[redacted] INHALATION:
EMBRYO-FETAL TOXICITY AND
TERATOGENICITY STUDY IN THE RAT

[redacted]

HASKELL LABORATORY REPORT NO. 881-81

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Dates: Initiation (breeding date) - April 27, 1981
Completion (sacrifice date)- July 9, 1981

[redacted]

Date Written: December 30, 1981

Date Issued: January 14, 1982

[redacted]

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QUALITY ASSURANCE DOCUMENTATION

STUDY: [redacted] HLR 881-81 [redacted] Inhalation: Embryo-Fetal Toxicity and Teratogenicity Study in the Rat

QUALITY ASSURANCE AUDITS

Audited: July/August 1981

[redacted]

Date Findings Reported to Management and Study Director: August 14, 1981

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CMB/mle
1/8/82

[redacted] INHALATION:
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[redacted] INHALATION:
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[redacted]

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I. SUMMARY

[redacted] was administered to rats by inhalation as a dust (whole body exposure) from Days 6 through 15 of gestation at nominal concentrations of 0, 0.1, 1.0, 10.0, and 25.0 mg/m^3. The actual mean concentrations achieved were 0 and about 0.14, 1.2, 9.9, and 21.0 mg/m^3, respectively. Maternal deaths occurred at the 25.0 mg/m^3 concentration only, but overt toxicity was evident among the surviving dams and among those of the 10.0 mg/m^3 group. A teratogenic response was not demonstrated upon sacrifice of the dams on Day 21 of gestation at any concentration of [redacted] tested. Embryo-fetal toxicity was noted only at the 25.0 mg/m^3 concentration which was overtly toxic to the dams. Other than for a temporary reduction in the body weight of the pups raised by additional dams in the 25.0 mg/m^3 group, no adverse effect that was concentration-related was noted among the dams or their offspring till scheduled sacrifice after weaning. At the 10.0 mg/m^3 level, [redacted] was still toxic but not lethal to the dams; however, no adverse [redacted] related effect was noted among their offspring.

Hence, in this study, [redacted] was not demonstrated to represent a unique hazard to the conceptus.

II. INTRODUCTION

A. Background

Du Pont obtains [redacted] 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 embryo-fetal toxicity and teratogenic potential of [redacted] was requested by E. D. Champney, Polymer Products Department, at a meeting held at Haskell Laboratory on April 9, 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 [redacted] and on several related chemicals.[1] The possible teratogenic activity reported to us by 3M included lens changes in the eyes of the

[redacted]

A. Background (cont)

near-term offspring of rats exposed to the test chemicals by gavage from Days 6 through 15 of gestation. It was not determined whether the lens changes persisted after birth of the rat offspring.

B. Protocol (Appendix A)

A draft protocol was distributed on April 24, 1981, and an MR request was signed on May 6, 1981; the protocol was issued on June 11, 1981, and it was amended on December 3 and on December 30, 1981.

C. Purpose

This study was designed to determine whether the reported teratogenicity of [redacted] in the rat would be expressed after exposure by the inhalation route and, if so, to establish an apparent "no-effect" concentration for the conceptus and to determine whether changes persist after birth. The information gained was to aid in establishment of workplace standards for women of childbearing potential.

III. MATERIALS AND METHODS

The study consisted of two experiments (Table I). Experiment I was a teratogenicity study in which [redacted] was given by inhalation, and the dams were sacrificed on the day before expected delivery (Day 21 G). The dams for Experiment II were exposed as in the first experiment, but were allowed to give birth and the offspring were maintained till 35 days postpartum (Day 35 PP).

A. Test Material

1. Physical characteristics - [redacted] is a white powder which sublimes at [redacted] its molecular weight is [redacted] and its structural formula is [redacted] The purity of the sample used was [redacted] and contaminants present were [redacted] and [redacted] no inhibitors, carriers, or additives were present).

2. Source - The [redacted] sample [redacted] was supplied by the Polymer Products Department. It was assigned Haskell Number 14,045.

3. Test concentrations - The Approximate Lethal Concentration of [redacted] in rats was 0.8 mg/L (800 mg/m^3) after a single 4-hr exposure period by inhalation (head only) as a dust. Liver enlargement and corneal opacity resulted(1).

[redacted] was administered to 7-8 week-old male Crl:CD® rats via inhalation as a dust at nominal concentrations of 0, 1, 8, and 80 mg/m^3. Exposure (head only) was for 6 hr/day, 5 days/week, for two weeks(2). At the highest exposure level, some rats died and among the survivors, body weight was decreased, and liver weight was increased; these effects were not noted at the 8 mg/m^3 concentration. In addition, the serum alkaline phosphatase level in the 8 and 80 mg/m^3 groups was elevated significantly above the control value (3).

Based upon this information and that gained from a brief pretest in non-pregnant female rats, the [redacted] exposure levels selected for testing in the current study were 25.0, 1.0, 0.1, and 0 mg/m^3. After the first of two "Runs" was conducted, the 25.0 mg/m^3 exposure concentration was replaced by one at 10.0 mg/m^3 in response to severe toxicity expressed at the initial concentration.

B. Animals

The rat was chosen for this test because previous toxicity testing on [redacted] was conducted in this species. The Crl:CD®(SD)BR strain was selected because the preliminary teratogenicity test on [redacted] conducted for 3M used the Sprague-Dawley (SD) derived rat obtained from the same supplier, and because extensive background information from previous teratogenicity testing at Haskell Laboratory exists on this rat strain.

B. Animals (cont)

Female rats about 55 days of age (nulliparous) were received from Charles River Breeding Laboratories, Inc., North Wilmington, Massachusetts. For Run I, they arrived on April 16, 1981, and weighed between 151.3 and 190.1 g. For Run II, they arrived on May 1, 1981, and weighed between 152.7 and 188.0 g. Male rats of the same strain and from the same supplier were used for cohabitation with the females. They ranged in age from that of the females to about one month older.

Upon arrival at Haskell Laboratory, each female rat was identified by a combination of toe clips and ear punches and by a cage card bearing its assigned number. The male rats were identified by ear slash and cage card. 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. 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. Animal room temperature was maintained between 72 and 77°F, and relative humidity was maintained between 36 and 70%.

Since the historical incidence of cataracts or opacities in adult CD rats is about 3% (personal communication with James 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.

C. Experimental Design and Procedures (Table I)

The female rats were quarantined for 11 days (Run I) or 12 days (Run II) after arrival at Haskell Laboratory and then they were mated to the males identified under III.B. on an as-needed basis. Mating was verified by

C. Experimental Design and Procedures (cont)

detection of spermatozoa in the vaginal lavage each morning following overnight cohabitation. The day on which spermatozoa were detected was designated as Day 1 of gestation (Day 1 G). For Run I, Day 1 G was April 28 and 29, 1981, for Breeding Lots A and B, respectively; for Run II, it was May 13, 14, and 15, 1981, for Breeding Lots A, B, and C, respectively. After the necessary number of females were bred for each "Run" and before exposure to [redacted] began, 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.

For Experiment I (females sacrificed before parturition), a total of 24 mated females were to have been assigned to each group (12 females/group/Run). However, due to the degree of maternal toxicity in evidence in the 25.0 mg/m^3 group in Run I, this concentration was reduced to 10.0 mg/m^3 for Run II, and 15 mated females were assigned to this new group. Furthermore, two more control groups (six mated females/group) were added to Run II; one was pair-fed to the 25.0 mg/m^3 group, and the other was pair-fed to the 10.0 mg/m^3 group.

For Experiment II (females allowed to give birth) in Run I, 12 mated female rats were distributed to each group. It was not intended that more dams be included in this experiment, but with the addition of the 10.0 mg/m^3 group in Run II, six mated females were added to both the control and the 10.0 mg/m^3 groups. These were all the mated females available unless the exposure period was extended for at least six more days.

The exposure of animals to [redacted] by inhalation was conducted by the Acute Investigations Section, Haskell Laboratory. The inhalation route was selected because it is the route by which Du Pont employees are likely to be exposed to [redacted]. The exposure period was 6 hr/day from Days 6-15 G. Neither feed nor water was available during the exposure period. The procedures used for generation of [redacted] exposure levels and measurement of the concentrations and particle size attained are attached (Attachment 1).

C. Experimental Design and Procedures (cont)

The test groups were exposed (whole body) to the [redacted] in 150-liter glass and stainless steel Rochester-type chambers within which the rats were housed individually in wire-mesh modules. Breeding lots within exposure levels were rotated within the chambers daily. Chamber concentrations of [redacted] were determined by gravimetric analysis either each one-half hour (intermediate and high levels) or each hour (low level), and by a spectrophotometric technique (on each low level sample, and on 5-6 samples per exposure day for the other levels tested). A cascade impactor was used to determine the particle size attained only for the high concentration group on the first and tenth exposure days for Run I, and on the seventh and tenth exposure days for Run II. Control rats were exposed to in-house air in the same type of chamber for the same duration of gestation. The temperatures of each chamber were recorded hourly for each day during the exposure period.

After each exposure session, the rats were housed in suspended, wire-mesh cages (two females/cage), and the racks holding these cages were placed in a walk-in hood. Since air-flow was vertical, the rats for each group were caged vertically such that the control group was on the end with the low level next to it. Baffles were present between cages, but, to further minimize the likelihood of airborne cross-contamination, a single baffle was placed between the rack containing the groups exposed to the two highest levels and the rack containing the low level group and the controls.

For Experiment I, the dams were weighed on the day of arrival, before breeding, and on 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. Feed consumption was measured during gestation (two females/cage due to space restriction). The dams were coded, from before sacrifice by cervical dislocation on Day 21 G, till all maternal and fetal data were collected and till all structural alterations noted among the fetuses were classified, so that personnel involved did not know the exposure group to which any dam or fetus belonged. The identity of each fetus was retained at least till the report was written. After sacrifice, the dams were

C. Experimental Design and Procedures (cont)

examined for gross pathologic changes, 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 (4); in addition, all stunted or malformed fetuses also were examined similarly. The heads of all fetuses examined for visceral alterations were fixed in Bouin's solution to permit examination as described by Barrow and Taylor (5), but only those from the 25.0 mg/m^3 and control groups (Run I) were sectioned free-hand and examined under a stereoscope. This included examination of a vertical cross-section through the center of the eyes as well as of one in front of the eyes and another through the widest portion of the head. Sections containing the eyes of three fetuses from each litter of the 25.0 mg/m^3 group and of two fetuses from each litter of the control group were processed histologically for examination. In Run II, one fetal head from each of four litters from the 10.0 mg/m^3 group and the control group were examined under a stereoscope after free-hand sectioning in front of and behind the eyes, and through the widest portion of the head. The eyes were left intact to minimize processing artifacts. The slices containing the intact eyes were processed histologically and examined. In addition, the heads from all fetuses in the group pair-

C. Experimental Design and Procedures (cont)

fed to the 25.0 mg/m^3 group and the heads from two fetuses with dark eyes detected during external examination (one from the 0.1 mg/m^3 group and one from the 10.0 mg/m^3 group) were processed by the method described for Run I. Histologic specimens were examined by light microscopy.

