 PP.

The pups from each dam were counted, weighed, and examined for external alterations toward the end of Day 1 PP. Pups with adverse signs were

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C. Experimental Design (cont)

marked for subsequent identification. Thereafter, each pup was weighed and inspected for adverse clinical signs on Days 4, 7, 14, and 22 PP. Neither standardization of litters nor cross-fostering was practiced. The eyes of the pups in both groups were examined on September 3, 1981 (between Days 27 and 31 PP). This examination was conducted with the exposure levels coded. The eyes were first dilated with atropine and then examined in a semidark room by focal illumination, indirect ophthalmoscopy, and, when indicated, by slitlamp microscopy. At sacrifice on Day 35 PP, each pup was exsanguinated after decapitation, and its eyes were removed and fixed in Bouin's solution.

D. Statistical Evaluation

The litter was used as the experimental unit for the purpose of statistical evaluation(5). The significance of differences in the incidence of pregnancy, clinical signs and maternal death was determined by use of Fisher's exact probability test(6). A two-way analysis of variance was used to detect differences in feed consumption among breeding lots and between groups. Dunnett's test(7) was used to test the statistical significance of differences between the control and C-8 group in maternal body weight, in body weight gain, and in feed consumption when the one-way analysis of variance was significant. The significance of differences in incidence of structural alterations between the control group and the C-8 group was determined by application of the Mann-Whitney U test(8). When more than 75% ties occurred in the data, the Fisher's exact probability test was applied(9). The level of significance selected was $p \leq 0.05$.

In addition, several reproductive indices were calculated for some results from Experiment II.

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IV. RESULTS

A. Eye Examination of Prospective Parents

The eyes of the male and female rats were examined on July 7, 1981; four males and four females were removed from the colony because ocular lesions were identified (Attachment 1).

B. Analysis of C-8 Suspensions

Five of the 14 suspensions of C-8 in corn oil prepared during the study were analyzed for fluoride ion content (Attachment 2). Calculations based upon the fluoride ion measurement indicated that the C-8 content of individual suspensions ranged from 2.04 to 3.14%; a C-8 content of 2.13% was expected.

C. Maternal Clinical Signs Observed

In Experiments I and II, six of the 37 dams given C-8 by gavage were either found dead (five) or had to be killed (one) in view of a moribund state before scheduled sacrifice, as opposed to none of the 37 dams given only corn oil. During the dosing period, all but one of the dams that subsequently died had wet perineal areas and were lethargic. Two also had chromodacryorrhea and chromorhinorrhea. Among the remaining dams, four developed alopecia, one had lung noise, and another developed diarrhea. In the control group, the only clinical sign noted was focal alopecia which developed in one dam.

D. Maternal Feed Consumption

Feed consumption was measured only for Experiment I. During the dosing period, the group given C-8 consumed significantly less feed than the control group (Table I). Feed consumption was similar to the control value in the post-exposure period.

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E. Maternal Body Weight Gain

For Experiments I and II, on Day 6 G the mean body weight of the dams to be given C-8 did not differ significantly from the value for the control dams, but in Experiment I, the mean gain in body weight from Days 1-5 G was significantly less in the group designated to be given C-8 than in the control group (Tables II and V). However, from Days 6-15 G, the group receiving C-8 in Experiment I gained about one-third less ($p \leq 0.05$) than the control group, and during the post-treatment period (Days 16-21 G), the body weight gain of the C-8 group significantly exceeded ($p \leq 0.05$) the control value (Table II). In Experiment II, the Day 16 G body weight was not taken; therefore, Days 6-15 G body weight gains were not calculated.

F. Gross Examination of Maternal Organs at Sacrifice

Mean maternal liver weight for the C-8 group was increased versus the control value, but the difference was not statistically significant (Table II). At sacrifice, one of the dams given C-8 was observed to have several red areas on the visceral surface of the median lobe of the liver.

In Experiment II, the dams were not examined for internal alterations, but they were sacrificed on Day 23 PP and discarded.

G. Reproductive Effects and Body Weight of the Offspring

In Experiment I, the maintenance of pregnancy, the incidence of resorptions, and fetal body weight were not adversely affected by C-8 administration (Table II). Similarly, in Experiment II, no adverse effect on reproductive performance or on pup viability or growth was demonstrated (Table V).

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H. Fetal Alterations

No fetal malformations were detected among the fetuses from dams given C-8 (Table III). In the control group, two littermates were malformed; one fetus had small kidneys and the other had multiple malformations. In addition to the malformations listed, these fetuses also had variations which were not listed in Table IV. The fetus with small kidneys also had subcutaneous hematomas, one misaligned sternebra, one or more doubled, dumbbelled, or small vertebral centra, a partially ossified vertebral arch, and oblong ovaries. The multiply-malformed fetus also had subcutaneous petechiae, a hematoma, one misaligned sternebra and another that was partially ossified, one or more doubled, dumbbelled, or partially ossified vertebral centra, a partially ossified supraoccipital bone, pubis, and vertebral arch, and an open eyelid.

The overall "average percent fetuses with variations" per litter did not increase significantly among the dams given C-8 versus those given only corn oil (Table IV). This also was the case for the two components of the total incidence v.i.z. "developmental variations" and "variations due to retarded development." Among the "developmental variations," the incidence of fetuses with ossification sites on the first lumbar vertebral arch was above the control value; the difference in incidence between the two groups was statistically significant only if the one-tailed Mann-Whitney U test was applied. Among the variations listed for the C-8 group, 12 occurred in a single fetus.

