C-8 ALTERNATES: MATERNAL AND DEVELOPMENTAL
TOXICITY IN THE RAT (PRELIMINARY STUDY, BY GAVAGE)

[redacted]

HASKELL LABORATORY REPORT NUMBER 441-84

Copies to: [redacted]

Company Sanitized. Does not contain TSCA CBI

FOR DU PONT USE ONLY

E. I. DU PONT DE NEMOURS & CO., INC.
HASKELL LABORATORY FOR TOXICOLOGY AND INDUSTRIAL MEDICINE
CENTRAL RESEARCH AND DEVELOPMENT DEPARTMENT
NEWARK, DELAWARE 19714

C-8 ALTERNATES[1]: MATERNAL AND DEVELOPMENTAL TOXICITY IN THE RAT (PRELIMINARY STUDY, BY GAVAGE)

[redacted]

HASKELL LABORATORY REPORT NUMBER 441-84

Dates: Initiation (breeding date) - July 13, 1981
Completion (sacrifice date) - August 19, 1981

Notebook Numbers: [redacted]

Date Written: September 26, 1984
Date Issued: October 2, 1984
No. pages in this report: 72

---

1 Du Pont Classified Information [redacted]

Company Sanitized. Does not contain TSCA (

C-8 ALTERNATES: MATERNAL AND
DEVELOPMENTAL TOXICITY IN THE RAT
(PRELIMINARY STUDY, BY GAVAGE)
[redacted]
HLR 441-84

Report by: Robert E Staples

R. E. Staples, Ph.D.
Study Director
Staff Teratologist
Haskell Laboratory

Approved by: James G. Aftosmis

J. G. Aftosmis, D.V.M.
Associate Director
Haskell Laboratory

RES:jy:MR4130.2

Company Sanitized. Does not contain TSCA CBI

C-8 ALTERNATES: MATERNAL AND DEVELOPMENTAL TOXICITY IN THE RAT (PRELIMINARY STUDY, BY GAVAGE)

[redacted]

HASKELL LABORATORY REPORT NUMBER 441-84

TABLE OF CONTENTS

		Page
Title Page		1
Signature Page		2
Table of Contents		3
I.	SUMMARY	6
II.	INTRODUCTION	6
	A. Background	6
	B. Protocol	7
III.	MATERIALS AND METHODS	7
	A. Test Chemicals	7
	B. Animals and Husbandry	8
	C. Dose Selection	9
	D. Animal Breeding and Distribution	10
	E. Test Chemical Administration	10
	F. Maternal and Fetal Examination and Sacrifice	11
	G. Statistical Tests	12
IV.	RESULTS	12
	A. Eye Examination of Prospective Parents	12
	B. Maternal Clinical Signs Observed	12
	C. Maternal Feed Consumption	13
	D. Maternal Body Weight Gain	14
	E. Gross Examination of Maternal Organs at Sacrifice	14
	F. Reproductive Effects and Fetal Body Weight	14
	G. Fetal Alterations	14
V.	DISCUSSION	15
VI.	CONCLUSION	16
VII.	ACKNOWLEDGEMENTS	16
VIII.	REFERENCES	17

Company Sanitized. Does not contain TSCA CBI

TABLE OF CONTENTS (CONT.)

Page

TABLES

I. Feed Consumption in Rats Given Test Chemicals by Gavage from Days 6-15 of Gestation 18

II. Reproduction and Fetal Development in Rats Given Test Chemicals by Gavage from Days 6-15 of Gestation 19

III. Fetal Malformations in Rats Given Test Chemicals by Gavage from Days 6-15 of Gestation 21

IV. Fetal Variations in Rats Given Test Chemicals by Gavage from Days 6-15 of Gestation 22

APPENDIX

A. Protocol for Maternal and Developmental Toxicity Study in Rats after Administration by Gavage with C-8 Alternates* 28

Amendment to the Protocol. 39

ATTACHMENTS

1. *Letter, from J. R. Barnes to R. E. Staples, 3/20/81 42

2. Letter, from J. M. Clinton to R. E. Staples, 7/7/81 54

3. Memorandum, from W. D. Kerns to R. E. Staples, 8/19/81 55

4. Memorandum, from W. D. Kerns to R. E. Staples, 1/29/82 56

---

* Du Pont classified information

Company Sanitized. Does not contain TSCA CBI

TABLE OF CONTENTS (CONT.)

Page

ATTACHMENTS (CONT.)

5. Memorandum, from W. D. Kerns to R. E. Staples, 10/5/81 including Memorandum from C. L. Lamontia to N. L. Chromey, 10/1/81, and the code for rats assigned to this study. 57

6. Memorandum, from W. D. Kerns to R. E. Staples, 12/18/81. . . . 67

7. Memorandum, from R. E. Staples to Teratology Section File, 10/30/81 71

8. Tabulation of Results of Dose Selection Based Upon Comparison of LD50 or ALD Values 72

Company Sanitized. Does not contain TSCA CBI

C-8 ALTERNATES: MATERNAL AND DEVELOPMENTAL TOXICITY IN THE RAT (PRELIMINARY STUDY, BY GAVAGE)
[redacted]
HLR 441-84

I. SUMMARY

[redacted] were administered to rats by gavage at 100, 500, 250, or 500 mg/kg body weight/day, respectively, from Days 6 through 15 of gestation to determine the degree of hazard to the developing conceptus. The dosage of [redacted] given was lethal to six of the seven rats tested, and the rat that survived did not yield live fetuses at term. Therefore, its potential hazard to the conceptus was not assessed. The remaining chemicals were tested at levels that were less toxic to the dams. None of the test chemicals were demonstrated to represent a unique hazard to the maintenance of pregnancy or to the developing 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. 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.

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.

[1] [redacted]

Company Sanitized. Does not contain TSCA CBI

A. Background (Cont.)

In addition, a report (2) on the C-8 portion of the current study was issued on January 14, 1982. The current report presents preliminary data on the maternal and developmental toxicity of four similar chemicals that were collected concurrently with the C-8 data.

The request for a proposal to conduct a maternal and developmental toxicity study on [redacted] was received from [redacted] (Haskell Coordinator for PPD), on March 23, 1981 (Attachment 1). On June 11, 1981, [redacted] orally requested that the developmental toxicity of [redacted] also be tested in a pilot study for comparison to C-8.

B. Protocol (Appendix A)

An MR request was sent to PPD 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.

III. MATERIALS AND METHODS

A. Test Chemicals*

1. 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] Its CAS Registry Number is [redacted] and it was assigned Haskell Number 14,045. The sample was received from the Polymer Products Department.

2. [redacted] is a white solid with greater than 10% solubility in water. Its structural formula is [redacted] Its molecular weight is 463, its CAS Registry Number is [redacted] and the Haskell Number assigned is 14,075. The sample was received from [redacted] PP&R, Washington Works.

---

* Du Pont classified information

Company Sanitized. Does not contain TSCA CBI

A. Test Chemicals (Cont.)

3. [redacted] is a white solid, with greater than 10% solubility in water. Its structural formula is [redacted]

Its molecular weight is 525 and the Haskell Number assigned is 14053. The sample was received from [redacted] PP&R, Washington Works, with a purity of [redacted]

4. [redacted] is an off-white powder with a density of 2.0, pH 7-9 and a 1-10% solubility in water. Its structural formula is [redacted] with a molecular weight of 550. Its assigned Haskell Number is 14,068 and the sample was received from [redacted] Polymer Products, E353/318 Experimental Station.

5. [redacted] is a beige powder with a density of about 2, pH 4-5 in water, and 1-10% solubility in water. Its structurual formula is [redacted]

Its molecular weight is 540 and its assigned Haskell Number is 14,008. The sample was [redacted] the [redacted] The sample was received from [redacted] PPD, ESL 269/306.

B. Animals and Husbandry

The Crl:CD®(SD)BR strain of rat was chosen for this test because previous toxicity testing on this class of chemicals was conducted in this species and strain, and because extensive background information from previous developmental toxicity tests at Haskell Laboratory exists on this rat strain.

Female rats about 56 days of age (nilliparous) were received from Charles River Breeding Laboratories, Inc., North Wilmington, Massachusetts. They arrived on July 2, 1981, and weighed 170±0.7 g (S.E.M.). Individual weights ranged from 154 to 198 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 and weighed 278±2.8 g (S.E.M.) with a range of 224 to 342 g.

Company Sanitized. Does not contain TSCA CBI

B. Animals and Husbandry (Cont.)

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. They were quarantined for 11 dyas. 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 ophthalomologist), 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.

C. Dose Selection

In a pretest, two nonpregnant 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 nonpregnant 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.