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.

For Experiment II, the procedures used till Day 21 G were the same as for Experiment I, except that the dams were weighed less frequently during gestation (only on Days 1, 6, and 21), feed consumption was not measured, and the identity of each offspring within litters was not retained. Before expected parturition, each dam was housed in a 13" x 15" polycarbonate cage (with a wire-mesh lid) that contained Bed-O-Cobs® (1/4" size). The bedding 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 22 PP.

The pups from each dam were counted, sexed, weighed, and examined for external alterations toward the end of Day 1 PP. Thereafter, each pup was weighed and inspected for adverse clinical signs on Days 4, 7, 14, and 22 PP; pups with adverse signs were marked for subsequent identification. Neither standardization of litters nor cross-fostering was practiced. The eyes of the pups in all groups of Run I (Experiment II) were examined by a consultant ophthalmologist on Days 15, 16, or 17 PP shortly

C. Experimental Design and Procedures (cont)

after the eyes opened. 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 slit-lamp microscopy. At sacrifice on Day 35 PP, each pup was exsanguinated with a guillotine, 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 (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 among test groups. The significance of differences in feed consumption among groups was determined by a one-way analysis of variance. Dunnett's test (8) was used to test the statistical significance of differences between the control and experimental groups in maternal body weight, in body weight gain, and in feed consumption when the one-way analysis of variance was significant. The presence of a concentration-related response in the incidence of structural alterations and other parameters was determined by Jonckheere's test (9). The significance of differences in incidence between the control group and individual experimental groups was determined by application of the Mann-Whitney U test (10). When more than 75% ties occurred in the data, the Fisher's exact probability test was applied (11). The level of significance selected was $p \leq 0.05$.

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

IV. RESULTS

A. Eye Examination of Prospective Parents

The eyes of 291 male and 357 female rats were examined for Run I on April 24, 1981; 11 males and seven females

A. Eye Examination of Prospective Parents (cont)

were removed from the colony because their eyes were not normal ophthalmoscopically (Attachment 2). For Run II, 210 females were similarly examined on May 5, 1981, and 13 were discarded (Attachment 3). The same males were used for both runs.

B. Maternal Exposure to [redacted]

The animal exposure levels achieved were reported in detail by the Acute Investigations Section (Attachment 1). In brief, for Run I, the nominal exposure concentrations for 6 hr/day from Days 6-15 G were 0, 0.1, 1.0, and 25.0 mg/m^3; the average daily concentrations achieved ($\bar{x}$ + S.D.) by gravimetric measurement were 0, 0.14 + 0.06, 1.2 + 0.5, and 21.0 + 9.7 mg/m^3, respectively. The average mean daily temperature in the chambers was about 76°F and ranged between 72.0 and 78.5°F.

For Run II, the nominal exposure levels were 0, 0.1, 1.0, and 10.0 mg/m^3; the values similarly achieved were 0, 0.12 + 0.04, 1.1 + 0.6, and 9.9 + 5.4 mg/m^3, respectively. The average mean daily temperatures in the chambers were between 76.4 and 77.3°F, and individual temperatures recorded ranged between 72.2 and 78.6°F.

For the two runs, between 73 and 88% of the [redacted] particles in the chambers were respirable (Attachment 1).

C. Clinical Signs Observed

In Experiment I, clinical signs noted during the period of exposure that were concentration-related appeared only in the dams of the 10.0 and 25.0 mg/m^3 groups. In both groups most of the dams developed wet abdomens, which began in the perineal area, had chromodacryorrhea and chromorhinorrhea, and were unkempt. In addition, three of the 12 dams in the 25.0 mg/m^3 group died, and four of the remainder became very lethargic toward the end of the exposure period. Focal alopecia was somewhat more prevalent among the dams of

C. Clinical Signs Observed (cont)

the 10.0 and 25.0 mg/m^3 groups (5/15 and 4/12) than among those of the control group (3/24), but the differences were not statistically significant.

In Experiment II, concentration-related clinical signs appeared only in the same two groups, and they were similar in type and incidence. Again, in the 25.0 mg/m^3 group, maternal deaths occurred (2/12 females).

No adverse clinical signs were noted among the dams of the pair-fed control groups.

D. Maternal Feed Consumption

Feed consumption was measured only in Experiment I. During the period of exposure to [redacted], the feed consumption of the dams in the 10.0 and 25.0 mg/m^3 groups was significantly less than that for the control group (Table II). Feed consumption returned to the control value in the post-exposure period. No significant differences were noted in this regard between the control group and the groups exposed to the two lowest concentrations of [redacted]

The average amount of feed consumed by the pair-fed control groups was not different statistically from that of the groups to which each was matched (Table II).

E. Maternal Body Weight Gain

1. Experiment I

The body weight gain of the dams exposed to [redacted] at the 25.0 mg/m^3 concentration was significantly less during the exposure period (Days 6-15 G) than for the control group (Table III). Although the 10.0 mg/m^3 group also gained less weight during the exposure period than the control group, the difference was not statistically significant. Body weight gain from Days 16-21 G was similar for the groups exposed to [redacted] and for the control group. The body

E. Maternal Body Weight Gain (cont)

1. Experiment I (cont)

weight gain achieved by both the 10.0 and 25.0 mg/m^3 groups after Day 6 G was at least as much as that gained by their respective pair-fed control groups.

2. Experiment II

From Days 6-21 G, the maternal body weight gain of the groups exposed to [redacted] that were not sacrificed at term was not significantly different from the control value (Table VI).

F. Maternal Deaths

In this study, five dams died; all were in groups exposed to [redacted] at the 25.0 mg/m^3 concentration (3/12 in Experiment I, and 2/12 in Experiment II).

In Experiment I, the dams were found dead on Days 12, 13, and 17 G (Table IV). The first was not necropsied; both of the others had grossly observable liver changes, and their uteri contained only resorbed fetuses. The probable cause of death of the dam found dead on Day 17 G was circulatory collapse. All three dams died after showing considerable body weight loss, an unkempt appearance, a wet abdominal surface, and chromodacryorrhea and chromorhinorrhea beginning on the first day of exposure to [redacted] or shortly thereafter.

In Experiment II, one dam was found dead on Day 9 G and the second was autopsied on Day 11 G when she became moribund (Table VI). The uteri of both contained implantations. Their clinical signs preceding death were similar to those described above.

G. Gross Examination of Maternal Organs at Sacrifice

In Experiment I, dark red mottling of the lungs was noted for two of the dams in the 0.1 mg/m^3 group and in one dam in the 10.0 mg/m^3 group. A hematoma between the rectum and pelvic muscles was noted in one dam in the 1.0 mg/m^3 group.

G. Gross Examination of Maternal Organs at Sacrifice (cont)

In addition, in the 25.0 mg/m^3 group, actual liver weight was significantly increased (Table III). The liver weights of the control groups that were pair-fed to the 10.0 and 25.0 mg/m^3 groups were significantly less than those for the [redacted] groups to which they were paired, and than that for the control group (Table III). On a relative weight basis (using corrected Day 21 G maternal body weights), the liver weights ($\bar{x}$ + S.E.M.) for the groups exposed to [redacted] at 10.0 and 25.0 mg/m^3 (5.42 + 0.125, and 6.46 + 0.222, respectively) were still significantly more (MWU - two-tailed) than that for their respective pair-fed control groups (4.58 + 0.164, and 4.62 + 0.103). On the same basis, all groups differed significantly from the control group.

In Experiment II, the dams were examined for clinical signs and weighed on Day 22 PP (Table VI); they were then sacrificed and discarded.

H. Reproductive Effects and Body Weight of the Offspring

In Experiment I, the maintenance of pregnancy and the incidence of resorptions among the surviving dams were not adversely affected by exposure to [redacted] at concentrations up to and including 25.0 mg/m^3 (Table III). In Experiment II, no adverse effect on reproductive performance was demonstrated that was [redacted] concentration-related (Table VI). Parameters examined included Fertility Index, Gestation Index, Viability Index, and Lactation Index.

The mean body weight of fetuses in the 25.0 mg/m^3 group was significantly decreased ($p = 0.002$) below the control value (Table III); but, this also was the case ($p = 0.001$) for the control group pair-fed to the 25.0 mg/m^3 group (Table III). The mean weight of the fetuses in the 10.0 mg/m^3 group, and in its pair-fed control group, were not significantly different from the control group ($p \geq 0.23$). In Experiment II, the neonates in the 25.0 mg/m^3 group also were significantly smaller than those in the control group ($p = 0.002$) but by Day 4 PP, the difference was no longer statistically significant (Table VI).

I. Fetal Alterations

1. Fetal Malformations

A [redacted] concentration-related increase in the incidence of external, visceral, or skeletal malformations was not detected (Table IV). No malformations were seen among the fetuses from dams exposed to [redacted] at 25.0 mg/m^3. Similarly, coded stereoscopic and histomorphologic examination of fetal eyes from heads that were fixed in Bouin's solution did not reveal concentration-related structural alterations (Attachments 4 and 5).

2. Fetal Variations

The overall "percent fetuses with variations" per litter did not increase significantly with increased concentration of [redacted] (Table V). This was the case also for the two specific components of the total incidence v.i.z. "developmental variations," and "variations due to retarded development." Among the "developmental variations" a positive concentration-response also was not obtained for the incidence of subcutaneous hematomas; however, in the 25.0 mg/m^3 group, their incidence was significantly increased ($p = 0.05$), but only upon application of the one-tailed Mann-Whitney U test. Among the "variations due to retarded development," only the incidence of partially ossified sternebrae in the 25.0 mg/m^3 group was similarly increased ($p = 0.04$). Again, the incidence of partially ossified sternebrae was not concentration-related (Jonckheere's test).