Stereoscopic examination of the bisected eyes from two fetal heads per litter (Bouin's fixed) from each group did not reveal malformations or variations (Talbes III and IV). The only eye alteration noted among the fetuses was an eye with a focal area of redness beneath the cornea in a fetus from the C-8 group that was detected during routine examination of the live fetuses for external alterations (Table IV). In addition, histo-morphologic examination of the eyes of three

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H. Fetal Alterations (cont)

fetuses per litter per group did not reveal any pathologic lesions. A postmortem artifact was recognized in the central anucleate portion of the fetal lens which was equally distributed in incidence between the C-8 and control groups (Attachment 3).

I. Pup Alterations

Examination of the neonates obtained in Experiment II did not reveal malformations (Table V). In vivo examination of their eyes between Days 27 and 31 PP revealed alterations only in two of the 266 pups still alive. One pup from the control group had focal retinal degeneration, and one pup from the C-8 group had corneal edema with superficial vascularization in the temporal quadrant (Attachment 4). According to the consultant ophthalmologist, the corneal edema was probably a normal response to healing of a corneal injury.

V. DISCUSSION

C-8 was overtly toxic to some of the dams when administered by gavage to rats from Days 6-15 G at a dose level of 100 mg/kg/day. Several died after developing lethargy and wet perineal areas, but most did not show adverse clinical signs other than for a substantial decrease in feed consumption and in body weight gain during the dosing period. Despite this degree of maternal toxicity, no evidence of C-8 related teratogenicity was detected.

Minimal evidence of embryo-fetal toxicity was detected in the C-8 group. This consisted of about a doubling in the incidence of fetuses with extra ossification sites on the first lumbar vertebral arch (26.0% in the C-8 group versus 14.0% in the concurrent control group. At Haskell Laboratory, from July, 1979 to January, 1982, cumulative

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V. DISCUSSION (CONT)

control incidence of rat fetuses with this alteration was 15.3% (352/2,296 fetuses) with a range among control groups from 5.8 to 23.0%. This alteration is regarded as a normal skeletal variant and not as a malformation(10). The effect is considered to be minimal since its incidence was significantly increased statistically ($p = 0.04$) only if tested by one-tailed analysis, and, therefore, it may represent a spurious finding, since it occurred at a dose level that was toxic to the dams, and since its presence would not likely have any significant adverse effect, if any, on viability, health, or functional processes. In general, its presence signifies that the dam is likely being stressed sufficiently to express the developmental instability inherent in the species(11,12).

This study did not demonstrate C-8 related effects in the eyes of the offspring obtained. Examination of fetal eyes within minutes after sacrifice of each dam revealed one eye with a focal area of redness beneath the cornea. This fetus was from a dam given C-8. Although this eye was not examined further by the time of this writing, based upon previous experience, it is anticipated that the discoloration was due to the presence of blood in the anterior chamber of the eye (hyphemia). This alteration occurred in seven of 7,201 fetuses (0.10%) from six of 720 litters among the control litters of past studies conducted at Haskell Laboratory since March, 1976. Among studies, the incidence ranged from 0 to 1.0%. In the published literature, this type of eye alteration was previously noted to occur spontaneously(13). Subsequent stereoscopic and histomorphologic examination of the eyes of several fetuses per litter did not reveal malformations, developmental variations, or pathologic lesions. Furthermore, ophthalmoscopic examination of the eyes of all live pups between Days 27 and 31 PP did not demonstrate C-8 related alterations. Therefore, at sacrifice of the pups on Day 35 G, all eyes were removed, fixed, and stored, but they were not processed further.

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V. DISCUSSION (CONT)

From Days 1-5 G, the mean maternal body weight gain of the dams designated to be given C-8 was significantly less than that for the control group. This finding was not compound-related, since the dosing period did not begin till Day 6 G, but was probably due to chance alone. In this regard, it is of interest to note that one dam in the control group gained more weight from Days 1-5 G than any other dam in the study. Had this dam not been assigned to the control group, the weight difference between the groups would not have been statistically significant.

VI. CONCLUSION

C-8 was not demonstrated to be teratogenic when administered to rats by gavage from Days 6 through 15 of gestation even at a dose level that was toxic to the dams. The increased incidence of fetuses with ossification sites on the first lumbar vertebra in the C-8 group versus the control group, at most, was indicative of mild embryo-fetal toxicity in response to the maternal toxicity. No adverse postpartum effects were noted among the offspring from dams of the C-8 group that were raised through the weaning period.

Hence, in this study, C-8 was not demonstrated to represent a unique hazard to the conceptus.

VII. ACKNOWLEDGEMENTS

The fluoride ion content of several of the C-8 suspensions in corn oil was measured by the Analytical Chemistry Section, Experimental Station, under the supervision of Charles R. Ginnard; Bruce M. Monroe, Ph.D., served as coordinator. Histologic specimens were prepared by the Pathology Section. Histomorphologic examination of the eyes and other tissues was conducted by William D. Kerns, D.V.M.,M.Sc. The in vivo eye examinations were conducted by James M. Clinton, V.M.D., Consultant in Comparative Ophthalmology. The remainder of the study was conducted by the Teratology Section, Haskell Laboratory.