Company Sanitized. Does not contain TSCA CBI

C. Dose Selection (Cont.)

Dose selection for the other chemicals was based upon LD50 and ALD data relative to that for C-8. The calculated dosages were 100, 500, 500, and 1500 mg/kg/day for [redacted] respectively. Due to toxicity observed in a pretest with two rats per group, the dose levels for the last two chemicals were reduced to 250 and 500 mg/kg/day, respectively.

D. Animal Breeding and Distribution

The female rats 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, in proportion to the total number of rats to be allocated to each group.

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

E. Test Chemical Administration

The test chemicals were given by gavage in corn oil. 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 test chemical required was removed from a sealed plastic bag, which was contained in a Fiberpak® carton. During the dosing period (Days 6-15G), suspensions were prepared daily such that the dose was delivered 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. The control group received 5 mL stripped corn oil/kg body weight for the same period of gestation. To minimize 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(3). Aflatoxin concentration contained in stripped corn oil received previously from the same source (Lot D 4-25) was determined to be <2 ppb(3).

---

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

Company Sanitized. Does not contain TSCA C

F. Maternal and Fetal Examination at Sacrifice

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

After sacrifice of the dams by cervical dislocation on Day 21G, 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 "nonpregnant" 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(4); in addition, all stunted or malformed fetuses 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(5). 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 microscopy by a pathologist. The histologic specimens were coded for this examination, and particular emphasis was placed upon the structural integrity of the lens.

Company Sanitized. Does not contain TSCA CBI

F. Maternal and Fetal Examination at Sacrifice (Cont.)

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.

G. Statistical Evaluation

The litter was used as the experimental unit for the purpose of statistical evaluation (6). The significance of differences in the incidence of pregnancy, clinical signs, and maternal death was determined by use of Fisher's exact probability test(7). A two-way analysis of variance was used to detect differences in feed consumption among breeding lots and between groups. Dunnett's test(8) was used to test the statistical significance of differences between the control and each experimental 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 each experimental group was determined by application of the Mann-Whitney U test(9). When more than 75% ties occurred in the data, the Fisher's exact probability test was applied(10). The level of significance selected was $p \leq 0.05$.

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 2).

B. Maternal Clinical Signs Observed

In the control group, the only clinical sign noted was focal alopecia which developed in one dam.

Company Sanitized. Does not contain TSCA CBI

B. Maternal Clinical Signs Observed (Cont.)

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. All but one of the dams that subsequently died (during the dosing period) 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.

No deaths or clinical signs were observed in the [redacted] group. Similarly, no deaths occurred in the [redacted] group and only one female had clinical signs which consisted of red discharge from the left eye from Days 6-13G and yellow stain of the perineal area from Days 10-16G.

In the [redacted] group overt toxicity was observed after the second day of dosing. Dosing was discontinued after Breeding Lot A received eight daily dosages, but even so five of the seven females died between Days 11-14G, and one was sacrificed in extremis on Day 12G. The only female that survived to scheduled sacrifice had no visible sign of being pregnant but nidations were detected after staining of the uterus with ammonium sulfide. Hence, fetal data for the [redacted] group are not available for tabulation.

In the [redacted] group five of the dams had clinical signs similar to those seen in the C-8 and [redacted] groups. Therefore, dosing was discontinued after breeding Lot A received eight daily dosages. One of the seven females in the group was found dead on Day 13G and two others were sacrificed in extremis before scheduled sacrifice (Days 12 and 14G). Three of the four that remained had live fetuses.

Microscopic evaluation of the pulmonary parenchyma of the rats that did not survive to scheduled sacrifice did not reveal evidence of iatrogenic pulmonary injury, hence the deaths were not found to be related to improper gavaging (Attachment 3).

C. Maternal Feed Consumption

During the dosing period, the groups given C-8, [redacted] consumed significantly less feed than the control group (Table I). Feed consumption was similar to the control value in the post-exposure period. Insufficient data were available for the [redacted] group to permit meaningful statistical comparison.

Company Sanitized. Does not contain TSCA CBI

D. Maternal Body Weight Gain

The mean gain in body weight from Days 1-5G was significantly less in the group designated to be given C-8 than in the control group (Table II). However, from Days 6-15G, the group receiving C-8 gained about one-third less ($p \leq 0.05$) than the control group, and during the post-treatment period (Days 16-21G), the body weight gain of the C-8 group significantly exceeded ($p \leq 0.05$) the control value. During the dosing period, the body weight gain of the [redacted] group was not significantly different from that of the control group, but during the postdosing period, the [redacted] group gained significantly more than did the control group. The [redacted] and [redacted] groups gained little during the dosing period, but, thereafter, the [redacted] group gained significantly more body weight than the control group.

E. Gross Examination of Maternal Organs at Sacrifice

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. The relative weight of the liver for the [redacted] group was significantly higher than that for the control group (Table II).

F. Reproductive Effects and Fetal Body Weight

The maintenance of pregnancy, the incidence of resorptions, and fetal body weight were not adversely affected by administration of C-8, [redacted] at the dosages tested (Table II).

G. Fetal Alterations

No more than one fetus was malformed in each of the groups given test chemicals (Table III). In the control group, two were malformed. None of the chemicals were demonstrated to increase the frequency of malformed fetuses.

However, the incidence of fetuses with variations was significantly increased above the control value in both the [redacted] groups (Table IV). The increase for the [redacted] group was statistically significant only if a one-tailed test was applied. In both groups the increase was not due to a statistically significant increase of any individual type of variation.

Company Sanitized. Does not contain TSCA CBI

G. Fetal Alterations (Cont.)

Stereoscopic examination of the bisected eyes from two fetal heads per litter (Bouin's fixed) from each group did not reveal malformations or variations (Tables III and IV). The only eye alteration noted among the fetuses was a focal area of redness beneath the cornea in a fetus from the C-8 group and in two fetuses of the [redacted] group that was detected during routine examination of the live fetuses for external alterations (Table IV). The redness was due to the presence of blood in the anterior chamber of each of the affected eyes (Attachment 4). In addition, histomorphologic examination of the eyes of three 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 among the experimental and control groups (Attachments 5-7).

V. DISCUSSION

Dose selecton based upon comparative LD50's or ALD's was not reliable even among chemicals with some similarity in chemical activity (see Attachment 8). Due to the maternal toxicity observed in the [redacted] groups extra unmated female rats from the same shipment were given lower dosages by gavage than were used in this study (4/group). Administration of [redacted] at 50 mg/kg or [redacted] at 150 mg/kg for a period of ten days still resulted in too much body weight loss to serve as the maximum tolerated doses. Therefore, it is recommended that dosage levels of 35 and 100 mg/kg, respectively, be the highest tested should additional toxicity testing be undertaken.

C-8 was previously evaluated in Haskell Laboratory Report No. [redacted] and will not be repeated here; the data on C-8 are presented in this report for comparative purposes only.

[redacted] was given at a dosage that reduced feed consumption but not to a degree sufficient to significantly reduce body weight gain during the dosing period. However, the dosage administered was toxic as indicated by the significant rebound effect measured after completion of the dosing period. Neither the reproductive outcome of the dams nor the survival, or body weight, of their fetuses were demonstrated to be adversely affected by exposure to [redacted] A slight, but statistically significant, increase in developmental variations was detectable only if a one-tailed rather than a two-tailed statistical test was applied. This effect was largely due to an increase in the incidence of extra ossification sites next to the vertebrae that was not significantly increased above the control value ($p>0.05$). In view of the concurrent toxicity present among the dams, [redacted] was not demonstrated to represent a unique hazard to the conceptus.

Company Sanitized. Does not contain TSCA CBI

V. DISCUSSION (CONT.)

[redacted] also was given at an effect level for the dam as evidenced by a significant decrease in feed consumption and in body weight gain during the dosing period, and by a significant increase in actual and relative liver weight. Neither the reproductive endpoints evaluated for the dams nor the developmental endpoints used for the fetuses were demonstrated to be adversely affected.

The dose level at which [redacted] was given was too high to permit evaluation of toxicity to the dam or their concepti. Subsequent range-finding studies in non-pregnant rats indicated that the dosage level should not exceed 35 mg/kg body weight, if given daily for 10 days, by gavage, in corn oil.

The [redacted] also was given at a dose level that was more toxic than desired. Three of the seven mated females in the group did not survive but three of the remainder did yield live young at scheduled sacrifice. Despite significantly reduced feed consumption and body weight gain vs. that for the control group, no additional adverse effects were demonstrated among the dams and changes detected among their offspring were very minor. The average percent of fetuses with variations per litter was significantly increased for developmental variations ($p<0.05$; two-tailed test) and for total variations ($p<0.05$; one-tailed test). These differences were due to slight increases in the degree of sternal ossification and in the number of misaligned sterebrae; the differences from control incidence for these individual variations were not statistically significant ($p>0.05$). [redacted] did not pose a unique hazard to the rat conceptus at the toxic level tested.