In the control group that was pair-fed to the 25.0 mg/m^3 group (Table V), the incidence of partially ossified sternebrae was significantly increased ($p = 0.04$ by the two-tailed MWU test) as was the incidence of variations regarded as being due to retarded development ($p = 0.02$).

J. Pup Alterations

Only one pup was observed to be malformed externally. It occurred in the 0.1 mg/m^3 group (Table VI). On

J. Pup Alterations (cont)

Day 19 PP, it was noted to have severe hydrocephaly and an abnormal gait. Since it was moribund, it was sacrificed the next day.

On Days 15, 16, or 17 PP, coded examination of the pups' eyes in vivo from Run I did not reveal concentration-related malformations. Eye changes were detected in six pups (Table VI and Attachment 6); three of these occurred in the control group and the remaining three occurred in the 0.1 mg/m^3 group. In view of these negative results, similar in vivo examination of the eyes of the pups from Run II was not conducted.

On Day 35 PP, all of the pups were sacrificed, and their eyes were fixed in Bouin's solution. Since concentration-related eye changes were not detected to this stage, in either the fetuses or the pups, pathologic examination of the eyes of the pups of Runs I and II was not conducted.

V. DISCUSSION

In this study, exposure to [redacted] at a concentration of 25.0 mg/m^3 for 6 hr/day from Days 6-15 G was overtly toxic to rats in that 5/24 did not survive to term, and most of the survivors had wet abdomens, reddish-brown discoloration around the eyes, nose, and mouth, lethargy, decreased feed consumption and body weight gain during the exposure period, and an unkempt appearance. None of the 21 dams exposed to [redacted] at 10.0 mg/m^3 died, but they showed similar clinical signs to a lesser degree than that seen at the 25.0 mg/m^3 level. Despite this degree of maternal toxicity, no evidence of [redacted]-related teratogenicity was detected.

The fetuses from the 25.0 mg/m^3 group were significantly smaller than those from the control group. This probably was due to the decrease in maternal feed consumption rather than to a direct response to [redacted] since the fetuses of the control group pair-fed to the 25.0 mg/m^3 group also were significantly smaller than the control fetuses. Similarly, decreased maternal feed consumption also was probably responsible for the significant decrease in weight of the neonates in Experiment II.

V. DISCUSSION (cont)

Maternal liver weight changes among groups on a relative weight basis indicated that exposure to [redacted] at 10.0 mg/m^3 or more resulted in significantly larger livers than in the control group. This effect also was noted in previous toxicity tests with [redacted] (1, 2, 12, 13, 14) and probably represented fatty degeneration accompanied by enzyme induction. This increase in liver weight occurred despite the significant decrease in body weight gain in these groups which significantly reduced the relative liver weights of the pair-fed control groups.

The incidence of resorptions was not increased above the control value at any test concentration of [redacted] or among the pair-fed control groups. The statistically significant decrease obtained in the 1.0 mg/m^3 group was not concentration-related and hence was not demonstrated to be due to [redacted].

The incidence of partially ossified sternebrae was significantly increased among the fetuses of the 25.0 mg/m^3 group by the Mann-Whitney U test (one-tailed). This variation is indicative of developmental delay. Since its incidence was not concentration-related it was not demonstrated to be directly due to [redacted] exposure. It also was probably related to the decreased feed consumption of the dams, since its incidence was even more frequent among the control dams pair-fed to the 25.0 mg/m^3 group.

Two fetuses were observed to have reddish eyes at sacrifice. Histomorphologic examination revealed hyphemia or the presence of blood in the anterior chamber of the eye (Attachment 7). Their incidence was not concentration-related since one occurred in the 0.1 mg/m^3 group and the other in the 10.0 mg/m^3 group. This alteration occurred in seven of 6706 fetuses (0.10%) from six of 681 litters (1.0%) among the control litters of past studies at Haskell Laboratory since March, 1976.

Subcutaneous hematomas were detected in all groups including the control groups. The difference in incidence between the 25.0 mg/m^3 group (7.6%) and the control group (4.0%)

V. DISCUSSION (cont)

was statistically significant but only upon application of a Mann-Whitney U one-tailed test. In addition, the incidence of fetuses with hematomas in the 25.0 mg/m^3 group did not exceed the range of incidence contained among the control groups of past studies (4.6-9.0%) at Haskell Laboratory. Therefore, it is not likely that the magnitude of this difference would be repeatable. Moreover, even if the increase was repeatable, its occurrence would likely depend more upon the presence of the overt toxicity in this group than upon a direct effect of [redacted] per se.

VI. CONCLUSION

[redacted] was not demonstrated to be teratogenic even when administered to rats at overtly toxic concentrations from Days 6 through 15 of gestation. Concentration-related embryo-fetal toxicity, expressed as decreased fetal weight, occurred only at the highest concentration tested (25.0 mg/m^3) which was overtly toxic to the dams. This decreased body weight persisted to Day 1 postpartum but not to Day 4 postpartum. After exposure to [redacted] at the 10.0 mg/m^3 concentration, which was still toxic but not lethal to the dams, no adverse [redacted] related effect was noted among their offspring. Among the fetuses of the 25.0 mg/m^3 group, a marginally significant increase in the incidence of hematomas also occurred; the increase was not demonstrated to be due to [redacted] per se.

Hence, in this study, [redacted] was not demonstrated to represent a unique hazard to the conceptus.

VII. ACKNOWLEDGEMENTS

The concentrations of [redacted] for inhalation were generated, monitored, and analyzed by the Acute Investigations Section, Haskell Laboratory. Histologic specimens were prepared by the Pathology Section, Haskell Laboratory. 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

EXPERIMENTAL DESIGN:
EXPOSURE LEVELS AND DISTRIBUTION OF MATED FEMALES AMONG TEST GROUPS

	Exposure Levels (mg/m^3)	Number Mated Females/Group		Exposure Period
		Experiment I[1]	Experiment II[2]	
RUN I	0	12	12	Days 6-15 G[3] (Air)
	0.1	12	12	Days 6-15 G [redacted]
	1.0	12	12	Days 6-15 G
	25.0	12	12	Days 6-15 G
RUN II	0	12	6	Days 6-15 G (Air)
	0.1	12		Days 6-15 G [redacted]
	1.0	12		Days 6-15 G
	10.0	15	6	Days 6-15 G
	PF10.0[4]	6		Days 6-15 G (Air)
	PF25.0[5]	6		Days 6-15 G (Air)

[1] all Experiment I females sacrificed on Day 21 G (Runs I and II)

[2] all Experiment II females allowed to give birth and to raise their offspring to at least Day 21 postpartum (Runs I and II)

[3] G - day of gestation

[4] control group pair-fed to group given [redacted] at 10.0 mg/m^3

[5] control group pair-fed to group given [redacted] at 25.0 mg/m^3

TABLE II

FEED CONSUMPTION[a] IN RATS EXPOSED TO [redacted] FROM DAYS 6-15 OF GESTATION

Experiment I	Exposure Concentrations[b] (mg/m³)					Pair-Fed[c]	
	0.0	0.1	1.0	10.0	25.0	10.0	25.0
Pre-exposure period (Days 1-5 G)	20.8±0.38	21.0±0.33	21.1±0.35	20.8±0.37	19.0±0.52	20.3±0.64	21.9±1.10
Exposure period[d] (Days 6-15 G)	23.4±0.38	24.0±0.52	23.0±0.45	21.8±0.37$^{\phi}$	18.4±0.46$^{\phi}$	20.6±0.57$^{\phi}$	17.2±0.77$^{\phi}$
Post-exposure period[d] (Days 16-20 G)	27.9±0.78	30.0±1.24	28.3±0.64	27.1±0.74	27.4±0.52	26.6±0.54	26.4±1.12

a - g±S.E.M.; values for non-pregnant rats were excluded
b - nominal concentrations of [redacted]
c - on each day of gestation, non-exposed dams were given the amount of feed consumed on the same gestation day by selected rats in the corresponding exposure group; if the exposed rats were housed two per cage, then their average consumption for each day was the amount offered to the pair-fed rat
d - during the exposure and post-exposure periods, the rats were caged in pairs; the experimental unit used was:

$$\frac{\text{total grams feed consumed per cage per day}}{\text{number of rats per cage}}$$

ϕ - significantly different from control value by Dunnett's test ($p \leq 0.05$)

TABLE III

REPRODUCTION AND FETAL DEVELOPMENT IN RATS EXPOSED TO [redacted] FROM DAYS 6-15 OF GESTATION

Experiment I	Exposure Concentrations[a] (mg/m³)					Pair-Fed[b]	
	0.0	0.1	1.0	10.0	25.0	10.0	25.0
Females							
No. pregnant[c]/ no. mated	23/24	24/24	23/24	15/15	8/12	6/6	5/6
No. deaths	0	0	0	0	3	0	0
No. litters	23	24	23	15	7	6	5
Mean no. corpora lutea	14.5±0.47[d]	14.3±0.51	15.6±0.58	15.2±0.65	15.1±1.42	13.8±0.48	14.4±1.29
Mean no. implants	14.0±0.51	13.4±0.41	13.8±0.28	14.2±0.22	14.0±0.62	14.0±0.63	14.0±1.10
Mean liver weight (g)[e,≠]	15.2±0.30	15.0±0.30	15.4±0.35	16.1±0.50	18.0±0.78[††]	12.8±0.50[††]	12.7±0.48[††]
Mean maternal weight gain (g)[e]							
Days 1-5	29.1±1.99	29.1±1.01	32.5±1.00	29.0±1.39	27.2±1.97	26.2±1.11	31.6±3.33
Days 6-15	57.6±1.69	57.6±2.06	56.7±1.95	50.9±2.21	36.4±5.33[ϕ]	34.9±3.21[ϕ]	22.7±6.93[ϕ]
Days 16-21	71.4±2.19	68.9±1.92	72.8±1.97	72.9±2.77	68.6±3.41	71.3±4.72	68.9±2.73
Days 6-21	129.0±3.39	126.5±3.28	129.5±3.12	123.8±2.86	105.0±7.26[ϕ]	106.2±4.70[ϕ]	91.6±6.66[ϕ]
Days 6-21C[f]	56.7±1.90	56.9±2.02	56.4±2.11	49.1±2.52	37.4±5.39[ϕ]	34.3±2.98[ϕ]	27.0±6.07[ϕ]

