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SUPPORT: FINAL REPORT, DEVELOPMENTAL TOXICITY EVALUATION OF CARBON BLACK OIL ADMINISTERED BY UNOCCLUDED CUTANEOUS APPLICATION TO CD (SPRAGUE-DAWLEY) RATS, W/CVR LTR DATED 2/19/98			
<b>Chemical Category</b>			
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OFFICE OF TOXIC SUBSTANCES  
CODING FORM FOR GLOBAL INDEXING

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4383

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**February 19, 1998**

8EHQ-85-576

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**Office of Toxic Substances**  
**U.S. Environmental Protection Agency**  
**401 M Street, S.W.**  
**Washington, D.C. 20460**

**Contains No CD**

**Subject: Final Report for a Previous TSCA Section 8(e) Notice on  
Carbon Black Oil (CAS# 64741-62-4; File 8EHQ-1185-0576)**

**In our letter dated June 5, 1997 (copy enclosed), the Atlantic Richfield Company (ARCO) submitted information on the preliminary results of a study in experimental animals to assess the developmental toxicity of a refinery stream. The material examined in this study was Carbon Black Oil (CAS# 64741-62-4).**

**The draft report has been finalized. The findings have not changed from the June submission, and the final report is enclosed for your files.**

**Sincerely yours,**

**Randy N. Roth**

**RNR/MDS/isc**  
**Enclosures**

**cc: C. O. Tillman**

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June 5, 1997

**Document Control Officer (TS-790)**  
**Attention: 8(e) Coordinator**  
**Office of Toxic Substances**  
**U.S. Environmental Protection Agency**  
**401 M Street, S.W.**  
**Washington, DC 20460**

**Subject: TSCA Section 8(e) Notice on Carbon Black Oil**  
**(CAS# 64741-62-4; File 8EHQ-1185-0576)**

**Dear Sir/Madam:**

**In accordance with the provisions of Section 8(e) of the Toxic Substances Control Act, the Atlantic Richfield Company (ARCO) is submitting information on the preliminary results of a study in experimental animals to assess the developmental toxicity of a refinery stream.**

**The material examined in this study was Carbon Black Oil (CBO) (CAS# 64741-62-4). This information supplements previous TSCA submittals on this material (8EHQ-1185-0576).**

**This is a follow-up study initiated by ARCO in response to previous reports by Mobil and ARCO (8EHQ-1185-0576) of adverse effects on rat fetuses after dermal exposure to this material.**

**In the study being submitted today, CBO was dermally administered to groups of pregnant rats on gestational days 5 through 11 at dose levels of 0, 50 or 250 mg/kg/day. In previous studies on this material by ARCO, animals were exposed either throughout gestation or for brief (3 day) intervals during gestation. This study was designed to determine if CBO induces maternal anemia that could be responsible for the fetal toxicity observed in previous studies. The animals were observed for signs of maternal toxicity (including anemia) during the gestational period. At Day 20 of gestation, animals were sacrificed, ovarian corpora lutea were counted and the status of uterine implantation sites (resorptions, dead fetuses, live fetuses) was recorded. Fetuses were counted, weighed, sexed and examined for external abnormalities. The dams were also evaluated for body, liver, spleen, thymus, kidney and gravid uterine weights.**

**As in previous studies, exposure to 250 mg/kg/day resulted in maternal and developmental toxicity. Maternal toxicity was evidenced by reduced maternal body weights and reduced**

June 5, 1997  
Document Control Officer  
Page 2

weight gain. Changes in some maternal organ weights were also observed (parameters not included in previous studies). Developmental toxicity was expressed as increased resorptions and therefore reduced live litter size. The hematological parameters measured did not indicate that maternal anemia would be the cause of the observed fetal toxicity.

In addition, this study showed a statistically significant decrease in ovarian corpora lutea in the 250 mg/kg/day dose group. This had not been observed in previous studies. Since there was no significant change among groups in preimplantation loss or number of total implants per litter, and since dosing in this study began on gestation day 5 (prior to completion of implantation in the rat) the investigator interpreted this finding as possible evidence of very early peri-implantation loss. The investigator indicated that corpora lutea involute (regress) when conceptuses die (and are resorbed in situ) although the concordance between reduced corpora lutea and reduced live conceptuses is not necessarily one-to-one. Therefore, the reduction in corpora lutea at term examination most likely is a reflection of the increased resorption rate (predominantly early resorptions) also observed in this group.