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TABLE I

FEED CONSUMPTION[a] IN RATS GIVEN C-8 BY GAVAGE FROM DAYS 6-15 OF GESTATION

Experiment I	Control[b] (5 mL corn oil/kg/day)	C-8[c] (100 mg/kg/day)
Pre-exposure period (Days 1-5 G)	21.0±0.59	20.6±0.32
Exposure period (Days 6-15 G)	21.9±0.48	17.2±0.37$^{\phi}$
Post-exposure period (Days 16-20 G)	28.1±0.58	29.0±0.52

a - grams/rat/day ±S.E.M.; values for females without litters were excluded
b - included data from 24 dams
c - included data from 22 dams
ϕ - significantly different from control value by Dunnett's test ($p \leq 0.05$)

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TABLE II

REPRODUCTION AND FETAL DEVELOPMENT
IN RATS GIVEN C-8 BY GAVAGE FROM DAYS 6-15 OF GESTATION

Experiment I	Control (5 mL corn oil/kg/day)	C-8 (100 mg/kg/day)
Females		
No. pregnant[a]/no. mated	25/25	22/25
No. deaths	0	3[b]
No. litters	24[c]	22
Mean no. corpora lutea	16.1±0.55[d]	16.7±0.87
Mean no. implants	13.6±0.57	13.8±0.64
Mean liver weight (g)[e]	15.4±0.34	16.2±0.44
Mean weight gain (g)[e]		
Days 1-5	33.2±1.12	30.0±1.09[ϕ]
Days 6-15	56.7±2.34	38.3±2.89[ϕ]
Days 16-21	72.6±1.31	84.5±3.00[ϕ]
Days 6-21	129.2±2.68	122.8±4.26
Days 6-21C[f]	57.8±2.35	49.8±2.75[ϕ]
Fetal Death		
Mean no. resorptions	0.7±0.16	0.6±0.13
Mean %	7.9±3.94	4.7±1.30
No. litters with total resorption	1	0

C-8 GAVAGE: EMBRYOTOXICITY AND TERATOGENICITY STUDY IN THE RAT
HLR 1-82

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TABLE II (CONT)

REPRODUCTION AND FETAL DEVELOPMENT
IN RATS GIVEN C-8 BY GAVAGE FROM DAYS 6-15 OF GESTATION

Experiment I	Control (5 mL corn oil/kg/day)	C-8 (100 mg/kg/day)
Fetuses		
No. live	322	292
Mean no. live	13.4±0.32	13.3±0.67
No. stunted	0	0
Mean weight (g)	3.8±0.08	4.0±0.05

a - all females had visible sign of pregnancy evident at autopsy
b - one was noted as being dead on Day 11 G, and two more on Day 12 G
c - one female had only two early resorptions in utero on Day 21 G
d - $\bar{x}$±S.E.M.
e - the mated female without a litter was excluded
f - Day 21C body weight denotes the body weight of females excluding the products of conception (i.e., Day 21 corrected body weight)
ϕ - significantly different from control value by Dunnett's test ($p \leq 0.05$)

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TABLE III

FETAL MALFORMATIONS IN RATS GIVEN C-8 BY GAVAGE FROM DAYS 6-15 OF GESTATION

Experiment I	Control (5 mL corn oil/kg/day)	C-8 (100 mg/kg/day)
External Malformations[a]		
No. examined	322/24[b]	292/22
Visceral Malformations		
No. examined	171/24	155/22
Innominate - none	1/1[c]	d
Kidneys - none	1/1[c]	
Kidneys - small	1/1[e]	
Spleen - small	1/1[c]	
Head Malformations[a]		
No. examined	221/24	198/22
Skeletal Malformations		
No. examined	322/24	292/22
Sternebrae - fused	1/1[c]	
Total with Malformations	2/1	
Avg. % Malformed Fetuses per Litter (±S.E.M.)	0.7±0.70	

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TABLE III (CONT)

FETAL MALFORMATIONS IN RATS GIVEN C-8 BY GAVAGE FROM DAYS 6-15 OF GESTATION

a - no malformations were noted
b - fetuses/litters
c - occurred in a single fetus
d - blanks represent zero incidence
e - occurred in a single fetus which was a littermate of the one noted in "c"

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TABLE IV

FETAL VARIATIONS[a] IN RATS GIVEN C-8 BY GAVAGE FROM DAYS 6-15 OF GESTATION

Experiment I	Control (5 mL corn oil/kg/day)	C-8 (100 mg/kg/day)
Developmental Variations		
External		
No. examined	322/24[b]	292/22
Hematoma	15/10	10/8
Petechia	61/19	34/15
Eye - red	c	1/1[d]
Visceral		
No. examined	171/24	155/22
Renal papilla -reduced	1/1[e]	
Renal pelvis -large	1/1[f]	2/1[g]
Pulmonary arteries -common trunk	3/2	9/6
Head[h]		
No. examined	221/24	198/22

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TABLE IV (CONT)

FETAL VARIATIONS[a] IN RATS GIVEN C-8 BY GAVAGE FROM DAYS 6-15 OF GESTATION

Experiment I	Control (5 mL corn oil/kg/day)	C-8 (100 mg/kg/day)
Developmental Variations (cont)		
Skeletal		
No. examined	322/24	292/23
Sternebra		
-misaligned(1)[i]	14/11	6/6
-misaligned(2+)[i]	7/7	9/8
-bipartite	1/1	
Centrum		
-bipartite	3/3	
-dumbbelled	2/2	4/4
Rib		
-extra ossification center	45/16	76/16[†]
-rudimentary	2/2	11/5
-extra		1/1
-thickened	1/1	
-calloused	1/1	1/1
-wavy		2/1[j]

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TABLE IV (CONT)