TABLE III (CONT)

REPRODUCTION AND FETAL DEVELOPMENT IN RATS EXPOSED TO [redacted] FROM DAYS 6-15 OF GESTATION

Experiment I	Exposure Concentrations[a] (mg/m^3)					Pair-Fed[b]	
	0.0	0.1	1.0	10.0	25.0	10.0	25.0
Fetal Death							
Mean no. resorptions	1.0±0.15	0.8±0.20	0.5±0.12††	0.7±0.21	0.8±0.55	0.7±0.49	0.8±0.58
Mean %	6.6±1.02	5.3±1.34	3.8±0.87†	5.2±1.47	5.9±3.50	4.1±2.94	5.3±3.54
No. litters with total resorption	0	0	0	0	0	0	0
Fetuses							
No. live	299	305	305	202	92	80	66
Mean no. live	13.0±0.47	12.7±0.39	13.3±0.28	13.5±0.34	13.1±0.70	13.3±0.21	13.2±0.97
No. stunted	1	0	0	0	1	0	0
Mean weight (g)[g,≠]	4.0±0.04	3.9±0.05	3.9±0.04	3.9±0.07	3.6±0.12††	3.9±0.12	3.5±0.09††

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TABLE III (CONT)

REPRODUCTION AND FETAL DEVELOPMENT IN RATS EXPOSED TO [redacted] FROM DAYS 6-15 OF GESTATION

a - nominal concentrations of [redacted]
b - on each day of gestation, non-exposed dams were given the amount of feed consumed on the same gestation day by selected rats in the corresponding exposure group; if the exposed rats were housed two per cage, then their average consumption for each day was the amount offered to the pair-fed rat
c - all females had visible sign of pregnancy evident at autopsy except for one in the 25.0 mg/m^3 group in which resorptions were detected only by ammonium sulfide staining; data from this female were excluded from all other calculations
d - $\bar{x}\pm$S.E.M.
e - non-pregnant animals were excluded
f - Day 21C body weight denotes the body weight of females excluding the products of conception (i.e., Day 21 corrected body weight)
g - stunted fetuses were excluded
≠ - significant dose-related response detected by Jonckheere's test ($p\leq0.05$)
ϕ - significantly different from control value by Dunnett's test ($p\leq0.05$)
† - significantly different from control value (one-tailed Mann-Whitney U test, $p\leq0.05$)
†† - significantly different from control value (two-tailed Mann-Whitney U test, $p\leq0.05$)

TABLE IV

FETAL MALFORMATIONS IN RATS EXPOSED TO [redacted] FROM DAYS 6-15 OF GESTATION

Experiment I	Exposure Concentrations[a] (mg/m^3)					Pair-Fed[b]	
	0.0	0.1	1.0	10.0	25.0	10.0	25.0
External Malformations							
No. examined	299/23[c]	305/24	305/23	202/15	92/7	80/6	66/5
Microphthalmia	d		1/1[e]				
Visceral Malformations							
No. examined	159/23	161/24	161/23	110/15	51/7	42/6	34/5
Great vessel malformation		2/2[f]					
Innominate artery-none				1/1[g]			1/1[h]
Renal papilla - none		1/1[i]					
Kidney - very small	1/1[j]						
Head Malformations[k]							
No. examined	90/17	1/1		19/6	51/7		34/5
Skeletal Malformations							
No. examined	299/23	305/24	305/23	202/15	92/7	80/6	66/5
Rib - fused	1/1[j]						
- missing	1/1[l]						

TABLE IV (CONT)

FETAL MALFORMATIONS IN RATS EXPOSED TO [redacted] FROM DAYS 6-15 OF GESTATION

Experiment I	Exposure Concentrations[a] (mg/m^3)					Pair-Fed[b]	
	0.0	0.1	1.0	10.0	25.0	10.0	25.0
Total with Malformations	2/2	3/3	1/1	1/1			1/1
Avg. % Malformed Fetuses per Litter (±S.E.M.)	0.6±0.44	1.0±0.53	0.4±0.40	0.4±0.41			1.4±1.42

a - nominal concentrations of [redacted]
b - on each day of gestation, non-exposed dams were given the amount of feed consumed on the same gestation day by selected rats in the corresponding exposure group; if the exposed rats were housed two per cage, then their average consumption for each day was the amount offered to the pair-fed rat
c - fetuses/litters
d - blanks represent zero incidence
e - fetus weighed 3.38 g; no variations detected
f - one fetus (3.44 g) also had a misaligned sternebra; the other fetus weighed 4.51 g
g - fetus weighed 3.91 g and also had petechiae
h - fetus weighed 3.25 g; no variations detected
i - fetus weighed 3.78 g and also had slight hydroureter
j - stunted fetus weighed 2.41 g and also had calloused, thickened, and partially ossified ribs, petechiae, partially ossified sternebra, misaligned sternebra, pulmonary arteries connected to main pulmonary artery by a common trunk
k - no head malformations were noted
l - fetus weighed 3.82 g; no variations detected

TABLE V

FETAL VARIATIONS IN RATS EXPOSED TO [redacted] FROM DAYS 6-15 OF GESTATION

Experiment I	Exposure Concentrations[a] (mg/m^3)					Pair-Fed[b]	
	0.0	0.1	1.0	10.0	25.0	10.0	25.0
External Variations							
No. examined	299/23[c]	305/24	305/23	202/15	92/7	80/6	66/5
Hematoma	12/10	16/12	11/7	5/4	7/5[†]	4/3	3/3
Petechia	31/13	38/17	30/16	33/14[††]	9/5	16/6[††]	11/3
Visceral Variations							
No. examined	159/23	161/24	161/23	110/15	51/7	42/6	34/5
Renal papilla -reduced	1/1[d]	e					
Renal pelvis -large	1/1				1/1		
Pulmonary arteries -common trunk	5/2	1/1	4/4	3/3		2/2	
Head Variations							
No. examined	90/17	1/1		19/6	51/7		34/5
Eye - blood in anterior chamber		1/1		1/1			

TABLE V (CONT)

FETAL VARIATIONS IN RATS EXPOSED TO [redacted] FROM DAYS 6-15 OF GESTATION

Experiment I	Exposure Concentrations[a] (mg/m^3)					Pair-Fed[b]	
	0.0	0.1	1.0	10.0	25.0	10.0	25.0
Skeletal Variations							
No. examined	299/23	305/24	305/23	202/15	92/7	80/6	66/5
Sternebra							
-misaligned(1)[f]	16/12	24/14	10/9	13/8	5/3		6/3
-misaligned(2+)[f]	6/5	12/9	13/11	6/5	3/3	3/1	4/3
Centrum							
-bipartite	4/4	4/4	6/4	1/1	1/1	1/1	1/1
-dumbbelled	9/6	3/3	7/6	4/3	1/1	2/2	1/1
Rib							
-extra ossification center	50/12[g]	41/15[h]	39/15[i]	26/6	22/7	11/4	3/2[g]
-rudimentary	7/6	3/3	3/3	3/3	2/2		
-extra		1/1		1/1			
-beaded					2/1		
-calloused		3/2					1/1
-wavy	1/1	1/1		1/1			1/1

TABLE V (CONT)

FETAL VARIATIONS IN RATS EXPOSED TO [redacted] FROM DAYS 6-15 OF GESTATION

Experiment I	Exposure Concentrations[a] (mg/m³)					Pair-Fed[b]	
	0.0	0.1	1.0	10.0	25.0	10.0	25.0
Skeletal Variations (cont)							
No. examined with heads	140/23	144/24	144/23	92/15	41/7	38/6	32/5
Skull bones partially ossified							
-parietal	3/3	7/4	2/1		1/1		3/2
-interparietal	2/2	12/4[l]	1/1	4/2		1/1	10/3
-supraoccipital	3/3	9/5	2/2		1/1	3/2	2/1
-squamosal	2/2		2/1		2/1		2/2
Zygoma							
-partially ossified		1/1	2/2		1/1	1/1	2/1
Maxilla							
-partially ossified		1/1	1/1				3/3
Hyoid							
-partially ossified	11/6	9/4	7/5	7/4		4/2	1/1
-unossified	4/2	15/6[m]	13/9		2/2	2/2	5/2[n]

TABLE V (CONT)

FETAL VARIATIONS IN RATS EXPOSED TO [redacted] FROM DAYS 6-15 OF GESTATION

Experiment I	Exposure Concentrations[a] (mg/m^3)					Pair-Fed[b]	
	0.0	0.1	1.0	10.0	25.0	10.0	25.0
Skeletal Variations (cont)							
Subtotal - Developmental Variations							
No. affected	118/22	123/24	100/23	76/15	44/6	31/6	26/5
Avg. % Affected Fetuses per Litter (±S.E.M.)	40.6±5.16	41.6±4.06	31.6±3.84	37.6±5.08	47.6±6.62	38.7±5.14	38.8±5.98
Sternebra							
-partially ossified	27/9	10/6	13/8	12/6	17/5[j,†]	1/1	25/4[k,††]
-unossified		3/2	3/2		4/2		
Centrum							
-partially ossified	2/2						
Rib							
-partially ossified		2/1					
Ischium							
-partially ossified	1/1	2/2					1/1
Pubis							
-partially ossified		4/3	1/1				2/2
-unossified							1/1

TABLE V (CONT)

FETAL VARIATIONS IN RATS EXPOSED TO [redacted] FROM DAYS 6-15 OF GESTATION

Experiment I	Exposure Concentrations[a] (mg/m³)					Pair-Fed[b]	
	0.0	0.1	1.0	10.0	25.0	10.0	25.0
Skeletal Variations (cont)							
Subtotal - Variations Due to Retarded Development							
No. affected	45/14	45/11	34/14	17/7	24/6	7/3	31/5
Avg. % Affected Fetuses per Litter (±S.E.M.)	14.3±4.01	14.2±4.35	10.9±2.84	8.4±3.03	25.3±8.50	8.5±3.93	49.8±16.07††
TOTAL							
No. Fetuses with Variations	144/22	140/24	123/23	87/15	55/6	34/6	42/5
Avg. % Fetuses with Variations per Litter (±S.E.M.)	48.7±5.07	49.4±4.42	40.1±3.43	42.8±5.14	58.4±5.98	42.2±5.91	64.5±10.42