VI. CONCLUSION

[redacted] were not demonstrated to represent a unique hazard to the conceptus after administration by gavage to rats during the period of major organogenesis at dosages toxic to the dam. [redacted] was given at a dose level that was too toxic to the dam to assess its potential hazard to the conceptus.

VII. ACKNOWLEDGEMENTS

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 vitro 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.

Company Sanitized. Does not contain TSCA CBI

VIII. REFERENCES

1. Unpublished Du Pont Data, Haskell Laboratory: [redacted]

2. Unpublished Du Pont Data, Haskell Laboratory: [redacted]

3. Unpublished Du Pont Data, Haskell Laboratory: [redacted]

4. Staples, R. E., "Detection of visceral alterations in mammalian fetuses." Teratology, 9:A37 (1974).

5. Barrow, M. V., and W. J. Taylor, "A rapid method for detecting malformations in rat fetuses." J. Morph., 127(3):291-306 (1969).

6. Haseman, J. K., and M. D. Hogan, "Selection of the experimental unit in teratology studies." Teratology, 12:165-172 (1975).

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

8. Steel, R. G. D., and H. H. Torrie, Principles and Procedures of Statistics, McGraw-Hill, New York, pp. 99-128 (1960).

9. Mann, H. G., and D. R. Whitney, "On a test of whether one or two random variables is stochastically larger than the other." Ann. Math. Stat., 18:50-60 (1947).

10. Haseman, J. K., and D. G. Hoel, "Tables of Gehan's generalized Wilcoxon test with fixed point censoring." J. Statist. Comput. Simul., 3:117-135 (1974).

 Company Sanitized. Does not contain TSCA CBI

[redacted]
HLR 441-84

TABLE I

C-8 ALTERNATES: MATERNAL AND DEVELOPMENTAL
TOXICITY IN THE RAT (PRELIMINARY STUDY, BY GAVAGE)

FEED CONSUMPTION[a] IN RATS GIVEN TEST CHEMICALS BY GAVAGE FROM DAYS 6-15 OF GESTATION (G)

	Corn oil (5 mL/kg/day)	C-8 (100 mg/kg/day)	[redacted] (100 mg/kg/day)	[redacted] (500 mg/kg/day)	[redacted] (250 mg/kg/day)	[redacted] (500 mg/kg/day)
Days 1-5G	21.0±0.59	20.6±0.32	21.8±0.79	21.3±0.52	19.3±0.0	19.6±1.67
Days 6-15G	21.9±0.48	17.2±0.37$^{\phi}$	18.3±0.25$^{\phi}$	13.7±0.40$^{\phi}$	11.7±0.0	13.3±1.90$^{\phi}$
Days 16-20G	28.1±0.58	29.0±0.52	30.5±1.04	27.9±0.52	18.5±0.0	26.7±1.78
Number of dams included	24	22	7	6	1	7

[a] grams/rat/day ±S.E.M.; values for non-pregnant females were excluded

$^{\phi}$ significantly different from control value by Dunnett's test ($p \leq 0.05$)

Company Sanitized. Does not contain TSCA CBI

[redacted]
HLR 441-84

TABLE II

C-8 ALTERNATES: MATERNAL AND DEVELOPMENTAL TOXICITY IN THE RAT (PRELIMINARY STUDY, BY GAVAGE)

REPRODUCTION AND FETAL DEVELOPMENT IN RATS GIVEN TEST CHEMICALS BY GAVAGE FROM DAYS 6-15 OF GESTATON

	Corn oil (5 mL/kg/day)	C-8 (100 mg/kg/day)	[redacted] (100 mg/kg/day)	[redacted] (500 mg/kg/day)	[redacted] (250 mg/kg/day)	[redacted] (500 mg/kg/day)
Females						
No. pregnant[a]/no. mated	25/25	22/25	7/7	6/7	0/7	3/7
No. deaths	0	3[b]	0	0	6[c]	3[d]
No. litters	24[e]	22	7	6	0	3
Mean no. corpora lutea	16.1±0.55[f]	16.7±0.87	15.3±1.19	15.3±0.67	--	17.3±2.8
Mean no. implants	13.6±0.57	13.8±0.64	14.6±0.65	15.2±0.54	--	14.7±0.88
Mean liver weight (g)[g]	15.4±0.34	16.2±0.44	17.5±0.68	22.9±1.20[ϕ]	--	14.9±1.12
Mean weight gain(g)[g]						
Days 1-5	33.2±1.12	30.0±1.09[ϕ]	35.4±2.08	32.1±2.75	--	34.6±2.07
Days 6-15	56.7±2.34	38.3±2.89[ϕ]	44.4±5.25	-1.5±8.95[ϕ]	--	12.5±1.87[ϕ]
Days 16-21	72.6±1.31	84.5±3.00[ϕ]	86.6±4.69[ϕ]	92.6±5.99[ϕ]	--	77.0±6.51
Days 6-21	129.2±2.68	122.8±4.26	131.0±7.78	91.0±4.29[ϕ]	--	89.5±7.79[ϕ]
Days 6-21C[h]	57.8±2.35	49.8±2.75[ϕ]	56.4±5.69	21.8±2.72[ϕ]	--	20.7±3.78[ϕ]
Fetal Death						
Mean no. resorptions/dam	0.7±0.16	0.6±0.13	1.0±0.38	0.8±0.17	--	1.3±0.33
No. litters with total resorption	1	0	0	0	0	0

Company Sanitized. Does not contain TSCA CBI

[redacted]
HLR 441-84

TABLE II (CONT.)

C-8 ALTERNATES: MATERNAL AND DEVELOPMENTAL
TOXICITY IN THE RAT (PRELIMINARY STUDY, BY GAVAGE)

REPRODUCTION AND FETAL DEVELOPMENT IN RATS GIVEN TEST CHEMICALS BY GAVAGE FROM DAYS 6-15 OF GESTATON

	Corn oil (5 mL/kg/day)	C-8 (100 mg/kg/day)	[redacted] (100 mg/kg/day)	[redacted] (500 mg/kg/day)	[redacted] (500 mg/kg/day)
Fetuses					
No. live	322	292	95	86	40
Mean no. live	13.4±0.32	13.3±0.67	13.6±0.95	14.3±0.61	13.3±0.88
No. stunted	0	0	0	0	0
Mean weight (g)	3.8±0.08	4.0±0.05	3.9±0.14	3.3±0.09	3.6±0.10

a all females had visible sign of pregnancy evident at autopsy
b one was noted as being dead on Day 11G, and two more on Day 12G
c rats were found dead on Days 10, 11, 12, 13, and 14G and one was sacrificed in extremis on Day 12G; the rat that survived had resorptions detected by stain only
d one rat was found dead on Day 13G; the others were sacrificed in extremis on Days 12 and 14G
e one female had only two early resorptions *in utero* on Day 21G
f x̄±S.E.M.
g females without a litter were excluded
h 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 ($p \leq 0.05$)

Company Sanitized. Does not contain TSCA CBI

[redacted]
HLR 441-84

TABLE III

C-8 ALTERNATES: MATERNAL AND DEVELOPMENTAL TOXICITY IN THE RAT (PRELIMINARY STUDY, BY GAVAGE)

FETAL MALFORMATIONS IN RATS GIVEN TEST CHEMICALS BY GAVAGE FROM DAYS 6-15 OF GESTATION

	Corn oil (5 mL/kg/day)	C-8 (100 mg/kg/day)	[redacted] (100 mg/kg/day)	[redacted] (500 mg/kg/day)	[redacted] (500 mg/kg/day)
External Malformations[a]					
No. examined	322/24[b]	292/22	95/7	86/6	40/3
Visceral Malformations					
No. examined	171/24[c]	155/22	49/7	45/6	22/3
Great vessels			1/1		
Innominate - none	1/1[d]		1/1		
Kidneys - none	1/1[d]				
Kidneys - small	1/1				
Spleen - small	1/1[d]				
Brain - ventricles enlarged					1/1
Head Malformations[a]					
No. examined	221/24	198/22	65/7	60/6	27/3
Skeletal Malformations					
No. examined	322/24	292/22	95/7	86/6	40/3
Sternebrae - fused	1/1[d]				
Total with Malformations	2/1		1/1		1/1
Avg. % Malformed Fetuses per Litter (±S.E.M.)	0.7±0.70		1.1±1.10		2.6±2.56

[a] no malformations were noted
[b] fetuses/litters
[c] blanks represent zero incidence
[d] occurred in a single fetus

Company Sanitized. Does not contain TSCA CBI

[redacted]
HLR 441-84

TABLE IV

C-8 ALTERNATES: MATERNAL AND DEVELOPMENTAL TOXICITY IN THE RAT (PRELIMINARY STUDY, BY GAVAGE)

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

	Corn oil (5 mL/kg/day)	C-8 (100 mg/kg/day)	[redacted] (100 mg/kg/day)	[redacted] (500 mg/kg/day)	[redacted] (500 mg/kg/day)
Developmental Variations					
External					
No. examined	322/24[b]	292/22	95/7	86/6	40/3
Hematoma	15/10	10/8	6/3	6/3	1/1
Petechia	60/19[c]	34/15	22/6	16/6	10/3
Eye - red		1/1[d]		2/2[d]	
Visceral					
No. examined	171/24	155/22	49/7	45/6	22/3
Renal papilla -reduced	1/1[e]				
Renal pelvis -large	1/1[f]	2/1[g]			1/1[h]
Pulmonary arteries -common trunk	3/2	9/6	3/2	3/2	5/3
Head[i]					
No. examined	221/24	198/22	65/7	60/6	27/3

Company Sanitized. Does not contain TSCA CBI

[redacted]
HLR 441-84

TABLE IV (CONT.)