FETAL VARIATIONS[a] IN RATS GIVEN C-8 BY GAVAGE FROM DAYS 6-15 OF GESTATION

Experiment I	Control (5 mL corn oil/kg/day)	C-8 (100 mg/kg/day)
Developmental Variations (cont)		
Subtotal - Developmental Variations		
-No. Affected	119/22	128/21
-Avg. % Affected Fetuses/Litter (±S.E.M.)	36.3±3.92	43.2±4.53
Variations Due to Retarded Development		
Visceral		
Renal papilla -slightly reduced		1/1[g]
Skeletal		
Sternebra		
-partially ossified	51/13	22/8
-unossified		
Rib -partially ossified		2/2[j]
Ischium -partially ossified	1/1	1/1[j]

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TABLE IV (CONT)

FETAL VARIATIONS[a] IN RATS GIVEN C-8 BY GAVAGE FROM DAYS 6-15 OF GESTATION

Experiment I	Control (5 mL corn oil/kg/day)	C-8 (100 mg/kg/day)
Variations Due to Retarded Development (cont)		
Skeletal (cont)		
Pubis -partially ossified	2/2	2/2[j]
No. examined with heads	101/24	94/22
Skull bones partially ossified		
-parietal	1/1	2/2[j]
-interparietal	4/3	5/5[j]
-supraoccipital	1/1	5/5[j]
-squamosal		2/2[j]
-frontal		1/1[j]
-zygoma	1/1	2/2[j]
Maxilla -partially ossified		1/1[j]
Hyoid		
-partially ossified	3/3	2/2
-unossified	5/4	4/3[j]

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TABLE IV (CONT)

FETAL VARIATIONS[a] IN RATS GIVEN C-8 BY GAVAGE FROM DAYS 6-15 OF GESTATION

Experiment I	Control (5 mL corn oil/kg/day)	C-8 (100 mg/kg/day)
Variations Due to Retarded Development (cont)		
Subtotal - Variations Due to Retarded Development		
-No. Affected	70/14	35/12
-Avg. % Affected Fetuses per Litter (±S.E.M.)	20.6±4.61	10.9±3.08
TOTAL		
No. Fetuses with Variations	154/23	145/21
Avg. % Fetuses with Variations per Litter (±S.E.M.)	46.5±5.18	48.5±4.35

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TABLE IV (CONT)

FETAL VARIATIONS[a] IN RATS GIVEN C-8 BY GAVAGE FROM DAYS 6-15 OF GESTATION

a - does not include those present in malformed fetuses as these fetuses were previously tabulated as being malformed
b - fetuses/litters
c - blanks represent zero incidence
d - blood subsequently noted in the anterior chamber of the eye at histomorphologic examination
e - this fetus weighed 2.89 g; hydroureter was not detected at visceral examination
f - this fetus weighed 3.58 g; hydroureter was not detected at visceral examination
g - one fetus (4.02 g) had the left kidney affected with associated hydroureter; the other, a littermate of the first (4.21 g), had both kidneys affected without associated hydroureter, but a slightly reduced papilla was present on the right side
h - no fetuses with developmental variations of head were detected
i - (1) and (2+) denote 1, or 2 or more sternebrae were misaligned, respectively
j - one fetus (3.26 g) had each of these variations
† - statistically significant difference detected by one-tailed Mann-Whitney U test ($p \leq 0.05$)

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TABLE V

REPRODUCTION AND DEVELOPMENT OF OFFSPRING
IN RATS GIVEN C-8 BY GAVAGE FROM DAYS 6-15 OF GESTATION

Experiment II	Control (5 mL corn oil/kg/day)	C-8 (100 mg/kg/day)
Females		
No. pregnant/no. mated	12/12	9/12
No. deaths	0	3[a]
No. litters	12	9
Fertility Index (%)[b]	100	100
Gestation Index (%)[c]	100	100
Mean maternal weight gain (g)		
Days 1-5 G[d]	29.2±1.74[e]	33.1±2.36
Days 6-21 G	129.2±4.23	117.0±5.00
Days 1 PP-22 PP[f]	9.6±4.28	4.6±8.73
Offspring		
At delivery (Day 1 PP)		
No. litters	12	9
No. live pups/litter	12.8±0.60[e]	12.8±0.55
No. live pups (%)	153(100)	116(99)
No. dead pups (%)	0(0)	1(1)

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TABLE V (CONT)

REPRODUCTION AND DEVELOPMENT OF OFFSPRING
IN RATS GIVEN C-8 BY GAVAGE FROM DAYS 6-15 OF GESTATION

Experiment II	Control (5 mL corn oil/kg/day)	C-8 (100 mg/kg/day)
Offspring (cont)		
After delivery[g]		
No. dead	0	1
No. cannibalized	1	1
No. live (Day 22 PP)	152	114
No. males/females	76/76	48/66
Viability Index (%)[h]	100	99.2
Lactation Index (%)[i]	99.5	99.2
Average body weight (g)		
Day 1 PP	6.9±0.12[f]	6.8±0.17
Day 4 PP	10.4±0.30	10.3±0.34
Day 7 PP	15.1±0.48	15.1±0.69
Day 14 PP	28.7±0.94	28.8±1.42
Day 22 PP	49.5±1.68	49.5±2.15
Alterations		
No. pups examined[j]	152/12[k]	114/9
No. malformed	0	0
No. pups with eyes examined in vivo[l]	152	114
No. with alterations	1[m]	1[n]

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TABLE V (CONT)