TABLE V (CONT)

FETAL VARIATIONS IN RATS EXPOSED TO [redacted] FROM DAYS 6-15 OF GESTATION

a - nominal concentrations of [redacted]
b - on each day of gestation, non-exposed dams were given the amount of feed consumed on the same gestation day by selected rats in the corresponding exposure group; if the exposed rats were housed two per cage, then their average consumption for each day was the amount offered to the pair-fed rat
c - fetuses/litters
d - slight hydroureter also was detected
e - blank cells indicate zero incidence
f - (1) and (2+) denote that 1, or 2 or more sternebrae were misaligned, respectively
g - one was located in the cervical region, all others were located in the lumbar region
h - 14 occurred in two litters
i - 16 occurred in two litters
j - 7 occurred in one litter
k - 18 occurred in two litters
l - 5 occurred in one litter
m - 11 occurred in two litters
n - 4 occurred in one litter
† - statistically significant difference detected only if Mann-Whitney U test is one-tailed ($p \leq 0.05$)
††- statistically significant difference detected by two-tailed Mann-Whitney U test ($p \leq 0.05$)

TABLE VI

REPRODUCTION AND DEVELOPMENT OF OFFSPRING OF RATS EXPOSED TO [redacted] FROM DAYS 6-15 OF GESTATION

Experiment II	Exposure Concentrations[a] (mg/m³)				
	0.0	0.1	1.0	10.0	25.0
Females					
No. pregnant/no. mated	18/18	10/12	11/12	6/6	11[b]/12
No. deaths	0	0	0	0	2[c]
No. litters	18	10	11	6	9
Fertility Index (%)[d]	100	83.3	91.7	100	91.7
Gestation Index (%)[e]	100	100	100	100	100
Mean maternal weight gain (g)					
Days 1-5 G	31.1±1.19[f]	31.8±2.08	31.7±2.16	28.2±2.44	34.8±2.38
Days 6-21 G	112.5±4.33	115.8±8.07	116.2±7.77	118.9±3.28	97.0±8.79
Days 1 PP-22 PP	-3.4±0.58	-2.8±0.75	-2.0±0.81	-3.1±0.78	-2.2±0.52
Offspring					
At delivery (Day 1 PP)					
No. litters	18	10	11	6	9
No. live pups/litter	12.3±0.60[f]	12.1±0.80	11.2±1.27	13.3±0.67	11.0±1.26
No. live pups (%)	222(99.6)	121(99.2)	123(98.4)	80(100)	99(99.0)
No. dead pups (%)	1(0.4)	1(0.8)	2(1.6)	0(0)	1(1.0)

TABLE VI (CONT)

REPRODUCTION AND DEVELOPMENT OF OFFSPRING OF RATS EXPOSED TO [redacted] FROM DAYS 6-15 OF GESTATION

Experiment II	Exposure Concentrations[a] (mg/m^3) 0.0	0.1	1.0	10.0	25.0
Offspring (cont)					
After delivery[g]					
No. dead	1	1[h]	0	0	0
No. cannibalized	0	2	0	0	2
No. live (Day 22 PP)	221[i]	118	123	80	97
No. males/females	115/106	62/56	70/53	44/36	45/52
Viability Index (%)[j]	100	99.2	100	100	99.2
Lactation Index (%)[k]	99.5	98.3	100	100	99.1
Mean weight (g)					
Day 1 PP[≠]	6.8±0.11[f]	7.0±0.18	6.7±0.18	6.6±0.23	6.1±0.15[††]
Day 4 PP	10.3±0.25	10.9±0.32	10.9±0.39	9.9±0.43	9.7±0.33
Day 7 PP	15.0±0.44	15.6±0.46	15.9±0.63	14.8±0.69	14.3±0.38
Day 14 PP	29.2±1.06	30.1±0.89	30.6±1.29	28.0±1.23	29.0±0.55
Day 22 PP	50.1±1.82	51.4±1.73	52.0±2.46	48.4±2.15	49.0±0.88
Alterations					
No. examined externally	222/18[l]	121/10	123/11	80/6	99/9
No. malformed		1/1[m]			
No. eyes (pairs) examined in vivo[n]	141/12	118/10	123/11	--	97/9
No. with alterations	3/3[o]	3/2[p]	0	--	0

TABLE VI (CONT)

REPRODUCTION AND DEVELOPMENT OF OFFSPRING OF RATS EXPOSED TO [redacted] FROM DAYS 6-15 OF GESTATION

a - nominal concentrations of [redacted]
b - two females with implants that did not survive the exposure period are included
c - one female was necropsied on Day 11 G since she was not expected to survive the entire exposure period (she had 12 implants); a second female was found dead on Day 9 G (she had 13 implants)
d - Fertility Index is the percentage of matings resulting in pregnancy (excludes females that did not survive to Day 21 G)
e - Gestation Index is the percentage of pregnancies resulting in the birth of live litters (excludes females that did not survive to term)
f - $\bar{x}$±S.E.M.
g - to Day 22 PP
h - pup with domed head was necropsied on Day 20; it was not expected to survive the lactation period
i - one pup was necropsied on Day 19 as a control for a pup from the 0.1 mg/m^3 group; it is counted as being alive for the Lactation Index
j - Viability Index is the percentage of animals born that survived four days or longer
k - Lactation Index is the percentage of animals alive on Day 4 PP that survived to Day 22 PP
l - pups/litters
m - one pup was found to have a domed head and abnormal gait on Day 19. Since it was not expected to survive the lactation period, it was necropsied on Day 20; it was hydrocephalic
n - all pups from Run I that were alive were examined on Day 15, 16, or 17 PP
o - opacities of posterior lens pole
p - two with pre-retinal hemorrhage and one with incomplete mydriasis and red oval opacity on the central endothelium
≠ - significant dose-related response detected by Jonckheere's test ($p \leq 0.05$)
†† - significantly different from control value (two-tailed Mann-Whitney U test, $p \leq 0.05$)

CENTRAL RESEARCH AND DEVELOPMENT DEPARTMENT
HASKELL LABORATORY FOR TOXICOLOGY
AND INDUSTRIAL MEDICINE
ELKTON ROAD
NEWARK, DELAWARE

APPENDIX A

PROTOCOL FOR INHALATION TERATOGENICITY STUDY IN RATS WITH [redacted]

I. BACKGROUND

This study was requested by [redacted], Polymer Products Department [redacted] at a meeting held at Haskell Laboratory on April 9, 1981. The need for such a study * emanated from TSCA Section 8(e)'s filed by 3M between the last part of 1980 and March 20, 1981. The adverse effects seen in studies conducted for 3M consisted of lens changes in the eyes of term offspring from rats exposed to the test chemicals by gavage from Days 6 through 15 of gestation. For additional information, see memos: [redacted], March 26, 1981, and [redacted], March 30, 1981.

For the development of this protocol, the draft of Haskell Laboratory Report [redacted] on the known toxicity of [redacted] was reviewed, and information gleaned from 3M also was considered.

II. MATERIALS AND METHODS

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

* Du Pont classified information

II. MATERIALS AND METHODS (CONT)

A. Test Chemical

[redacted], received from the Polymer Products Department, was assigned Haskell Number 14,045.

B. Animals

Crl:CD®(SD)BR female rats 48-54 days of age (nulliparous), weighing about 170 g will be ordered from Charles River Breeding Laboratories, Inc., North Wilmington, Massachusetts. The rats will be quarantined for at least 1 week after arrival by air-conditioned truck. Upon arrival each will be identified by a combination of ear punches and toe clips, and by a unique identification number on a cage card. They will be mated to mature males of the same strain. Mating will be verified by detection of spermatozoa in the vaginal lavage each morning (Day 1 of gestation) following overnight cohabitation.

The rat was selected for this study because 3M reported a positive teratogenic response in this species, and we wish to duplicate this finding at Haskell Laboratory. In addition, the degree of toxicity of this chemical 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 only on this rat strain.

Since the historical incidence of cataracts or opacities in CD rats is 3%, according to consulting opthalmologist [redacted] all animals will be screened for these alterations before breeding. The screening procedure will include examination of both eyes and elimination of affected rats.

C. Exposure by Inhalation

The inhalation route was selected since it is the route by which Du Pont employees are likely to be exposed to [redacted]

C. Exposure by Inhalation (cont)

The test groups will be exposed to the [redacted] in Rochester Chambers; free moving rats will be given whole body exposure for 6 hours/day from Days 6 through 15 of gestation. Chamber concentrations of [redacted] will be determined about every hour by gravimetric analysis and at least twice daily by a spectrophotometric technique. Control rats will be exposed to in-house air in the same type of chamber for the same duration of gestation.

After each exposure session, the rats will be housed in suspended wire-mesh cages (two females per cage), and the racks holding these cages will be placed in a walk-in hood. The rats for each group will be caged vertically starting with the highest exposure level and progressing to the lowest exposure level and then the control group. To minimize the likelihood of airborne cross-contamination, a baffle will be placed between the rack containing the groups exposed to the two highest levels and the rack containing the low level group and the controls. In addition, in the latter rack, baffles will be inserted between each cage containing control rats and those containing rats exposed to the lowest concentration of the test chemical.

D. Exposure Levels

Concentrations recommended for testing are 25.0, 1.0, 0.1, and 0 mg/m^3. Based on the limited toxicity data available at Haskell Laboratory and the results reported by 3M, these concentrations are expected to yield an "effect" level and an apparent "no-effect" level of exposure.

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 exposure, then minimal switching within breeding dates will be used to alleviate the statistically significant differences. A total of 24 mated females will be assigned to each group for Experiment I. About 12 females will be assigned to each group for Experiment II. Mated rats that are ill or that do not gain weight properly will be discarded before distribution

F. Husbandry

Upon arrival at Haskell Laboratory, the female rats will be housed two per 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, the 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 will be maintained for each animal except that feed consumed daily will be noted as a total for the animals caged together. The final report and raw data will be forwarded to the Information Section of Haskell Laboratory for archiving.

H. Safety Precautions and Disposal of Waste Material

Access to the inhalation laboratory will be limited during the periods that rats are being placed into or removed from the chambers. Chamber exhausts will be filtered through cotton filters and MSA charcoal filters before venting the air to the hood. Waste material containing [redacted] will be packaged in polyethylene-lined Fiberpaks® or plastic-lined waste bags for incineration at Stine Laboratory. All persons handling the test material or exposed to the study animals will wear the protective equipment and follow the safety procedures presented to the Process Hazards Review Committee.