Statistical analyses indicated no differences among groups in the incidence of pooled external fetal malformations or variations in this study. It was noted that one fetus in the 250 mg/kg/day dose group exhibited external malformations (anasarca, cleft palate and micromelia) and another fetus in a different litter exhibited a common external variation (specifically hematomas on the head and forelimb). The investigator did not conclude that either of these observations were treatment related.

Our current Material Safety Data Sheet on this material is being reviewed with these preliminary results in mind.

We are attaching the draft report on this study. EPA will be sent a copy of the final report once it is received by ARCO.

Sincerely yours,

  
Randy N. Roth

RNR/MDS/mds  
Enclosure



FINAL REPORT

**TITLE:** Developmental Toxicity Evaluation of Carbon Black Oil Administered by Unoccluded Cutaneous Application to CD® (Sprague-Dawley) Rats

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**SPONSOR'S REPRESENTATIVE:** Mark Saperstein, D. Env.  
ARCO

**STUDY INITIATION DATE:** December 20, 1996

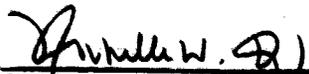
**IN-LIFE PERFORMANCE DATES:** February 10, 1997 - March 13, 1997

**LABORATORY COMPLETION DATE:** April 17, 1997

**FINAL REPORT DATE:** September 12, 1997

**RTI IDENTIFICATION NO.:** 65C-6766-100

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RTI Project No.: 65C-6766-100  
RTI Protocol No.: RTI-598

Developmental Toxicity Evaluation of Carbon Black Oil  
Administered by Unoccluded Cutaneous Application to CD® (Sprague-Dawley) Rats

Sponsor: ARCO Corporate Health and Safety

\*\*\*\*\*

ABSTRACT

Timed-pregnant CD® (Sprague-Dawley) rats were exposed to the test chemical, carbon black oil, administered by unoccluded cutaneous application on the dorsum for six hours daily, on gestational days (gd) 5 through 11 at doses of 0, 50 or 250 mg/kg/day (equivalent to 0.0, 0.05 or 0.24 ml/kg/day). There were 25 sperm-positive females per group. The dosing volume for the treated groups was adjusted based on each animal's most recent body weight. Clinical observations were taken daily, except during the dosing period when they were made at least twice daily. Draize scoring on the dosing site was also done twice daily during dosing, at the time of application and removal of the test material, and once daily during the post-dosing period. Maternal body weights were taken on gd 0, 5, 7, 9, 11, 13, 15, 17 and 20. Feed consumption was measured for the intervals gd 0-5, 5-7, 7-9, 9-11, 11-13, 13-15, 15-17, and 17-20. Each dam was bled from the lateral tail vein on gd 5 (immediately prior to the first application), 7, 9, 11 (immediately prior to the last application), 13 and 20 (immediately prior to scheduled sacrifice), 200 µl (0.2 ml) of blood removed each time, for hematologic assessment. At scheduled sacrifice on gd 20, the dams were evaluated for body, liver, spleen, thymus, kidneys(2), and gravid uterine weights. Maternal liver, spleen, kidneys and any gross lesions were retained in fixative for possible subsequent histologic examination. Ovarian corpora lutea were counted and the status of uterine implantation sites (*i.e.*, resorptions, dead fetuses, live fetuses) was recorded. All fetuses were dissected from the uterus, counted, weighed, sexed and examined for external abnormalities, euthanized by intraperitoneal injection of sodium pentobarbital, and retained in fixative with appropriate individual identification.