REPRODUCTION AND DEVELOPMENT OF OFFSPRING
IN RATS GIVEN C-8 BY GAVAGE FROM DAYS 6-15 OF GESTATION

a - one female found dead on Day 8 G, and another on Day 11 G; the third female was killed on Day 14 G as she was moribund
b - Fertility Index is the percentage of matings resulting in pregnancy; females that did not survive to term were excluded
c - Gestation Index is the percentage of pregnancies resulting in the birth of live litters; females that did not survive to term were excluded
d - G = gestation
e - $\bar{x} \pm$S.E.M.
f - PP = postpartum
g - to Day 22 PP
h - Viability Index is the percentage of animals born that survived to Day 4 PP or longer
i - Lactation Index is the percentage of animals alive on Day 4 PP that survived to Day 22 PP
j - only examined externally as neonates
k - pups/litters
l - all 266 pups that were alive were examined on September 3, 1981 (Days 27-31 PP)
m - the right eye of one male pup had a band of focal retinal degeneration ventral to the disc
n - the left eye of one female pup had corneal edema with superficial vascularization present in the temporal quadrant

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HASKELL LABORATORY FOR TOXICOLOGY
AND INDUSTRIAL MEDICINE
ELKTON ROAD
NEWARK, DELAWARE

PROTOCOL FOR TERATOGENICITY STUDY IN RATS AFTER ADMINISTRATION BY GAVAGE WITH [redacted]

I. BACKGROUND

*

On June 11, 1981, [redacted] orally requested that [redacted] * be tested in response to a * TSCA 8(e) notice from the 3M Company for [redacted] The oral LD50 and ALD estimates for the rat were provided by the Acute Section at Haskell Laboratory on June 12, 1981.

The purpose of this study is to test the teratogenicity of [redacted] in the rat after administration by gavage to determine whether we can repeat the preliminary findings reported by 3M, *

II. MATERIALS AND METHODS

This study consists of two experiments. Experiment I is a teratogenicity study by gavage with the dams sacrificed the day before expected delivery. The dams for Experiment II will be dosed as in the first experiment, but will be allowed to give birth and to raise their offspring to at least 22 days post partum.

* [redacted]

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A. Test Chemical

* received from the Polymer Products Department *

- o [redacted] (C-8)
  [redacted] H-14,045

*

B. Animals

Crl:CD®(SD)BR female rats (nulliparous) 50-60 days old weighing between 170-180 g will be ordered from Charles River Breeding Laboratories, Inc., North Wilmington, Massachusetts. The rats will be quarantined for at least one week after arrival by air-conditioned truck. Upon arrival, each will be assigned a unique identification number that will be written on a cage card and by a combination of ear punches and toe clips. The females will be mated by overnight cohabition to mature males of the same strain and mating will be verified by detection of spermatozoa in the vaginal lavage each morning (Day 1 of gestation).

The rat was selected for this study because 3M reported a positive teratogenic response to C-8 in this species and we intend to confirm this finding. Also, the degree of toxicity of C-8 * was determined in this species at Haskell Laboratory. The Crl:CD®(SD)BR strain was chosen because extensive background teratogenicity data exists at Haskell Laboratory on this rat strain.

Since the historical incidence of eye alterations including cataracts and lens opacities in CD rats is 3% (according to consulting opthalmologist James Clinton) all prospective parents for this study will be screened for these alterations before breeding. During the screening procedure, both eyes will be examined and all affected rats will be eliminated.

* [redacted]

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C. Route of Administration

Administration of the test chemical will be by gavage since this was the route used in the 3M study. The test chemicals will be suspended in stripped corn oil[1] just before being administered each morning from Days 6 through 15 of gestation. The body weight most recently recorded will be used to calculate the dose to be given to each rat. Periodically, samples of each solution will be retained for possible analysis of concentration and uniformity of mixture. A vehicle control group in each experiment will receive 5 ml stripped corn oil/kg body weight for the same period of gestation. To minimize oxidation during the dosage period, the corn oil from opened cans will be stored at 4°C in red bottles stoppered with ground glass tops.

D. Dose Levels

The recommended dosage levels are:

Experiment I

5 ml/kg Stripped corn oil
100 mg/kg C-8

*

Experiment II

5 ml/kg Stripped corn oil
100 mg/kg C-8

The C-8 level is recommended because it is the maximum level that can be tolerated by the dams by gavage as determined by range-finding testing; *

E. Animal Distribution

Before exposure, mated females will be ranked by body weight and assigned to groups by rotation in order of rank. The dose group the first animal is assigned to will be selected randomly. If the distribution process results in statistically significant differences in body weight among groups before

[1] Eastman Kodak, Rochester, NY,

* [redacted]

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exposure, then minimal switching within breeding dates will be used to alleviate the statistically significant differences.

For Experiment I, about 25 mated females will be assigned to the vehicle control group and to the test group receiving C-8. *

About 12 mated females will be assigned to each test group for Experiment II.

F. Husbandry

Upon arrival at Haskell Laboratory, the female rats will be housed two/cage in suspended wire-mesh steel cages. Purina Certified Rodent Chow® #5002, Checkers, and water from Wilmington Suburban Water Corporation (WSWC) will be provided ad libitum.

The potential effects of dietary contaminants were considered and, on the basis of the manufacturer's data contaminant levels are believed to be within acceptable ranges. No other contaminants reasonably anticipated to be present in the feed are expected to interfere with the results of this study. The potential effects of water contaminants reported by WSWC were considered and appear to be within acceptable ranges. To supplement the WSWC data, Haskell Laboratory initiated an analytical program that monitors these and other contaminants reasonably anticipated to be present in its water supply.