I. Parameters to be Studied

Experiment I - Full 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. 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

e. 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

f. Corpora lutea - counted and recorded for each ovary

g. 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

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

I. Parameters to be Studied (cont)

2. Fetuses

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

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 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 of all fetuses examined for soft tissue alterations will be fixed in Bouin's solution. Each 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 as described by Barrow and Taylor ('69).[1] The sections containing the eyes of at least the control and high exposure levels will be processed by the Histology group for examination 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); heads of fetuses examined for soft tissue alterations will be excluded.

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

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

I. Parameters to be Studied (cont)

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, breeding daily, at least on 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)

I. Parameters to be Studied (cont)

Experiment II - Extended Teratogenicity Study

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 and for each dead pup. The sex ratio of pups alive 22 days post partum also will be presented.

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

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 and processed by the Histology group for examination by a pathologist. The identity of eyes from pups previously marked will be retained. Otherwise, all eyes from pups will be identifiable only by dam number.

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 $p \leq 0.05$. In addition, the reproductive indices given earlier will be calculated.

IV. CRITICAL DATES

Starting Date (breeding): April 27, 1981

Completion: November 15, 1981

REPORT BY: R. E. Staples

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

B. A. Burgess
Research Toxicologist
Acute Investigations
Toxicology and Pathology

APPROVED BY: J. G. Aftosmis

J. G. Aftosmis
Associate Director
Toxicology and Pathology

G. L. Kennedy
Chief
Acute Investigations
Toxicology and Pathology

RES/BAB/mle
6/9/81

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: [redacted]

An amendment to the protocol for the teratogenicity study of [redacted] is attached. Please note that deviation #3 in the audit report [redacted] for this study no longer pertains; all skeletal specimens were examined for alterations.

RES/mle
Attachment

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

AMENDMENT TO THE
PROTOCOL FOR INHALATION TERATOGENICITY STUDY IN
RATS WITH [redacted]

The following changes were made after initiation of the study:

Page 6

II. MATERIALS AND METHODS

I. Parameters to be Studied

Experiment I - Full Teratogenicity Study

2. Fetuses

d. Soft tissue alterations

As stated in the protocol, the heads of all fetuses examined for soft tissue alterations were fixed in Bouin's solution and retained for subsequent examination. For Run I, all head specimens in the 0.0 mg/m^3 and 25.0 mg/m^3 groups were examined grossly after being sliced in vertical cross-section in front of the eyes, through the center of the eyes, and through the widest portion of the head. These specimens were embedded, and two specimens from each litter in the 0.0 mg/m^3 group and three from each litter in the 25.0 mg/m^3 group were examined by a pathologist. Since no compound-related effects were detected grossly or microscopically, the remaining specimens were not examined.

For Run II, one specimen from each of four litters in the 0.0 mg/m^3 and 10.0 mg/m^3 groups was examined grossly. They were sliced in vertical cross-section in front

d. Soft tissue alterations (cont)

of and behind the eyes and through the widest portion of the head. The eyes were left intact to minimize processing artifacts. These specimens were processed and examined microscopically by a pathologist. Because no compound-related effects were detected grossly or microscopically, the remaining specimens were not examined.

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II. MATERIALS AND METHODS

I. Parameters to be Studied

Experiment II - Extended Teratogenicity Study

2. Offspring

g. Eye examinations

No compound-related effects were detected *in vivo* by the ophthalmologist among the pups from Run I, and the highest exposure level in Run II (10.0 mg/m^3) was lower than that for Run I (25.0 mg/m^3). Therefore, the eyes of pups from Run II were not examined *in vivo* by an ophthalmologist.

As stated in the protocol, each pup was exsanguinated at sacrifice and its eyes were removed, fixed, and identified. However, since no compound-related effects were detected in the previous test specimens during clinical, gross, or microscopic examination, the eye specimens were not processed or examined but were retained for storage.

Page 9

II. MATERIALS AND METHODS

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.

REPORT BY: _____

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

_____

B. A. Burgess
Research Toxicologist
Acute Investigations
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APPROVED BY: _____

J. G. Aftosmis
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_____

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RES/mle
12/3/81

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

R E V I S E D

[redacted]

December 30, 1981

M E M O R A N D U M

TO: QUALITY ASSURANCE COMMITTEE - C. M. BARBA

FROM: R. E. STAPLES RES

SUBJECT: [redacted]: PROTOCOL AMENDMENT FOR THE INHALATION TERATOGENICITY STUDY

An amendment to the protocol for the teratogenicity study of [redacted] is attached.

RES/mle
Attachment(1)

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R E V I S E D

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
INHALATION TERATOGENICITY STUDY IN RATS
WITH [redacted]

The following changes were made after initiation of the study:

II. MATERIALS AND METHODS

D. Exposure Levels

- After severe clinical signs and mortality occurred in females in the 25.0 mg/m^3 exposure group (Run I), the exposure level was reduced to 10.0 mg/m^3 for Run II. The 10.0 mg/m^3 exposure group now consists of 12 of the females originally scheduled for the 25.0 mg/m^3 group (Run II), and an additional nine females. Fifteen of these females will be used for Experiment I, six for Experiment II.

- Females in the 25.0 mg/m^3 exposure group consumed significantly less feed on the exposure days than did the control group. To help determine whether the reduced intake of feed would be responsible for any adverse effects that might be noted among the fetuses, control groups pair-fed to the 25.0 and the 10.0 mg/m^3 groups were added to Run II.

AMENDMENT TO THE PROTOCOL
FOR INHALATION TERATOGENICITY
STUDY IN RATS WITH [redacted]

REPORT BY: R. E. Staples

R. E. Staples
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B. A. Burgess
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APPROVED BY: J. G. Aftosmis

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G. L. Kennedy
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RES/mle
12/30/81

FOR DU PONT USE ONLY

E. I. du Pont de Nemours and Co., Inc.
Haskell Laboratory for Toxicology and Industrial Medicine
Elkton Road, P. O. Box 50,
Newark, Delaware 19711

HASKELL LABORATORY IN HOUSE REPORT [redacted]

Material Tested	Haskell No.	Other Codes
[redacted]	14,045	None

Study Initiated/Completed
5/2/81-5/13/81 - Part I
5/18/81-5/29/81 - Part II

Material Submitted by
[redacted]
Washington Works
Polymer Products Department

IN-HOUSE REPORT: INHALATION EXPOSURE RESULTS FOR THE

[redacted] TERATOLOGY STUDY - PART I AND PART II

I. Introduction

This report summarizes the methodologies for generation and analysis of exposure atmospheres for the inhalation teratology study of [redacted] This test was conducted in two parts.

II. Procedures

A. Protocol

Pregnant Crl:CD® rats for this study were supplied and cared for by Haskell's teratology group. Dams were mated in lots, two for Part I and three for Part II, by caging them overnight with males. Mating was verified by detection of spermatozoa in a vaginal lavage each morning following overnight cohabitation.

Mated rats were assigned to the following test groups:

Part I
Group I - Air Control
Group II (low) - Design Level of 0.1 mg/m^3
Group III (intermediate) - Design Level of 1.0 mg/m^3
Group IV (high) - Design Level of 25.0 mg/m^3

Part II

Group V - Air Control
Group VI (low) - Design Level of 0.1 mg/m^3
Group VII (intermediate) - Design Level of 1.0 mg/m^3
Group VIII (high) - Design Level of 10.0 mg/m^3

These groups were exposed to the indicated design concentrations for 6 hours a day on days 6 through 15 of gestation. Because of different breeding dates a total of 11 exposures were necessary for Part I, and 12 exposures for Part II, to expose all animals on these gestation days.

Dams were exposed in 150 L glass and stainless steel chambers. During exposure, dams were housed in compartmentalized, stainless steel, wire mesh modules. These were stacked one over the other. X-Cold Bricks®, placed on the outside surface of the chambers, were used to control chamber temperature.

B. Atmospheric Generation

Dust atmospheres of [redacted] were generated with an airtight, two stage glass apparatus composed of a round bottom reservoir and a cyclone shaped elutriator. A generation air stream introduced at the bottom of the reservoir carried dust particles upward to the elutriator. Dilution/carrier air entered tangentially into the top of the elutriator and swept airborne dust particles into the exposure chamber. Attached to the lower reservoir was a surface contact vibrator, which vibrated the entire apparatus minimizing buildup on apparatus walls.

The dust atmosphere was vented from the bottom of the chamber, passed through a hand-made cotton fiber filter, and an MSA cartridge filter. Air, thus filtered, was then exhausted into the hood.

C. Atmosphereic Analysis

Two methods were employed to determine chamber concentrations of [redacted]

- Gravimetric samples were taken from all chambers at regular intervals (low @ 1 hr; intermediate and high @ 1/2 hour). A known volume of chamber air was drawn though preweighed Gelman glass fiber filters (Type AE, 25mm). Filters were reweighed and [redacted] concentration calculated from filter weight gain. Filters were weighed on a Cahn® Model 26 Automatic Electrobalance.

- Chamber atmospheres were also analyzed by a spectrophotometric technique**. [redacted] absorbed to filters was desorbed with an acidified aqueous methylene blue solution. The [redacted] methylene blue complex formed under these conditions was extracted into chloroform and [redacted] concentrations determined spectrophoto-

metrically by comparison with standards prepared in the same manner. Samples were analyzed using a Bausch and Lomb Model 710 Spectrophotometer at a wavelength of 627.6 um.

All samples taken from the low level were analyzed by this procedure. Due to the excessive labor involved however, only 5-6 samples per exposure were analyzed from the intermediate and high levels. Filter samples taken from the control chamber were also periodically analyzed.

A mean concentration (± standard deviation) was calculated for each exposure.

Chamber temperature was monitored each hour with a thermometer.

Oxygen depletion is not a problem with "all-air" systems, so oxygen was not monitored during these exposures.

Due to the low design concentrations, particle size could be obtained from the high levels only. An eight stage Sierra Cascade Impactor Model #218K was used to determine particle size.

III. Results

Test concentrations were not visible to the naked eye but particulate could be seen by turning off room lights and passing a flashlight beam through the darkened chamber.

Control samples were indistinguishable from blanks.