C-8 ALTERNATES: MATERNAL AND DEVELOPMENTAL TOXICITY IN THE RAT (PRELIMINARY STUDY, BY GAVAGE)

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

	Corn oil (5 mL/kg/day)	C-8 (100 mg/kg/day)	[redacted] (100 mg/kg/day)	[redacted] (500 mg/kg/day)	[redacted] (500 mg/kg/day)
Developmental Variations (Cont.)					
Skeletal					
No. Examined	322/24	292/23	95/7	86/6	40/3
Sternebrae					
-misaligned(1)[j]	14/11	6/6	2/2	5/4	1/1
-misaligned(2+)[j]	7/7	9/8	2/2		5/2
-bipartite	1/1				
Centrum					
-bipartite	3/3		1/1	3/3	1/1
-dumbbelled	2/2	4/4	2/2		
Rib					
-extra ossification center	45/16	76/16*	30/16	14/5	8/3
-rudimentary	2/2	11/5	1/1	1/1	
-extra		1/1			
-thickened	1/1			2/1	
-calloused	1/1	1/1		4/1	
-wavy		1/1[j]		2/1	

Company Sanitized. Does not contain TSCA CBI

[redacted]
HLR 441-84

TABLE IV (CONT.)

C-8 ALTERNATES: MATERNAL AND DEVELOPMENTAL TOXICITY IN THE RAT (PRELIMINARY STUDY, BY GAVAGE)

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

	Corn oil (5 mL/kg/day)	C-8 (100 mg/kg/day)	[redacted] (100 mg/kg/day)	[redacted] (500 mg/kg/day)	[redacted] (500 mg/kg/day)
Developmental Variations (Cont.)					
Subtotal - Developmental Variations -No. Affected -Avg. % Affected	119/22	128/21	51/7	40/6	22/3
Fetuses/Litter (±S.E.M.)	36.3±3.92	43.2±4.53	53.7±9.41*	46.7±6.87	55.7±5.85**
Variations Due to Retarded Development					
Visceral					
Renal papilla -slightly reduced		1/1[g]			
Skeletal					
Sternebra -partiall ossified	51/13	22/8	2/2	6/4	13/3
-unossified	10/6	1/1	2/1	2/1	1/1
Rib -partially ossified		2/2[j]			
Ischium -partially ossified	1/1	1/1[j]			
Centrum -partially ossified				1/1	
-unossified				1/1	

Company Sanitized. Does not contain TSCA CBI

[redacted]
HLR 441-84

TABLE IV (CONT.)

C-8 ALTERNATES: MATERNAL AND DEVELOPMENTAL TOXICITY IN THE RAT (PRELIMINARY STUDY, BY GAVAGE)

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

	Corn oil (5 mL/kg/day)	C-8 (100 mg/kg/day)	[redacted] (100 mg/kg/day)	[redacted] (500 mg/kg/day)	[redacted] (500 mg/kg/day)
Variations Due to Retarded Development (Cont.)					
Skeletal (Cont.)					
Pubis					
-partially ossified	2/2	2/2[k]			1/1
No. examined with heads	104/24	94/22	30/7	26/6	13/3
Skull bones					
partially ossified					
-parietal	1/1	2/2[k]		3/1	
-interparietal	4/3	5/5[k]		1/1	
-supraoccipital	1/1	5/5[k]		1/1	
-squamosal		2/2[k]		1/1	
-frontal		1/1[k]			
-zygoma	1/1	2/2[k]			
Maxilla					
-partially ossified		1/1[k]			
Hyoid					
-partially ossified	3/3	2/2		2/2	
-unossified	5/4	4/3[k]			

Company Sanitized. Does not contain TSCA CBI

[redacted]
HLR 441-84

TABLE IV (CONT.)

C-8 ALTERNATES: MATERNAL AND DEVELOPMENTAL TOXICITY IN THE RAT (PRELIMINARY STUDY, BY GAVAGE)

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

	Corn oil (5 mL/kg/day)	C-8 (100 mg/kg/day)	[redacted] (100 mg/kg/day)	[redacted] (500 mg/kg/day)	[redacted] (500 mg/kg/day)
Variations Due to Retarded Development (Cont.)					
Subtotal - Variations Due to Retarded Development					
-No. Affected	70/14	35/12	4/7	14/6	14/3
-Avg. % Affected Fetuses per Litter (±S.E.M.)	20.6±4.61	10.9±3.08	4.9±2.82	16.5±4.36	36.2±21.04
TOTAL					
No. Fetuses with Variations	154/23	145/21	53/7	41/6	29/3
Avg. % Fetuses with Variations per Litter (±S.E.M.)	46.5±5.18	48.5±4.35	56.0±8.80	47.9±6.84	73.5±11.27*

Company Sanitized. Does not contain TSCA CBI

[redacted]
HLR 441-84

TABLE IV (CONT.)

C-8 ALTERNATES: MATERNAL AND DEVELOPMENTAL
TOXICITY IN THE RAT (PRELIMINARY STUDY, BY GAVAGE)

FETAL VARIATIONS[a] IN RATS GIVEN TEST CHEMICALS 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 examiantion
g - one fetus (4.02 g) with left kidney affected had associated hydroureter; the other, a littermate (4.21 g), had both kidneys affected without associated hydroureter, but a slightly reduced papilla was present on the right side
h - one fetus (3.47 g); both renal pelves slightly enlarged
i - no fetuses with developmental variations of head were detected
j - (1) and (2+) denote 1, or 2 or more sternebrae were misaligned, respectively
k - one fetus (3.26 g) had each of these variations
* - statistically significant difference by one-tailed Mann-Whitney U test ($p \leq 0.05$)
** - statistically significant difference detected by two-tailed Mann-Whitney U test ($p \leq 0.05$)

Company Sanitized. Does not contain TSCA CBI

C-8 & alternates

APPENDIX A

ES- 575 REV. 1-75

DUPONT

ESTABLISHED 1802

E. I. DU PONT DE NEMOURS & COMPANY
INCORPORATED
WILMINGTON, DELAWARE 19898
CENTRAL RESEARCH & DEVELOPMENT DEPARTMENT

HASKELL LABORATORY
FOR
TOXICOLOGY AND INDUSTRIAL MEDICINE

CC: [redacted]

July 23, 1981

M E M O R A N D U M

TO: [redacted]
POLYMER PRODUCTS DEPARTMENT
CUSTOMS HOUSE SQUARE 314

[redacted]
CHEMICALS AND PIGMENTS DEPARTMENT
BRANDYWINE BUILDING 6210

FROM: R. E. STAPLES RES

SUBJECT: C-8 AND PROSPECTIVE ALTERNATES: PROTOCOL FOR TERATOGENICITY TESTING

The protocol for the teratogenicity testing of C-8 and its alternates is attached [redacted] If you have questions concerning this study, please let me know (366-5302).

RES/mle
Attachment (1)

Company Sanitized. Does not contain TSCA CBI

BETTER THINGS FOR BETTER LIVING . . . THROUGH CHEMISTRY

E. I. DU PONT DE NEMOURS & CO.
CENTRAL RESEARCH AND DEVELOPMENT DEPARTMENT
HASKELL LABORATORY FOR TOXICOLOGY
AND INDUSTRIAL MEDICINE
ELKTON ROAD
NEWARK, DELAWARE

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

[redacted]

I. BACKGROUND

A request for a proposal to conduct a teratogenicity study on [redacted] was received from [redacted] (Haskell Coordinator for PPD), on March 23, 1981. This request was made in response to a TSCA 8(e) notice from the 3M Company for [redacted]. On June 11, 1981, E. D. Champney orally requested that [redacted] also be tested in response to a second 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, and to conduct a preliminary teratogenicity test on the other chemicals listed above for comparative purposes.