G. Records Maintenance

Records for parameters in the protocol will be maintained for each animal. When the study is completed and the final report is issued, the raw data will be forwarded to the Information Section of Haskell Laboratory for archiving.

H. Safety Precautions and Disposal of Waste Material

All personnel will wear flock-lined latex gloves[2] when handling test compounds or mixtures. Kevlar® gloves will be worn when study animals are handled and latex examination gloves will be worn during autopsy. Contaminated waste material will be packaged in polyethylene-lined Fiberpaks® for incineration at Stine Laboratory.

---

[2] Golden Thumb Glove Company, Glove #L-61, 18 mils thick

* [redacted]

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I. Parameters to be Studied

Experiment I - Teratogenicity Study

1. Dams

a. Body weight - weighed on the day of arrival, before breeding, and on the morning of Days 1, 6, 9, 13, 16, and 21 of gestation

b. Feed consumption - the average amount of feed consumed daily per rat for each group will be determined for the pre-exposure, exposure, and post-exposure time periods. The measurements will be taken at the same time each morning.

c. Clinical signs - observed upon arrival, at breeding, and daily at least from Days 6 through 15 of gestation

d. Viscera of dams will be examined immediately after sacrifice by cervical dislocation.

e. Liver weight - absolute weights will be taken and, if indicated, liver weight will be presented relative to the corrected maternal body weight at the time of sacrifice

f. Uterine weight - the intact and empty uterus of each dam having one or more fetuses will be weighed to permit calculation of actual maternal body weight gain during gestation

g. Corpora lutea - counted and recorded for each ovary

h. Implantation sites - counted and recorded for each pregnant rat; the uterus of each apparently "non-pregnant" rat will be stained with ammonium sulfide to detect very early resorptions

i. Resorptions - counted and recorded for each rat (not those detected by stain only)

2. Fetuses

a. Number, location, and condition - recorded for each fetus in each litter

* [redacted]

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b. Fetal weight - recorded for all live fetuses and those classified as "Dead" fetuses

c. External alterations - detected and recorded for all live fetuses.

d. Soft tissue alterations - detected[3] and recorded for the first live fetus and thereafter for every other live fetus of each litter; all stunted fetuses and all live fetuses with external malformations also will be examined for soft tissue alterations.
The heads[4] of all fetuses examined for soft tissue alterations and sufficient of the remainder to total two-thirds of each litter will be fixed in Bouin's solution. Two of the Bouin's fixed heads of each litter will be sliced in vertical cross-section in front of the eyes, through the center of the eyes, and through the widest portion of the head; the remaining Bouins's fixed heads of each litter will be sectioned immediately in front of and behind the eyes, rather than through the eyes to permit processing for histologic evaluation if desired. It is expected that at least three of the Bouins's fixed heads with uncut eyes from each litter of the C-8 and Control groups will be processed and examined histologically by a pathologist. Particular emphasis will be placed on lens structure during examination.

e. Skeletal alterations - detected and recorded for all fetuses (including "Dead" fetuses); the fetal heads that were fixed in Bouin's solution will be excluded

All groups will be coded from just before sacrifice until all raw data are collected.

---

[3] Staples, R. E., Teratology, 9:A37-A38 (1969).

[4] Barrow, M. V., and W. J. Taylor, J. Morph., 127:291-306 (1969).

*[redacted]

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Experiment II - Extended Teratogenicity Study

1. Dams

   a. Body Weight - weighed on the day of arrival, before breeding, Days 1, 6, and 21 of gestation and 1, 7, 14, and 22 days post partum

   b. Clinical signs - observed upon arrival, at breeding, daily from Days 6 through 15 of gestation, and on 1, 7, 14, and 22 days post partum. Adverse effects observed at any other time will be noted.

   c. Date of delivery - noted for each dam

   d. Reproductive indices

      For each test group:

      - o Fertility index (% matings resulting in pregnancy)
      - o Gestation index (% matings resulting in live births)

      For each litter:

      - o Viability index (% animals born that survived 4 days or more)
      - o Lactation index (% animals alive at 4 days that survived to 22 days post partum)

2. Offspring

   a. The number of live pups per litter and the number of dead or cannibalized pups per litter will be recorded on 1, 4, 7, 14, and 22 days post partum.

   b. Sex ratio - recorded for each litter on the date of delivery. The sex ratio of pups alive 22 days post partum also will be presented. The sex of each pup found dead will be recorded.

   c. Body weights - weighed 1, 4, 7, 14, and 22 days post partum

*[redacted]

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d. Clinical signs - observed 1, 4, 7, 14 and 22 days post partum. Pups with adverse signs noted will be marked for subsequent identification within the litter.

e. External alterations - detected and recorded for all live pups

f. Soft tissue and skeletal alterations - pups will not be examined for these types of alterations unless otherwise indicated

g. Eye examinations - the eyes of pups in all groups will be examined by an ophthalmologist shortly after the eyes open. If eye alterations are detected that appear to be compound-related, a second examination may be conducted. The groups will be coded for all examinations. Pups with eye alterations will be marked for identification at sacrifice.

At sacrifice, each pup will be exsanguinated and its eyes will be removed. All eyes will be fixed to permit processing by the Histology group for examination by a pathologist if indicated. The identity of eyes from pups previously marked will be retained. Otherwise, all eyes from pups will be identifiable only by dam number. At least the eyes of two pups from each litter will be processed and examined by a pathologist.