Particle size samples were taken from the first and tenth exposures during Part I. Mass median diameters for these samples were 1.4 and 2.8 u, with 90 and 88% total respirable content. Particle size samples were taken from the seventh and tenth exposures during Part II. Mass median diameter for these samples were 9.4 and 8.6 u, with 73 and 94% total respirable content. The data would indicate that these atmospheres were of highly respirable character.

Analytical results are summarized in Tables I through IV (Part I) and V throught VIII (Part II):

TABLE I

Low Level Analytical Data (Part I)

[redacted]

Exposure #	Mean Chamber Concentration (mg/m^3 + S.D.) Gravimetric	Spectrophotometric
1	0.12 ± 0.06	0.08 ± 0.05
2	0.14 ± 0.07	0.08 ± 0.03
3	0.18 ± 0.08	0.12 ± 0.04
4	0.13 ± 0.05	0.12 ± 0.06
5	0.12 ± 0.08	0.09 ± 0.04
6	0.18 ± 0.10	0.13 ± 0.09
7	0.13 ± 0.06	0.09 ± 0.04
8	0.12 ± 0.02	0.10 ± 0.02
9	0.13 ± 0.03	0.10 ± 0.04
10	0.11 ± 0.05	0.12 ± 0.03
11	0.12 ± 0.04	0.14 ± 0.02
Overall Mean† ± S.D.	0.14 ± 0.06	0.11 ± 0.05

† Mean and S.D. of all samples throughout exposure period.

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TABLE II

Intermediate Level Analytical Data (Part I)

[redacted]

Exposure #	Mean Chamber Concentration (mg/m^3 + S.D.) Gravimetric	Spectrophotometric
1	1.3 ± 0.4	1.3 ± 0.5
2	1.3 ± 0.5	1.1 ± 0.5
3	0.9 ± 0.2	1.0 ± 0.2
4	1.1 ± 0.3	1.0 ± 0.2
5	1.1 ± 0.3	1.3 ± 0.3
6	1.3 ± 0.7	1.5 ± 1.0
7	1.1 ± 0.8	0.8 ± 0.4
8	1.1 ± 0.2	1.1 ± 0.2
9	1.2 ± 0.5	1.3 ± 0.7
10	1.4 ± 0.5	1.5 ± 0.5
11	1.2 ± 0.8	1.1 ± 0.2
Overall Mean† ± S.D.	1.2 ± 0.5	1.2 ± 0.5

† Mean and S.D. of all samples throughout the exposure period.

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TABLE III

High Level Analytical Data (Part I)

[redacted]

Exposure #	Mean Chamber Concentration (mg/m^3 + S.D.) Gravimetric	Spectrophotometric
1	18.0 ± 15.7	24.6 ± 23.2
2	15.9 ± 5.9	12.0 ± 3.0
3	23.8 ± 19.4	26.7 ± 16.8
4	18.2 ± 5.1	18.8 ± 1.9
5	22.0 ± 6.5	22.7 ± 6.6
6	23.0 ± 4.7	19.9 ± 5.7
7	21.9 ± 6.4	21.9 ± 6.6
8	21.0 ± 8.8	18.5 ± 6.4
9	21.7 ± 7.7	23.3 ± 7.4
10	21.7 ± 10.0	14.8 ± 5.2
11	23.3 ± 6.2	24.1 ± 7.7
Overall Mean† ± S.D.	21.0 ± 9.7	20.5 ± 10.0

† Mean and S.D. of all samples throughout the exposure period.

TABLE IV

Mean Chamber Temperature Data (°F+S.D.) (Part I)

Exposure #	Control	Low	Intermediate	High
1	72.0 ± 0.0	77.5 ± 0.6	77.1 ± 0.6	75.8 ± 1.3
2	78.0 ± 1.4	77.0 ± 1.7	77.2 ± 2.2	75.8 ± 2.9
3	77.3 ± 0.8	75.0 ± 1.3	77.0 ± 1.1	75.5 ± 1.0
4	78.0 ± 1.1	76.3 ± 0.8	76.6 ± 0.7	76.8 ± 1.2
5	77.3 ± 1.5	76.2 ± 1.3	76.0 ± 1.2	78.2 ± 1.2
6	76.6 ± 0.9	76.6 ± 0.5	76.7 ± 0.7	76.2 ± 1.1
7	77.4 ± 0.5	75.8 ± 1.6	75.8 ± 1.6	75.8 ± 2.5
8	78.5 ± 0.8	77.7 ± 1.5	77.7 ± 1.2	77.0 ± 0.9
9	76.4 ± 1.1	76.6 ± 1.8	75.8 ± 1.6	77.6 ± 1.1
10	77.3 ± 2.2	75.8 ± 1.5	76.8 ± 1.6	77.1 ± 1.3
11	72.3 ± 1.9	73.7 ± 1.5	74.5 ± 0.8	75.0 ± 1.1
Overall Mean† ± S.D.	76.5 ± 2.4	76.2 ± 1.7	76.5 ± 1.5	76.4 ± 1.7

† Mean and S.D. of all values throughout exposure period.

TABLE V

Low Level Analytical Data (Part II)

[redacted]

Exposure #	Mean Chamber Concentration (mg/m^3 + S.D.) Gravimetric	Spectrophotometric
1	0.11 ± 0.05	0.13 ± 0.05
2	0.11 ± 0.03	0.11 ± 0.04
3	0.14 ± 0.03	0.12 ± 0.02
4	0.11 ± 0.03	0.10 ± 0.03
5	0.10 ± 0.05	0.07 ± 0.02
6	0.13 ± 0.03	0.11 ± 0.02
7	0.15 ± 0.01	0.14 ± 0.01
8	0.13 ± 0.04	0.11 ± 0.02
9	0.11 ± 0.04	0.07 ± 0.02
10	0.15 ± 0.04	0.13 ± 0.02
11	0.12 ± 0.05	0.09 ± 0.05
12	0.12 ± 0.04	0.12 ± 0.04
Overall Mean†	0.12 ± 0.04	0.11 ± 0.04

† Mean and S.D. of all samples throughout exposure period.

TABLE VI

Intermediate Level Analytical Data (Part II)

[redacted]

Exposure #	Mean Chamber Concentration (mg/m^3 + S.D.) Gravimetric	Spectrophotometric
1	1.1 ± 1.20	0.67 ± 0.32
2	1.1 ± 0.56	0.96 ± 0.47
3	1.2 ± 0.47	1.1 ± 0.29
4	1.3 ± 0.27	1.2 ± 0.21
5	1.1 ± 0.37	0.80 ± 0.37
6	1.2 ± 0.66	1.0 ± 0.33
7	1.2 ± 0.24	1.2 ± 0.17
8	1.1 ± 0.61	1.1 ± 0.45
9	0.78 ± 0.25	0.82 ± 0.08
10	0.75 ± 0.39	0.80 ± 0.17
11	0.97 ± 0.33	0.85 ± 0.16
12	1.3 ± 0.59	1.3 ± 0.71
Overall Mean†	1.1 ± 0.56	0.98 ± 0.37

† Mean and S.D. of all samples throughout exposure period.

TABLE VII

High Level Analytical Data (Part II)

[redacted]

Exposure #	Mean Chamber Concentration (mg/m^3 + S.D.) Gravimetric	Spectrophotometric
1	8.4 ± 3.74	10.3 ± 2.45
2	8.8 ± 3.16	8.1 ± 2.58
3	8.3 ± 3.80	10.0 ± 4.99
4	10.8 ± 6.56	11.1 ± 7.69
5	9.5 ± 5.07	9.9 ± 6.60
6	12.5 ± 6.58	13.6 ± 6.27
7	10.3 ± 5.30	11.8 ± 6.09
8	10.9 ± 8.66	11.1 ± 4.97
9	9.9 ± 6.41	14.6 ± 7.35
10	9.8 ± 5.19	12.5 ± 6.21
11	8.7 ± 3.84	7.9 ± 3.86
12	10.7 ± 1.35	12.2 ± 1.65
Overall Mean†	9.9 ± 5.41	11.1 ± 5.3

† Mean and S.D. of all samples throughout exposure period.

Company Sanitized. Does not contain TSCA CBI

TABLE VIII

Mean Chamber Temperature Data (°F +S.D.) (Part II)

Exposure #	Control	Low	Intermediate	High
1	78.4 ± 0.5	74.7 ± 1.4	77.5 ± 1.5	78.0 ± 1.1
2	77.2 ± 1.0	76.7 ± 1.2	72.2 ± 3.4	74.3 ± 1.0
3	78.3 ± 1.3	75.9 ± 2.6	77.1 ± 1.5	77.0 ± 2.3
4	77.3 ± 1.0	75.6 ± 1.4	77.0 ± 2.0	78.4 ± 0.8
5	77.0 ± 1.5	76.8 ± 1.7	77.7 ± 1.0	78.1 ± 1.3
6	76.8 ± 2.3	77.7 ± 2.3	78.0 ± 1.2	77.6 ± 1.5
7	77.1 ± 1.3	76.3 ± 1.0	77.5 ± 0.5	77.5 ± 1.2
8	77.6 ± 1.0	77.5 ± 0.8	77.2 ± 0.8	77.3 ± 1.7
9	78.6 ± 0.5	77.9 ± 1.1	75.9 ± 1.9	77.6 ± 1.9
10	77.2 ± 1.3	76.8 ± 1.3	77.2 ± 0.8	78.0 ± 1.8
11	77.0 ± 1.7	76.0 ± 0.6	76.1 ± 0.7	76.2 ± 1.8
12	75.3 ± 0.8	74.8 ± 0.8	76.0 ± 0.9	75.3 ± 1.0
Overall Mean† ±S.D.	77.3 ± 1.4	76.4 ± 1.7	76.6 ± 2.1	77.1 ± 1.8

† Mean and S.D. of all values throughout the exposure period.

During exposure no clinical observations were noted in Groups I, II, III, V, VI, or VII. A dry red discoloration around the mouth was observed the morning following the first exposure in Group IV and VIII rats, which persisted for the remainder of the exposure period. No other overt clinical signs were noted in rats exposed to [redacted]

During Part I, four Group IV rats died during the exposure phase of the test. One rat was found dead each day, on the mornings following the 3rd, 5th, 7th, and 8th exposures.

No deaths occurred during Part II.

Summary

The purpose of this study was to determine the teratogenic effects of [redacted] on pregnant Crl:CD® rats. The test was conducted in two parts, in which dams were exposed 6 hours/day on days 6 through day 15 of gestation.