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.

Company Sanitized. Does not contain TSCA CBI

A. Test Chemical

The following chemicals were received from the Polymer Products Department and assigned Haskell Numbers:

- [redacted] (C-8) H-14,045
- [redacted] H-14,075
- [redacted] H-14,053
- [redacted] H-14,068
- [redacted] H-14,008

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, [redacted] 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.

Company Sanitized. Does not contain TSCA CBI

C. Route of Administration

Administration of the test chemicals 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
100 mg/kg	[redacted]
500 mg/kg	
250 mg/kg	
500 mg/kg	

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; the level selected for each of the alternate chemicals was determined similarly.

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,

Company Sanitized. Does not contain TSCA CBI

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. Each of the remaining test groups in this experiment will be assigned about 7 mated females. 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

Company Sanitized. Does not contain TSCA CBI

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

Company Sanitized. Does not contain TSCA CBI

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).

Company Sanitized. Does not contain TSCA CBI

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:

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

      For each litter:

      - Viability index (% animals born that survived 4 days or more)
      - 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

Company Sanitized. Does not contain TSCA CBI

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.

Company Sanitized. Does not contain TSCA CBI

IV. CRITICAL DATES

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

Company Sanitized. Does not contain TSCA CBI

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

Company Sanitized. Does not contain TSCA CBI

[redacted]

CENTRAL RESEARCH AND DEVELOPMENT DEPARTMENT
HASKELL LABORATORY FOR TOXICOLOGY
AND INDUSTRIAL MEDICINE

cc: [redacted]

December 3, 1981

M E M O R A N D U M

TO: QUALITY ASSURANCE COMMITTEE - C. M. BARBA

FROM: R. E. STAPLES RES

SUBJECT: PROTOCOL AMENDMENT FOR TERATOGENICITY STUDY: C-8 AND PROSPECTIVE ALTERNATES [redacted]

An amendment to the protocol for the teratogenicity testing of C-8 and its alternates is attached.

RES/mle
Attachment (1)

Company Sanitized. Does not contain TSCA CBI

E. I. DU PONT DE NEMOURS & CO., INC.
CENTRAL RESEARCH AND DEVELOPMENT DEPARTMENT
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] OR ITS ALTERNATES
[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.

Company Sanitized. Does not contain TSCA CBI

PREPARED BY: C. L. Lamontia

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

R. E. Staples

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

APPROVED BY: J. G. Aftosmis

J. G. Aftosmis - Associate Director
Toxicology and Pathology

CLL/RES/mle
12/3/81

Company Sanitized. Does not contain TSCA CBI

CENTRAL RESEARCH AND DEVELOPMENT DEPARTMENT
HASKELL LABORATORY FOR TOXICOLOGY
AND INDUSTRIAL MEDICINE

cc: [redacted]

R. E. STAPLES

MAR 23 1981

RECEIVED

ATTACHMENT 1

March 20, 1981

M E M O R A N D U M

TO : R. E. Staples

FROM: J. R. Barnes

TERATOGENIC STUDY WITH [redacted]

Polymer Products Department would like Haskell Laboratory to prepare a protocol and cost estimate for an oral teratology study with [redacted] The purpose of the study is to determine whether [redacted] produces effects similar to those reported by the 3M Company for [redacted] in a TSCA 8(e) notice. Some details of the 3M Company protocol were obtained by PPD and are attached for your information.

The project should be addressed to [redacted] PPD, Customs House Square-314.

JRB:mjh

Attach.

Company Sanitized. Does not contain TSCA CBI

Z-69 REV 12-79

E. I. DU PONT DE NEMOURS & COMPANY
INCORPORATED
WILMINGTON, DELAWARE 19898

POLYMER PRODUCTS DEPARTMENT

cc: [redacted]

February 11, 1981

TO: E. D. CHAMPNEY
D-11070

FROM: J. B. ARMITAGE
CHS-314 JBA

TSCA SECTION 8(e) REPORT FROM 3M

Under the Freedom of Information Act, we obtained from the EPA the attached TSCA 8(e) notice on the [redacted] submitted by 3M, which was noted in my December 12, 1980 letter to J. R. Barnes.

Some details of the oral teratology protocol are given, which may already have been disclosed to McKusick and Kennedy by 3M at their January 16, 1981 meeting in Chicago, but of which I was unaware.

JBA/skd

Attachment

Company Sanitized. Does not contain TSCA CBI

There's a world of things we're doing something about

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

REC'D
PSRA
FEB 10 1981

JAN 30 1981

OFFICE OF
PESTICIDES AND TOXIC SUBSTANCES

Mr. J. B. Armitage
E.I. duPont de Nemours & Company, Inc.
Patents and Regulatory Affairs Division
Wilmington, Delaware 19899

Re: Freedom of Information Request Act
(A-101) RIN-247-81

Dear Mr. Armitage:

In response to your letter dated January 23, 1981, enclosed is a nonconfidential copy of substantial risk notification 8EHQ-1180-0374S, submitted by 3M Company in compliance with Section 8(e) of the Toxic Substances Control Act.

There is no charge for the enclosed material. In accordance with Agency regulation, we charge $.20 per page for duplication and $5.00 per hour for search time if a total charge of $10.00 or more is incurred.

If you have any questions, please contact Ms. Janet L. West of my staff at (202) 755-8050. Please use the reference number RIN-247-81 when inquiring about your request.

Sincerely,

Edward J. Cull III

Edward J. Cull, Chief
Information Control Branch

Enclosure

Company Sanitized. Does not contain TSCA CBI

8EHQ-1180-03745

General Offices/3M
3M Center
St. Paul, Minnesota 55101
612/733 1110

Certified Mail - Return Receipt Requested

November 19, 1980

Document Control Officer
Chemical Information Division
Office of Toxic Substances (WH-557)
Environmental Protection Agency
401 M Street, S. W.
Washington, D. C. 20460

8EHQ-1180-03745
86-8000148

Gentlemen:

Subject: Section 8(e) Toxic Substances Control Act
Potassium Salt of Perfluoroalkyl Sulfonates

FPA INFO. CONTROL BRANCH
80 NOV 24 PM 4:29

Please find attached the following information relating to the subject chemicals:

(1) Protocols for Oral Teratology Study in Rats.
(2) Preliminary report, 3M memo, dated November 12, 1980.
(3) 3M Technical Report entitled "Analysis of Selected Decatur Employee Serum for Sulfonic and Carboxylic Fluorochemicals".

Preliminary information from the ongoing teratology study cited in (2) above indicates that the subject chemicals are teratogenic in rats. This information and the findings described in (3) above, indicating the presence of fluorochemicals in the blood of some of our plant employees, leads us to submit this information pursuant to Section 8(e) of the Toxic Substances Control Act and EPA's statement of interpretation published in the FEDERAL REGISTER, March 16, 1978.

As noted on page 584 of an article published in the October 1980 American Industrial Hygiene Journal, entitled "Health Status of Plant Workers Exposed to Fluorochemicals--A Preliminary Report", this publication and the attached information reflect, in part, 3M's testing and monitoring program designed to "...evaluate the overall impact of exposure of fluorochemicals on the health of workers". Although this preliminary information cited in (2) indicates that the subject chemicals are most probably animal teratogens, our employee records and the epidemiology data described in the aforementioned publication indicate that to date no human health problems have been observed nor disease patterns detected which are attributable to

Company Sanitized. Does not contain TSCA CBI

related to fluorochemical exposure. This publication also described the industrial hygiene measures undertaken in further reducing employee exposure.

"Potassium salts of perfluroalkyl sulfonates" is a generic chemical name for a mixture of homologs which can be expressed by the general formula $C_nF_{2n+1}SO_3K$. These homolog were reported on the TSCA inventory

This homologous mixture, or the corresponding ammonium salts, is currently sold as various products containing from 100 percent "solids" to 0.58 percent of the mixture. These products and their customer uses are described as follows:

FLUORAD^R Brand Etching Bath Additive FC-93 (correponding ammonium salt of the subject chemical)	Electronic Manufacturing
FLUORAD^R Brand Fluorochemical Surfactant, FC-95	Chrome Plating
FLUORAD^R Brand Fluorochemical Surfactant, FC-99 (corresponding amine salt of subject chemical)	Chrome Plating
LIGHT WATER^R Brand Aqueous Film Forming Foam FC-203	Fire Suppression
LIGHT WATER^R Brand Aqueous Film Forming Foam FC-203A	Fire Suppression
LIGHT WATER^R Brand Aqueous Film Forming Foam, FC-206A	Fire Suppression
LIGHT WATER^R Brand Aqueous Film Forming Foam Alcohol Type Concentrate, FC-600	Fire Suppression
AFFF 6% Concentrate, FC-780B	Fire Suppression

Approximately of perfluoroalkyl sulfonates are produced per year domestically at our Decatur production facility located at P.O. Box 2206, Decatur, Alabama 35602, with 48 employees potentially exposed on an intermittent basis. Chemical reaction occurs in a closed system. Approximately of the are processed at our Chemolite production site located at Highway 61 & Washington County Rd. 19, St. Paul, Minnesota 55133, with 37 employees potentially exposed on an intermittent basis.