Pregnancy rates were 100.0, 84.0 and 100.0% at 0, 50, and 250 mg/kg/day, respectively, with four females not pregnant at scheduled sacrifice at 50 mg/kg/day. No dams died, aborted, delivered early or were removed from study. All pregnant females had one or more live fetuses at sacrifice, except for one fully resorbed litter at 250 mg/kg/day; the numbers of litters (fetuses) examined were 25 (332), 21 (281), and 24 (233) at 0, 50 and 250 mg/kg/day, respectively. Maternal body weights were significantly reduced at 250 mg/kg/day for gd 7, 9, 11, 13, 15, 17 and 20; maternal weight gains were significantly reduced at 250 mg/kg/day for gd 5-7, 9-11, 17-20, gd 5-11 and 0-20. There were no effects of treatment at 50 mg/kg/day on maternal body weights or weight changes. At 250 mg/kg/day, gravid uterine weight was significantly reduced, maternal relative (but not absolute) liver and kidney weights were significantly increased, maternal absolute and relative spleen weights were significantly increased; maternal absolute and relative thymus weights were unaffected. Maternal clinical observations included chromodacryorrhea and clinical weight loss ( $\geq 5$  g per weigh period) initially in a dose-related incidence, and transient very slight through moderate edema and slight through well-defined erythema at 250 mg/kg/day on gd 8 through gd 11 (and only very slight edema in one dam on the afternoon of gd 11 at 50 mg/kg/day). Maternal feed consumption as g/kg/day was significantly reduced at 250 mg/kg/day for gd 5-7, 7-9, 9-11 and 5-11. At 50 mg/kg/day, maternal

feed consumption as g/kg/day was significantly reduced for gd 0-5 (prior to the onset of dosing, most likely due to biologic variability), 5-7 and 5-11. Maternal hematologic assessment resulted in a number of statistically significant changes at 50 and 250 mg/kg/day, but no consistent pattern of anemia between groups at any timepoint or over time. There was a pattern within groups consistent with acquisition of anemia over time (*i.e.*, over the gestational period) which is commonly characterized as "anemia of pregnancy." Gestational parameters exhibited a consistent pattern of prenatal loss at 250 mg/kg/day, including reduced number of corpora lutea (with no changes in preimplantation loss or number of implants per litter), increased number of resorptions (predominantly early), increased number of nonlive resorptions (dead plus resorbed, due to resorptions; no dead fetuses were observed in this study) and adversely affected samples (nonlive plus malformed due predominantly to resorptions) per litter. The number of live fetuses per litter was significantly reduced at 250 mg/kg/day (due to resorptions). Fetal sex ratio (% males) per litter and fetal body weights were statistically equivalent across groups, although fetal body weights per litter at 250 mg/kg/day were 95-98% of the control group values (for total fetuses and separately by sex). Only two fetuses exhibited external findings: one fetus in one litter at 250 mg/kg/day with anasarca (whole body edema), cleft palate and micromelia (short limbs), all classified as external malformations and one fetus in another litter at 250 mg/kg/day with hematomas on the head and forelimb, classified as external variations.

In conclusion, carbon black oil administered by unoccluded cutaneous application during major organogenesis to CD® (Sprague-Dawley) rats resulted in maternal toxicity at 50 and 250 mg/kg/day. The maternal toxicity at 50 mg/kg/day consisted only of slight edema at the dosing site on one dam on gd 11 and reduced feed consumption during the dosing period. The maternal toxicity at 250 mg/kg/day consisted of significant reductions in body weights, weight gain and feed consumption, edema and erythema at the dosing site during the dosing period, and increased relative liver and kidney weights and absolute and relative spleen weights. Developmental toxicity, expressed as increased resorptions and therefore reduced live litter size, was observed at 250 mg/kg/day. Maternal hematologic assessment immediately prior to the exposure period, and during the exposure and post-exposure periods indicated little or no evidence of any treatment-related anemia. Hematologic changes over the course of the gestational period in all groups were consistent with development of a mild "anemia of pregnancy."

## OBJECTIVES

The present study was designed to provide maternal and developmental toxicity data relative to a seven-day dosing regimen of carbon black oil by unoccluded cutaneous application during early organogenesis (gestational days 5 through 11) in gravid rats, and to evaluate the maternal hematologic status during and after dosing.