Atmospheric concentrations were determined gravimetrically and spectrophotometrically. Mean gravimetric concentrations were 0, 0.14, 1.2 and 21.0 mg/m^3 in Part I and 0, 0.12, 1.1, and 9.9 mg/m^3 in Part II. These results were confirmed by spectrophotometric analysis.

In Part I, no clinical observations were noted in rats exposed to 0, 0.14, or 1.2 mg/m^3. Four rats died following exposure to 21.0 mg/m^3 of [redacted] Rats surviving exposure to 21.0 mg/m^3 exhibited dry red discoloration around the mouth following each exposure.

During Part II, a dry red discoloration was observed around the mouths of rats exposed to 9.9 mg/m^3 following each exposure. No other clinical signs were noted in any other test group.

[redacted]

**Reference:

L. F. Percival. "Determination of C_8 and C_9 Dispersing Agents, Methylene Blue Method". E. I. du Pont de Nemours & Company. Plastics Polymer Products Department Code No. W 240.240, issued 10/9/69. Washington Works Technical Library, 1st Circulation 5-20-68.

Work and Report by: Clarence Hutt
Clarence Hutt
Technician

Stephen D. Nash
Stephen D. Nash
Technician

Supervised by: Joseph C. Hamill
Joseph C. Hamill
Technologist

Reviewed by: Bruce A. Burgess
Bruce A. Burgess
Research Toxicologist

Approved by: Gerald L. Kennedy
Gerald L. Kennedy, Jr.
Section Supervisor
Acute Investigations

CH:SDN:tac:WP:12.2
Date Issued: December 18, 1981

[redacted]

DIPLOMATE: AMERICAN COLLEGE
OF VETERINARY OPHTHALMOLOGISTS

ATTACHMENT 2

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
Robert E. Staples, Ph.D.
Study: [redacted]

Exam Date:
24 April 1981

Ophthalmoscopic Summary

Both eyes of all of the male and female rats in the colony were examined by focal illumination and indirect ophthalmoscopy. Mydriasis was achieved with 1% Atropine (1% Atropisol, Cooper, B3036, exp. 7/82), and darkness maintained until the following morning. Both eyes of all rats were ophthalmoscopically normal except as follows:

FEMALE RATS

Rat Number	Observations
301112	Left Eye: Posterior synechiae.
301116	Right Eye: Focal retinal degeneration.
301335	Right Eye: Focal retinal degeneration.
301194	Right Eye: Focal retinal degeneration.
301305	Right Eye: Anterior synechiae from 2 to 4 o'clock.
301231	Right Eye: Corneal opacities and subjacent anterior synechiae from 11 to 1 o'clock.
301251	Right Eye: Marked ventral entropion, blepharospasm.

MALE RATS

Rat Number	Observations
298494	Left Eye: Superficial corneal vascularization.
298514	Bilateral superficial corneal vascularization.
298429	Right Eye: Anterior synechiae from 1 to 3 o'clock.
298305	Left Eye: Anterior synechiae.
298299	Left Eye: Anterior synechiae.
298128	Right Eye: Superficial corneal vascularization.
298083	Right Eye: Anterior synechiae from 9 - 11 o'clock.
298148	Right Eye: Anterior synechiae at 9 o'clock.
300068	Bilateral anterior synechiae, nasal quadrant.
300130	Left Eye: Anterior synechiae.
300177	Left Eye: Anterior synechiae.

COMMENTS

The 18 rats described above were removed from the colony and euthanatized by Supervisor Alice Erwin. All of those rats remaining are ophthalmoscopically normal and suitable for use in the forthcoming study.

James M. Clinton, V.M.D.

DIPLOMATE: AMERICAN COLLEGE
OF VETERINARY OPHTHALMOLOGISTS

ATTACHMENT 3

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: [redacted]
Robert E. Staples, Ph.D.

Exam Date:
5 May 1981

Ophthalmoscopic Examination

Both eyes of 210 female rats were examined by focal illumination, indirect ophthalmoscopy and, when indicated, slit-lamp microscopy. Mydriasis was produced with 1% atropine solution and the eyes examined in subdued light. The subdued light was maintained until the following morning.

With just 13 exceptions, both eyes of all of the rats in the colony were ophthalmoscopically normal. The exceptions are listed below:

Rat Number	Observations
302217	Left Eye: Very pale ocular fundus.
302240	Bilateral pale ocular fundi.
302194	Right Eye: Focal retinal degeneration.
302201	Right Eye: Anterior synechiae 5-6 o'clock.
302248	Right Eye: Anterior synechiae, nasal quadrant, with corneal opacity.
302319	Right Eye: Anterior synechiae from 11-1 o'clock.
302321	Left Eye: Very pale ocular fundus.
302339	Left Eye: Very pale ocular fundus.
302291	Right Eye: Anterior synechiae from 11-1 o'clock.
302356	Left Eye: Pale ocular fundus.
302357	Pale ocular fundi.
302361	Pale ocular fundi.
303360	Pale ocular fundi.

Comments

As soon as they were identified, the 13 rats described above were removed from the colony.

James M. Clinton

James M. Clinton, V.M.D.

ATTACHMENT 4

HASKELL LABORATORY

December 18, 1981

MEMORANDUM

TO: R. E. Staples

FROM: W. D. Kerns

SUBJECT: [redacted]

All animals listed in the attached table 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

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

ATTACHMENT 5

HASKELL LABORATORY

R E V I S E D

December 21, 1981

MEMORANDUM

TO: R. E. Staples

FROM: W. D. Kerns

SUBJECT: H-14045 - FETAL OPHTHALMIC PATHOLOGY

There were no lesions present in either run that were considered to be related to the administration of the test compound.

The fetal heads from run two produced much better histological preparations than those from run one.

WDK:wfd

Company Sanitized. Does not contain TSCA CBI

TABLE 1

INDIVIDUAL ANIMAL HISTOPATHOLOGY DATA

	Run:	I														
	Exposure Level (mg/m^3) :	0.00														
Tissue* and	Dam Number:	40	35	35	38	12	12	15	15	11	11	7	7	8	8	23
Observation**	Fetus Number:	03	03	13	13	03	11	11	05	03	10	03	05	03	09	01
Fetal Eyes		N	N	N	N	N	N	N	O	O	N'	O	N'	N	N	O
Hyphema, focal																

* Tissue Accounting Code: N = Both eyes (including lens) were within normal limits for a developing fetus.
N'= Only one lens was present for histomorphologic evaluation and it was within normal limits.
O = Lens was not present in the sections submitted for evaluation.
L = Lesion observed

** Degree of Change (severity): 1 = Minimal
2 = Mild
3 = Moderate
4 = Marked
5 = Severe

TABLE 1 (Continued)

Part 2

INDIVIDUAL ANIMAL HISTOPATHOLOGY DATA

Run:	I														
Exposure Level (mg/m³) :	0.00									25.0					
Tissue* and Observation** / Dam Number:	23	25	25	30	30	40	43	43	38	45	9	9	9	9	3
Fetus Number:	09	05	11	03	09	05	01	09	05	09	01	06	07	09	03
Fetal Eyes	N'	N	N'	N'	N	N	N	N	N	N	N	N'	O	O	N'
Hyphema, focal															

Code: See Table 1.

H-14045

[redacted]

TABLE 1 (Continued)

Part 3

INDIVIDUAL ANIMAL HISTOPATHOLOGY DATA

Tissue* and Observation**	Run:	I															
	Exposure Level (mg/m³):	25.0															
	Dam Number:	3	3	6	6	6	19	19	19	22	22	22	26	26	26	45	45
	Fetus Number:	07	13	01	05	11	01	11	13	03	07	09	01	05	10	01	13
Fetal Eyes		N'	N'	0	N'	N'	0	N'	N	N	N	N'	N	N	N	N	N
Hyphema, focal																	

Code: See Table 1.

H-14045 [redacted]

TABLE 2

INDIVIDUAL ANIMAL HISTOPATHOLOGY DATA

Tissue* and Observation**	Run:	II									
	Exposure Level (mg/m^3) :	0.00				0.10	10.0				
	Dam Number:	78	75	101	55	99	105	74	64	93	64
	Fetus Number:	04	06	13	07	09	02	05	07	07	14
Fetal Eyes		N	N	N	N	L	N	N	N	N	L
Hyphema, focal						2					2

Code: See Table 1.

DIPLOMATE: AMERICAN COLLEGE
OF VETERINARY OPHTHALMOLOGISTS

ATTACHMENT 6

JAMES M. CLINTON, V. M. D.
ANIMAL EYE CLINIC AT SOUTH JERSEY ANIMAL HOSPITAL
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MEDFORD, NEW JERSEY 08055

TELEPHONE (609) 654-0304

Haskell Laboratories
[redacted]
R. E. Staples, Ph.D.

Exam Date:
5 June 1981

Ophthalmoscopic Examination
F1 Generation of Ophthalmoscopically Normal Parents
First Run

Both eyes of all of the male and female rat pups obtained from the parents already determined to be ophthalmoscopically normal were examined by focal illumination, indirect ophthalmoscopy and, when indicated, slit-lamp microscopy. Mydriasis was produced with 1% atropine ophthalmic solution, and the eyes examined in semi-darkness. Semi-darkness was maintained until the following morning. The dose levels and group identities were not disclosed to me.

Both eyes of all of the male and female rat pups were ophthalmoscopically normal except as follows:

Litter No.	Offspring No.	Observations
301260	1	Prominent filamentous opacities of each posterior lens pole.
301154	1	Prominent filamentous opacities of each posterior lens pole.
301179	1	Prominent filamentous opacities of each posterior lens pole.
301117	1	Left Eye: Focus of pre-retinal hemorrhage of 2 disc diameters.
301117	2	Right Eye: Incomplete mydriasis and red oval opacity on central endothelium.
301072	1	Right Eye: Pre-retinal hemorrhage at peripheral nasal quadrant.

Comments

In my opinion, the lesions noted might typically occur in any well managed colony of rats. Although some of these young rats' eyes were too small to be completely visualized by the means described, I believe that the anterior and posterior segments of most of the eyes were readily visualized.

At this juncture, I do not believe that the test material, as evaluated in this study, has produced ocular changes in the F1 generation.

James M. Clinton

James M. Clinton, V.M.D.