III. STATISTICS

Experiments I and II

The litter will be used as the experimental unit. The Fisher's exact test will be used to determine the significant differences in the incidence of pregnancy, and maternal pup mortality. Jonckheere's test will be used to determine the presence of a dose response. Dunnett's test will be used for testing the significance of differences in maternal body weight and body weight gain. A two-way analysis of variance will be used to detect interaction between breeding lots and test groups. For all other parameters, the Mann-Whitney U test will be applied to detect significant differences between the control group and individual experimental groups. The level of significance will be $p < 0.05$. In addition, the reproductive indices given earlier will be calculated.

*[redacted]

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IV. CRITICAL DATES

Starting Date (breeding): July 13, 1981
Completion: January 18, 1982

*[redacted]

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Prepared by: C. L. Lamontia

C. L. Lamontia - Biologist
Teratology Section
Toxicology and Pathology

R. E. Staples

R. E. Staples - Staff Teratologist
Study Director
Teratology Section
Toxicology and Pathology

Approved by: James G. Aftosmis

J. G. Aftosmis
Associate Director
Toxicology and Pathology

* [redacted]

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HASKELL LABORATORY FOR TOXICOLOGY AND INDUSTRIAL MEDICINE
ELKTON ROAD
NEWARK, DELAWARE

AMENDMENT TO THE
PROTOCOL FOR TERATOGENICITY TESTING IN RATS
AFTER ADMINISTRATION BY GAVAGE WITH [redacted]

[redacted]

The following changes were made after initiation of the study:

Page 8

II. MATERIALS AND METHODS

I. Parameters to be Studied

Experiment II - Extended Teratogenicity Study

2. Offspring

g. Eye examinations

Because no compound-related effects were detected grossly or microscopically in the fetal eyes from Experiment I, the eyes from all offspring in Experiment II were fixed, identified, and retained but none were processed or examined microscopically by a pathologist.

J. Retention of Specimens

All skeletal, head, and selected visceral specimens, as well as histologic preparations, will be retained till issuance of the final report. Thereafter, they will be retained for as long as the quality of the material affords proper evaluation.

*[redacted]

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PREPARED BY: C. L. Lamontia

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APPROVED BY: J. G. Aftosmis

J. G. Aftosmis - Associate Director
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CLL/RES/mle
12/3/81

*[redacted]

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JAMES M. CLINTON, V. M. D.
ANIMAL EYE CLINIC AT SOUTH JERSEY ANIMAL HOSPITAL
ROUTE 541 ABOVE CHURCH ROAD -- P. O. BOX 115
MEDFORD, NEW JERSEY 08055

TELEPHONE (609) 654-0304

Haskell Laboratories
Study C-8
[redacted]

Exam Date:
7 July 1981
Robert Stapels, Ph.D.

Initial Ophthalmoscopic Examination

Both eyes of all of the male and female rats in the colony were examined by focal illumination, indirect ophthalmoscopy and, when indicated, slit-lamp microscopy. Mydriasis was achieved with 1% Atropine (1% Atropisol, Cooper Laboratories, lot B3039, 12/82), and the eyes examined in subdued light. Semi-darkness was maintained until the following morning.

Ocular lesions were identified in female rats 306514, 306518, 306440 and 306448 and in male rats 305275, 305289, 305184 and 304846. They were removed from the colony and euthanatized by technologists S. Vivian and E. Wollenburg.

The remaining male and female rats are ophthalmoscopically normal and suitable for use in the forthcoming study.

James M. Clinton

James M. Clinton, V.M.D.

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Central Research and
Development Department

Copy to: [redacted]

October 20, 1981

MEMORANDUM FOR R. E. STAPLES
CR&D - HASKELL

FROM C. R. PERROTTO

FLUORINE ANALYSIS OF CORN OIL SAMPLES

The corn oil samples, containing the C-8 additive, were analyzed by our standard procedure for organic compounds. Since the samples had two visible layers in them they were taken out of the refrigerator, in which they were stored, and put on a mechanical shaker overnight. An aliquot was taken immediately and weighed into a gelatin capsule.

The samples, in the capsules, were decomposed in an oxyhydrogen flame using the standard Wickbold apparatus(1). Using this technique, the material is vaporized into a stream of oxygen, then led into the oxyhydrogen flame. The vapors condense in a water cooled quartz container, and finally rinsed into an absorption chamber containing a 1.0N NaOH solution.

The fluoride ion content is measured by a thorium nitrate titration of the above solution to a visual end point. Excess thorium forms a red-colored complex with alizarin red indicator. This end point is detected in the presence of methylene blue under illumination from a mercury vapor lamp(2).

A sample of m-carboxybenzotrifluoride is burned and titrated with every batch to check on accuracy. Calculations are as follows:

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$$\%F = \frac{(\text{mls of } Th(NO_3)_4)(K \text{ factor})}{(\text{wt of sample in mg})} \times 100\%$$

The K factor is determined by titrating an aliquot of a standard solution of NaF.

$$K \text{ factor} = \frac{(0.4525)(\text{Mls of aliquot})(\text{conc. of NaF in g/l})}{(\text{mls of } Th(NO_3)_4)}$$

Raw data are recorded in a notebook during the analysis then transferred onto the analytical slip. This slip is stored for twenty years in E228/237. The person who is currently responsible for this is Mrs. D. Ewing. Data recorded includes sample weight, K factor used, dilutions of sample solution, and mls of $Th(NO_3)_4$ used. Also on the analytical slip is sample number, description and designation of sample, submitter's name and location, dates received and completed, and initials of the person who performed the analysis and supervising chemist.

The laboratory technician who did the analysis was M. P. Balaguer and the supervising chemist was Carol R. Perrotto. Results of the analysis are given below.