Company Sanitized. Does not contain TSCA CBI

We plan to inform, by mid-December, all those customers and 3M employees who have a potential, through certain uses and/or processing, of significant exposure to the subject chemicals. At that time we will summarize these findings and outline our recommendations for handling and using thes products. We are by copy of this letter advising NIOSH of these new but preliminary teratogenic findings. When significant new information becomes available to us, we pla to advise these customers and employees accordingly.

In line with our ongoing testing and monitoring program in fluorochemicals, this is to advise the Agency of the following work planned for initiation in the near future:

(1) A second teratogenic study designed to further evaluate these findings in both rats and rabbits.

(2) Developing industrial hygiene procedures designed to further reduce the exposure to plant employees.

Since certain of the information provided herein is considered confidential business information, we are providing a sanitized version of this report for the public file. In addition, we have deleted from the confidential submission inconsequential information such as the names of 3M employees for the purpose of protecting their privacy.

The teratology study referred to herein is based on feeding FC-95, the mixture of potassium salts of perfluoroalkyl sulfonates described as above. When the study is complete, a final written report will be provided to EPA. In the interim, should additional correspondence be necessary on this matter, please contact:

Larry Magill
Manager, Regulatory Affairs Department
Commercial Chemicals Division
3M
3M Center, 223-6S-04
Saint Paul, MN 55144
Telephone: 612/733-7062

Yours truly,

L. D. DeSimone
Group Vice President

dea
Attachments

c: Anthony Robbins, M.
Director, NIOSH
Park Lawn Building
5600 Fishers Lane
Rockville, MD 20855

Company Sanitized. Does not contain TSCA CBI

November 12, 1980

cc:

TO:

FROM:

SUBJECT: Teratogenicity of Two Fluorochemical Compounds in Rats
(Study numbers 680TR0008 and 680TR0010)

Preliminary results from rat teratology studies on FC-95 and indicate a teratogenic effect on the embryonal eye. The developmental eye abnormality was seen at all dose levels except the control groups. The range of morphologic appearances observed under the dissecting microscope varied from a slight discoloration on the lens near the anterior margin to a dark colored oval area, often containing a cleft, extending from beneath the lens epithelium to halfway through the lens posteriorly. Histologically, the discolorations were primarily due to the presence of lens vesicle remnants or abnormal primary lens fibers in the embryonal nucleus of the lens.

Company Sanitized. Does not contain TSCA CBI

TITLE: Protocol for Oral Teratology Study of FC-95 in Rats (Experiment Number 0680TR0008).

OBJECTIVE: A teratology study will be used to evaluate the embryotoxic and teratogenic effects of orally administered FC-95 to pregnant rats during the period of organogenesis. The procedure complies with the general recommendations of the FDA issued in January, 1966 ("Guidelines for Reproduction Studies for Safety Evaluation of Drugs for Human Use"). The study will be conducted according to the 1978 Good Laboratory Practice regulations and Safety Evaluation Laboratory's Standard Operating Procedures.

SPONSOR: 3M Commercial Chemical Division, St. Paul, Minnesota.

TESTING FACILITY: Safety Evaluation Laboratory
St. Paul, Minnesota.

STUDY DIRECTOR:

START OF DOSING: Mid July, 1980.

TEST SYSTEM: Eighty-eight sexually mature, time mated Sprague-Dawley derived female rats from Charles River Breeding Laboratory will be housed in hanging stainless steel cages with wire mesh floors and fronts in a temperature and humidity controlled room. This strain of rats will be used because of historical control data and time mated females are readily available. Purina Laboratory Chow and water will be available ad libitum. The lights will be on a 12 hour light/dark cycle.

TEST SYSTEM IDENTIFICATION: Each animal will be ear tagged and that number will be indicated on the outside of the cage.

RANDOMIZATION: The animals will be assigned cages according to a computer-generated random number table.

CONTROL ARTICLE: Corn oil.

TEST ARTICLE: FC-95.

ANALYTICAL SPECIFICATIONS: The test article, composition and purity will be determined by the Sponsor (3M Commercial Chemical group) prior to the start of the study and at the end of dosing.

DOSAGE LEVELS AND EXPERIMENT DESIGN: The test article will be suspended in corn oil daily. The test article suspension and control article will be administered by oral intubation to the rats on days 6 through 15 of gestation according to the following:

Company Sanitized. Does not contain TSCA CBI

Dose Group	Dose Level	Group Size
High	10 mg/kg/day	22 ♀
Mid	5 mg/kg/day	22 ♀
Low	1 mg/kg/day	22 ♀
Control	0 mg/kg/day	22 ♀

The oral route of administration will be used because of metabolism studies showed radiolabeled PC-95 was well absorbed. No dietary contaminants are known to interfere with the test article.

The animals will be observed daily from day 3 through day 20 of gestation for abnormal clinical signs. Body weights will be recorded on days 3, 6, 9, 12, 15 and 20 of pregnancy and the rats dosed accordingly using a constant dose volume of 5 ml/kg of body weight.

The females will be killed on day 20 and the ovaries, uterus and its contents will be examined to determine: number of corpora lutea, number of fetuses (live and dead), number of resorption sites, number of implantation sites, pup weight and gross abnormalities. Approximately one-third of the pups will be fixed in Bouin's solution for subsequent free-hand sectioning by the Wilson technique to determine any visceral abnormalities using a dissecting microscope. The remaining approximately two-thirds of the pups will be fixed in ethyl alcohol for subsequent skeletal examination after clearing and staining with alizarin red.

DATA ANALYSIS AND FINAL REPORT: The proposed statistical methods to be used for analysis of the data are: Dunnett's t test for dam and pup weights, number of fetuses, number of resorption sites, number of implantation sites and number of corpora lutea; Chi square for percent abnormalities. The proposed date for the final report is 2-3 months after detailed pup examinations have been completed (approximately fourth quarter, 1980).

6/26/80
Date

6/26/80
Date

6/26/80
Date

6/26/80
Date

Company Sanitized. Does not contain TSCA CBI

3M TECHNICAL REPORT

Report Number:

Date: October 4, 1979

TITLE: Analysis of Selected Decatur Employee Serum for Sulfonic and Carboxylic Fluorochemicals

AUTHOR:

ABSTRACT:

The precence of sulfonic acids was detected in certain blood samples using both electron capture and microwave plasma detector methods

Company Sanitized. Does not contain TSCA CBI

Report No. _____

Date October 4, 1979

Subject: Characterization of Decatur RF Values

Requestor: _____ Dept. Name Commercial Chemicals Proj. No. ___

Request No. _____ Dated October 3, 1979

Report:

The serum of 10 selected 3M Decatur employees was submitted (A72754) in June, 197 for analysis of organic fluoride levels and the total organic fluoride values wer reported in Analytical Report 7194. Subsequent to that work, it became of intere to further characterize the fluoride serum value and attempt to identify the pres of 3M fluorochemicals.

Initial analysis (7/18/79) by the microwave plasma detector (MPD) established the presence of the expected perfluoro sulfonate and perfluorooctanoate anions in the serum extract of . -25 and . -22. However, the observation of perfluoro- sulfonate anion in the above extracts was unexpected and it therefore becam necessary to spend additional analytical development time in order to measure the 3 fluorochemicals in the same serum extract sample. During this time, the method was evaluated on the electron capture (EC) gas chromatograph. The EC detector would be expected to have greater sensitivity than the MPD for these fluorochemica but with less specificity since the MPD can be operated on the fluorine channel eliminating any confusion by other EC active compounds.

We selected the 5 highest fluorine containing Decatur samples and analyzed the serum extract for perfluoro sulfonate, perfluoro sulfonate, and perfluor octanoate anion. We used both the MPD and EC detection systems and report the following values:

		% FOUND①			
Person	RF (ppm)②	Perfluoro- sulfonate	Perfluoro- sulfonate	Perfluoro- octanoate	Total
-22	10.1	5%	60%	30%	95%
-23	5.7	15%	70%	20%	105%
-24	9.4	5%	80%	15%	100%
-25③	11.8	5%	55%	20%	80%
-26	4.1	10%	65%	25%	100%

①The analysis was conducted on a single determination (1 ml of serum) due to limit availability of sample and time and represents our interpretation of the average of the EC and MPD values. No internal standard was used; expect ±10-20% accuracy

②AR 6962, 7028, 7194

③Previously identified as -21(Z) due to mis-labeling by medical personnel.