CR Number	Designation	Results (%F)
81-9263	1	1.63
81-9264	4	2.12
81-9265	7	2.37
81-9266	10	0.79*
81-9267	13	1.80
81-9268	14	0.46**
81-10554	10	1.84,1.79
81-10555	14	0.29,0.26

*Sample would not mix, so second analysis was performed in duplicate with special attention to sampling.
**Sample was too small for accurate analysis and was repeated in duplicate with larger sample size.

If you need more information, my phone number is 772-2339.

C.R Perrotto

dre

1) Bock, Rudolf; "Decomposition Methods in Analytical Chemistry"; p. 185-6, International Textbook Co. Ltd.; London, 1979
2) Williams, W. John; "Handbook of Anion Determination; p. 349-350; Butterworth & Co. (Publishers) Inc.; Boston, 1979

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cc: [redacted]

HASKELL LABORATORY

October 5, 1981

MEMORANDUM

TO: R. E. Staples

FROM: W. D. Kerns WDK

RE.: [MR-4130]

Fetal lenses from this gavage study (Table 1) were evaluated for the presence of microscopic lesions. The evaluations were completed without knowledge of group assignments or dose.

There were no pathological lesions in any of these fetal eyes. A peculiar postmortem artifact was recognized and it was equally distributed among the high-dose and control groups. This alteration was present in the central anucleate portion of the fetal lens. In this area, the lens fibers were more eosinophilic and there was separation from the anterior lens capsule in some cases. In others, the lens material in this area had been fractured by the microtome.

*

All microscopic lens alterations seen thus far are interpreted as postmortem artifact and cannot be associated with test agent administration.

* [redacted]

WDK:ljm

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TABLE 1

Dam Code	Fetus Number	Dam Code	Fetus Number
2	1, 5, 9	34	3, 7, 12
4	3, 5, 9	37	3, 7, 9
5	3, 4, 7	38	1, 8, 11
6	1, 8, 13	39	1, 8, 11
7	1, 5, 11	40	5, 11, 15
8	1, 5, 11	41	3, 8, 17
9	3, 7, 13	42	1, 7, 12
10	4, 7, 12	43	5, 11, 15
14	5, 8, 11	45	4, 13, 15
15	3, 5, 15	46	3
17 /	5, 9, 15	49	1, 9, 12
18	4, 9, 15	50	4, 9, 11
19	1, 8, 13	51	1, 7, 13
21	3, 5, 7	53	5, 9, 11
22	1, 4, 11	55	1, 5, 12
23	1, 5, 9	57	1, 9, 13
24	4, 7, 11	59	3, 9, 13
25	1, 7, 11	60	5, 9, 10
26	5, 9, 13	61	4, 9, 15
29	4, 9, 15	62	3, 11, 13
30	1, 11, 15	64	3, 7, 13
32	5, 7, 9	65	1, 7, 11
33	3, 8, 13	66	4, 7, 11

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TABLE 2

*

*

Company Sanitized. Does not contain TSCA CBI

TABLE 3

*

*[ ]

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JAMES M. CLINTON, V. M. D.
ANIMAL EYE CLINIC AT SOUTH JERSEY ANIMAL HOSPITAL
ROUTE 541 ABOVE CHURCH ROAD -- P. O. BOX 115
MEDFORD, NEW JERSEY 08055

TELEPHONE (609) 654-0304

Exam Date:
3 September 1981

Haskell Laboratories
Study: C-8 [redacted]
Robert Staples, Ph.D.

Ophthalmoscopic Examination
Post-partum Day 31-35

Both eyes of 266 rats were examined by focal illumination, indirect ophthalmoscopy and, when indicated, slit-lamp microscopy. Mydriasis was produced with 1% atropine solution (Atropisol 1%, Cooper Laboratories, lot B3039, 12/82) and the eyes examined in subdued light. Semi-darkness was maintained until the following day. The dose level identifications were not disclosed to me until after my examinations.

The summary follows:

Dam No.	No. in Litter	Observations
306333	12	No ocular lesions.
306378	16	No ocular lesions.
306388	12	No ocular lesions.
306431	10	No ocular lesions.
306453	11	No ocular lesions.
306533	15	In the right eye of one male, a focal band of retinal degeneration was present ventral to the disc.
306437	12	No ocular lesions.
306504	10	No ocular lesions.
306545	12	No ocular lesions.
306337	14	No ocular lesions.
306345	13	No ocular lesions.
306552	15	No ocular lesions.
306359	15	No ocular lesions.
306364	11	No ocular lesions.
306343	13	No ocular lesions.
306418	13	No ocular lesions.
306349	12	No ocular lesions.
306403	11	No ocular lesions.
306339	11	No ocular lesions.
306450	14	In the left eye of one female, corneal edema with superficial vascularization was present in temporal quadrant.
306527	14	No ocular lesions.

James M. Clinton

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Exam Date:
3 September 1981

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Robert Staples, Ph.D.

Comments

With the exception of the 2 rats described above, both eyes of all rats were ophthalmoscopically normal. When the rats presenting with ocular lesions were identified, they were marked with gentian violet. Focal retinal degeneration is a spontaneously occurring lesion characterized by sharply demarcated bands in which the outer retinal layers are most involved. The cause is unknown. The corneal edema noted in the female is most likely to be a normal response to healing of a corneal injury. Both lesions are commonly found in well managed rat colonies.

In my opinion, the test materials, as evaluated in this study, do not produce ocular changes in Rattus norvegicus.

James M. Clinton

James M. Clinton, V.M.D.

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