Company Sanitized. Does not contain TSCA CBI

The above results are necessarily somewhat approximate due to the lack of an inter standard and continual refinements in the derivatization and GC of the correspondi methyl ester. Sufficient sample remains for additional analysis. Further improve in the analysis are and will continue to be made as time permits. We thought it best to report these values now, especially since we identified the presence of perfluoro sulfonate in human serum extract and no toxicology studies to date have been completed on this fluorochemical.

Three years ago (10-18-76) we reported that Decatur employee . serum extract contained perfluoro sulfonate (based on F/NMR) and a minor fluorine containin component now identified as perfluorooctanoate. Therefore, the presence of perfluoro sulfonate in this individual has been known for some time. Now, with improved analytical methods, we have greater sensitivity and can determine the presence of perfluoro sulfonate in addition to perfluoro sulfonate anion

Company Sanitized. Does not contain TSCA CBI

- Data + Interim Rpts

DIPLOMATE: AMERICAN COLLEGE
OF VETERINARY OPHTHALMOLOGISTS

ATTACHMENT 2

R. E. STAPLES

JUL 22 1981

RECEIVED

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 and Alternates
[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. Hollenburg.

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.

Company Sanitized. Does not contain TSCA CBI

CENTRAL RESEARCH AND DEVELOPMENT DEPARTMENT
HASKELL LABORATORY FOR TOXICOLOGY
AND INDUSTRIAL MEDICINE

ATTACHMENT 3

R. E. STAPLES
AUG 21 1981
RECEIVED

August 19, 1981

M E M O R A N D U M

TO : R. E. Staples

FROM: W. D. Kerns WDK

H-14045 - [redacted] PULMONARY PATHOLOGY

The pulmonary parenchyma was evaluated microscopically from several female rats (Table I) in order to determine if the death of the animals was technique related.

There was no microscopic evidence of iatrogenic pulmonary injury in any of the rats that were examined.

TABLE I

Animal No.	
306409	306363
306498	306401
306477	306551
306469	306519
306508	306338
306529	306354
306346	306486
306330	

WDK:mjh

Company Sanitized. Does not contain TSCA CBI

DUPONT

HASKELL LABORATORY

[redacted]

1/29/82

ATTACHMENT 4

R. E. STAPLES

JAN 29 1982

RECEIVED

MEMORANDUM

DATE: January 29, 1982
TO: R. E. Staples
FROM: W. D. Kerns
SUBJECT: [redacted] WDK

Three fetuses were examined microscopically (12-12, 13-6, 30-6) for a histomorphological correlate to a red area in either the right or left eye. All three fetuses had focal areas of hyphema. This lesion was consistantly located adjacent to the drainage angle in all fetuses.

WDK:smk

Company Sanitized. Does not contain TSCA CBI

ES-3606

ATTACHMENT 5

cc: [redacted]

DUPONT

HASKELL LABORATORY

October 5, 1981

MEMORANDUM

TO: R. E. Staples

FROM: W. D. Kerns WDK

RE.: [redacted]

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.

Six additional fetuses [redacted] were evaluated for the presence of microscopic lesions. Five of these animals had macroscopic observations of brown discoloration in the central lens (Table 2). Microscopically, (Table 3) alterations in these animals were similar in their morphological characteristics to the postmortem alterations that occurred in the gavage (C-8) study. The lens alterations in these animals additionally contained brownish-green birefringent particulates similar to acid hematin. This pigmentation may correlate with the macroscopic observation of brown discoloration. I suspect that this alteration is in some manner an effect of acidic Bouin's fixation and is similar to a brownish pigment (acid hematin) produced in tissue fixed in nonbuffered formalin.

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

WDK:ljm

Company Sanitized. Does not contain TSCA CBI

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

Company Sanitized. Does not contain TSCA CBI

TABLE 2

Dam Code	Fetus Number	Observations During Head Examination
52	11	"Pale brown area in lens; right lens ~ 1 mm, left lens ~ 0.5 mm"
35	12	"Light brown area in center of lens of right eye ~ 0.5 mm"
55	11	No alterations detected
266842	2	Slightly dark area in center of left lens. Currently, this is the slightest degree that will be noted as an effect.
253340	1	Dark area in center of both lenses
"6"	"6"	Very small dark area in the right lens between "y" suture and epithelium

Company Sanitized. Does not contain TSCA CBI

TABLE 3

MICROSCOPIC OBSERVATIONS

D52F11	Unilateral alteration (artifact) with brownish-green refractile particulates
D35F12	Bilateral alteration (artifact) with brownish-green refractile particulates
D55F11	No significant lesions observed (NSLO)
D266842F2	NSLO
D253340F1	Unilateral alteration (artifact) with brownish-green refractile particulates
D"6"F"6"	Unilateral alteration (artifact) with brownish-green refractile particulates

Company Sanitized. Does not contain TSCA CE

Correspondence

CENTRAL RESEARCH AND DEVELOPMENT DEPARTMENT
HASKELL LABORATORY FOR TOXICOLOGY
AND INDUSTRIAL MEDICINE

cc: [redacted]

October 1, 1981

M E M O R A N D U M

TO: N. C. CHROMEY

FROM: C. L. LAMONTIA CLL

SUBJECT: HISTOLOGIC PREPARATION AND EVALUATION OF FETAL EYES: C-8 TERATOGENICITY STUDIES - [redacted]

Please have these two groups of fetal specimens (attachment) embedded, sectioned, and examined. Embed one-half with the cut surface of the lens up. Since evaluation of the lenses of these fetuses is particularly important, each specimen should be sectioned so as to obtain cross-sections which include the largest diameter of each lens. Stain selected sections from both groups with hematoxylin and eosin and send them to W. D. Kerns for evaluation. Please contact W. D. Kerns when the specimens are sectioned. Also, the slides should remain separated by groups.

Please send any information concerning these specimens directly to R. E. Staples (Study Director) or me.

CLL/mle
Attachments (2)

Company Sanitized. Does not contain TSCA CBI

SPECIMENS TO BE PREPARED
FOR EVALUATION BY W. D. KERNS

[redacted]

The following fetuses had no eye alterations detected during external examination:

Dam Code	Fetus Number	Observations during Head Examination
68	1	Not sufficient discoloration to be considered an alteration
54	7	Not sufficient discoloration to be considered an alteration
58	9	Not sufficient discoloration to be considered an alteration
58	11	Not sufficient discoloration to be considered an alteration

CLL/as
9/30/81

Company Sanitized. Does not contain TSCA CBI

SPECIMENS TO BE PREPARED
FOR EVALUATION BY W. D. KERNS

[redacted]

The following fetuses had no eye alterations detected during external examination:

Dam Code	Fetus Number	Observations during Head Examination
35	1	Faint brown area in anterior half of lens of left eye ∿0.5x0.4mm.
35	3	No alterations detected
35	4	Faint brown area in anterior half of lens of left eye ∿0.6x0.4mm
35	7	Light brown area in anterior half of lens of left eye ∿0.4x0.3mm; Very faint brown area in anterior half of lens of right eye ∿0.4x0.2mm.
35	9	Light brown area in anterior half of lens of right eye ∿0.6x0.2mm
35	11	Very light brown area in anterior half of lens of both eyes; Left ∿0.4x0.4mm, right ∿0.4x0.4mm.
52	1	Light brown area in anterior half of lens of both eyes; Left ∿0.8x0.5mm, right ∿0.6x0.4mm
52	3	Light brown area in anterior half of lens of both eyes; Left ∿0.5x0.3mm, right ∿0.5x0.3mm
52	7	Light brown area in anterior half of lens of both eyes; Left. ∿0.4x0.4mm, right ∿0.5x0.3mm
52	8	Very faint brown area in anterior half of lens of both eyes; Left ∿0.4x 0.4mm, right ∿0.5x0.4mm

Company Sanitized. Does not contain TSCA CBI

SPECIMENS TO BE PREPARED
FOR EVALUATION BY W. D. KERNS (cont'd)

[redacted]

Dam Code	Fetus Number	Observations during Head Examination
52	9	Light brown area in anterior half of lens of both eyes; Left ∿0.6x0.4mm, right ∿0.6x0.4mm
52	13	No alterations detected
52	15	Light brown area in anterior half of lens of both eyes; Left ∿0.3x0.3mm, right ∿0.5x0.4mm
35	5	Light brown area in center of lens of right eye ∿0.5mm
52	4	No alterations detected
52	5	Light brown area in anterior half of lens of both eyes; Left ∿0.6x0.5mm, right ∿0.5x0.5mm

Note - Alterations listed by severity:

Very faint brown<faint brown<very light brown<light brown

CLL/as
9/30/81

Company Sanitized. Does not contain TSCA CBI

R. E. STAPLES
SEP 30 1981
RECEIVED

C-8 AND ALTERNATES:
CODE FOR ROUTINE TERATOGENICITY TEST IN RATS BY GAVAGE

[redacted]

for decoding
F. heads examined
by Reprs. Effects
division

Corn Oil (5 ml/kg)		C-8 (100 mg/kg)	
Dam Code	Animal Number	Dam Code	Animal Number
2	306376	4	306365
5	306385	8	306426
6	306541	14	306465
7	306571	15	306558
9	306523	18	306494
10	306443	21	306341
17	306501	23	306539
19	306475	25	306464
22	306482	29	306517
24	306471	30	306472
26	306368	33	306414
28	306458	37	306547
32	306447	39	306557
34	306397	41	306399
38	306352	43	306416
40	306556	46	306425
42	306438	51	306532
45	306391	53	306374
49	306433	57	306481
50	306370	61	306540
55	306384	62	306375
59	306563	65	306524
60	306382	--	306409
64	306406	--	306498
66	306538	--	306477

---

[a] Females without dam codes died or were sacrificed before Day 21

Division by Breeding Lots

	Dam Code
Lot A	1 through 5
Lot B	6 through 24
Lot C	25 through 44
Lot D	45 through 66

CLL/mle
8/17/81

Company Sanitized. Does not contain TSCA CBI

C-8 AND ALTERNATES:
CODE FOR ROUTINE TERATOGENICITY TEST IN RATS BY GAVAGE

[redacted]

C-9 (100 mg/kg)		[redacted] (500 mg/kg)		[redacted] (250 mg/kg)		[redacted] (500 mg/kg)	
Dam Code	Animal Number	Dam Code	Animal Number	Dam Code	Animal Number	Dam Code	Animal Number
1	306340	3	306355	54	306373	16	306470
11	306420	12	306484	--[a]	306354	35	306473
20	306439	13	306503	--	306486	52	306336
31	306516	27	306342	--	306469	63	306396
36	306367	44	306390	--	306346	--	306363
48	306412	47	306334	--	306551	--	306519
56	306421	58	306353	--	306338	--	306508

[a] Females that were not assigned dam codes died or were sacrificed before the usual sacrifice period.

Division by Breeding Lots

	Dam Code
Lot A	1 through 5
Lot B	6 through 24
Lot C	25 through 44
Lot D	45 through 66

CLL/mle
8/13/81

Company Sanitized. Does not contain TSCA CBI

DUPONT

HASKELL LABORATORY

December 18, 1981

MEMORANDUM

TO: R. E. Staples

FROM: W. D. Kerns

SUBJECT: [redacted]

All animals listed in the attached tables were evaluated microscopically for the presence of histomorphologic lesions in the fetal lens.

There were no microscopic lesions detected in any of the sections that were evaluated. Frequently, lenses contained artifacts that were attributed to trimming and processing.

WDK:wfd

Company Sanitized. Does not contain TSCA CBI

SPECIMENS TO BE PREPARED
FOR EVALUATION BY W. D. KERNS

[redacted]

The following fetuses had no eye alterations detected during external examination:

Dam Code	Fetus Number	Observations during Head Examination
68	1	Not sufficient discoloration to be considered an alteration
54	7	Not sufficient discoloration to be considered an alteration
58	9	Not sufficient discoloration to be considered an alteration
58	11	Not sufficient discoloration to be considered an alteration

CLL/as
9/30/81

Company Sanitized. Does not contain TSCA CBI

SPECIMENS TO BE PREPARED
FOR EVALUATION BY W. D. KERNS

[redacted]

The following fetuses had no eye alterations detected during external examination:

Dam Code	Fetus Number	Observations during Head Examination
35	1	Faint brown area in anterior half of lens of left eye ∿0.5x0.4mm.
35	3	No alterations detected
35	4	Faint brown area in anterior half of lens of left eye ∿0.6x0.4mm
35	7	Light brown area in anterior half of lens of left eye ∿0.4x0.3mm; Very faint brown area in anterior half of lens of right eye ∿0.4x0.2mm.
35	9	Light brown area in anterior half of lens of right eye ∿0.6x0.2mm
35	11	Very light brown area in anterior half of lens of both eyes; Left ∿0.4x0.4mm, right ∿0.4x0.4mm.
52	1	Light brown area in anterior half of lens of both eyes; Left ∿0.8x0.5mm, right ∿0.6x0.4mm
52	3	Light brown area in anterior half of lens of both eyes; Left ∿0.5x0.3mm, right ∿0.5x0.3mm
52	7	Light brown area in anterior half of lens of both eyes; Left. ∿0.4x0.4mm, right ∿0.5x0.3mm
52	8	Very faint brown area in anterior half of lens of both eyes; Left ∿0.4x 0.4mm, right ∿0.5x0.4mm

Company Sanitized. Does not contain TSCA CBI

SPECIMENS TO BE PREPARED
FOR EVALUATION BY W. D. KERNS (cont'd)

[redacted]

Dam Code	Fetus Number	Observations during Head Examination
52	9	Light brown area in anterior half of lens of both eyes; Left ∿0.6x0.4mm, right ∿0.6x0.4mm
52	13	No alterations detected
52	15	Light brown area in anterior half of lens of both eyes; Left ∿0.3x0.3mm, right ∿0.5x0.4mm
35	5	Light brown area in center of lens of right eye ∿0.5mm
52	4	No alterations detected
52	5	Light brown area in anterior half of lens of both eyes; Left ∿0.6x0.5mm, right ∿0.5x0.5mm

Note - Alterations listed by severity:

Very faint brown<faint brown<very light brown<light brown

CLL/as
9/30/81

Company Sanitized. Does not contain TSCA CBI

ES-3253 REV. 6-62

ATTACHMENT 7

DON'T SAY IT—WRITE IT

DATE 10/30/81

TO TERATOLOGY SECTION FILE - MEMO

AT

FROM R. E. STAPLES RES

CC: [redacted]

SUBJECT: C-8 ALTERNATES - TELEPHONE CALL FROM D. P. CORDS, 10/30/81

I received a telephone call from Phil Cords (C&P) this morning and responded to several questions asked concerning the report on C-8 given to representatives of several departments yesterday morning.

He also asked about the findings on the alternates and in particular, [redacted] I relayed that the dose levels selected for both the [redacted] were too high and as a result, no fetuses were obtained from the [redacted] and only three litters were obtained from the [redacted] even though exposure was stopped before the completion of the intended treatment period. I relayed also that the lenses of the eyes of most of the fetuses of two of the three litters had apparent discoloration of the lenses as seen under the dissecting scope. Subsequent processing of the fetuses of these two litters for histologic examination revealed only changes seen in the control groups.

Phil Cords asked that he be notified of any meetings scheduled to consider further teratogenicity studies conducted on the alternates and that a notation be included in the file on this point. This memorandum is intended to comply with his request.

RES/mle

Company Sanitized. Does not contain TSCA CBI

THE JOB YOU SAVE MAY BE YOUR OWN—P.I.P.

[redacted]
HLR 441-84

ATTACHMENT 8

C-8 ALTERNATES: MATERNAL AND DEVELOPMENTAL TOXICITY IN THE RAT (PRELIMINARY STUDY, BY GAVAGE)

TABULATION OF RESULTS OF DOSE SELECTION
BASED UPON COMPARISON OF LD50 OR ALD VALUES

Data available at the time this study was started indicated that the approximate LD50 and ALD values in the male rat were as follows:

	C-8	[redacted]	[redacted]	[redacted]	[redacted]
LD50	435	691	2250	3000	7500
ALD	300	550	1500	1500	4000

Therefore, on the basis of demonstrated toxicity, the relative ratios were about as follows:

Toxicity Ratios	1	1	5	5	15
Initial dose levels tested (2 rats/group)	150*	150*	750*	750*	2250**
2nd Pre-test (2 rats/group)	100	100	500	500**	1000**
Used for the main study (7/23/81)	200	100	500	250**	500**
1st Additional test (4 rats/group), Dosed for 5 days				125*	250*
2nd Additional test (4 rats/group), Dosed for 10 days				50[1]	150[1]
Estimated maximum tolerated dose levels for 10 days of dosing				35	100

** severe toxicity including deaths
* decreased body weight gain and adverse clinical signs
[1] body weight gain still too low but no adverse clinical signs

Company Sanitized. Does not contain TSCA CBI