## MATERIALS AND METHODS

### Test Chemical, Dosage Formulations and Analyses

The test material, carbon black oil, is described as "a C13 to C50+ hydrocarbon liquid which is highly aromatic and contains 1 to 2 wt % sulfur. This material may become a sticky solid at ambient temperatures." (MSDS attached to the protocol in Appendix IV). It is also designated as Petrobase 100, IFO 180, Clarified Oil, Cat Slurry Oil, Petrobase Oil, FCCU Clairoil, FCCU Decant Oil, FCCU Slurry Oil, and Catalytic Cracked Clarified Oil. It has a CAS No. 64741-32-4. A single one gallon metal drum with top bung of the test material (gross weight 4782.7 g) was received at Research Triangle Institute (RTI) Materials Handling Facility (MHF) from the ARCO Products Company, Division of Atlantic Richfield Company, 1055 W. Seventh Street, Los Angeles, CA 90051, on November 5, 1996. The supplier's Lot Number was "F-281 (Carbon Black Oil - FCCU)" and it received the RTI Log Book No. 8705-04-01. The material was a dark blue-green to red-brown colored liquid, with a slightly cracked or burnt odor. It was stored in the MHF at room temperature under controlled conditions, away from heat, acids, alkalis and oxidizers. The Sponsor requested that the material be applied "neat" (undiluted) so there was no formulation per se, and no analysis of the test material at RTI. The Sponsor provided characterization of the test material to the performing laboratory. The test material was considered to be 100% pure for purposes of administration. The Sponsor indicated that the test material was stable at room temperature, so it was distributed into aliquots for ease of use during dosing and stored at room temperature. Since the test material was applied "neat," the dosing volume in ml/kg/day differed between the two dosed groups based on target mg/kg/day; the dosing volume was also adjusted within each group based on each animal's most recent body weight. Archival samples of the test material were retained until the end of the dosing period, and then will be shipped to a destination approved by the Sponsor, or discarded with the concurrence of the Sponsor. All information on test material characterization from the Sponsor and information on dosing volumes applied is maintained in the Study Records.

The Sponsor indicated that the specific gravity of the test material was 1.05 g/ml ( $H_2O = 1.0$  g/ml) so that the dosing volumes, in ml/kg/day, to provide the selected doses in mg/kg/day were calculated as follows (also see Table 1):

$$(x) \text{ ml/kg/day} = \frac{\text{mg/kg/day}}{\text{specific gravity in mg/ml}}$$

$$\text{e.g., for 50 mg/kg/day, } x = \frac{50}{1050} = 0.048; \text{ i.e., } 0.05 \text{ ml/kg/day}$$

$$\text{for 250 mg/kg/day, } x = \frac{250}{1050} = 0.24; \text{ i.e., } 0.24 \text{ ml/kg/day}$$

## Animal Husbandry

The test animals were Caesarean-originated, Virus Antibody Free (VAF) CD $\phi$  (SD)BR outbred albino rats supplied by Charles River Laboratories, Inc., Raleigh, NC.

One hundred twenty-five (125) nulliparous female rats, age 63 days (date of birth December 9, 1996), ordered for this developmental toxicity study, were received at RTI on February 10, 1997. One hundred ten (110) male rats of the same strain to be used as breeders (76 days old upon arrival at RTI; date of birth November 26, 1996), ordered from the same supplier and location as the females, were also received at RTI on February 10, 1997. All animals came from the Charles River Facility, Raleigh, NC. Female rats were 10 weeks of age and 214.8 - 255.8 grams in weight on gestational day (gd) 0. Seventy-five (75) sperm-positive female rats were used in this study (i.e., three groups of 25 sperm-positive dams each).

During a seven-day quarantine period for the females, animals were randomly assigned to cages. Males were housed singly in solid bottom polycarbonate cages (8" x 19" x 10.5") with stainless steel wire lids. Non-mated females were group housed (maximum 3 per cage) and mated females were singly housed in solid bottom polycarbonate cages (8" x 19" x 10.5") with stainless steel wire lids (Laboratory Products, Rochelle Park, NJ). Sani-Chips $\phi$  cage litter (P. J. Murphy Forest Products Corp., Montville, NJ) were used in all cages. Pelleted feed (No. 5002 Certified Rodent Diet $\phi$ ; PMI Feeds, Inc., St. Louis, MO) and deionized/filtered tap water from the Durham, North Carolina water system in plastic bottles with stainless steel sipper tubes were available ad libitum throughout the study. During the mating period, cohabited males and females received drinking water ad libitum via an automated watering system (Edstrom Industries, Inc., Waterford, WI). The analysis of the rodent chow for chemical composition and possible chemical contamination was provided by the supplier and the analysis of the Durham City tap water was provided by the supplier; analyses were documented in the study records. Levels of contaminants in both feed and water were below the maximum certified or allowable levels and did not affect the design, conduct or conclusions of this study. Rat chow was stored at approximately 60-70°F and the period of use did not exceed six months from the milling date. All animals were maintained and treated at all times in compliance with the NIH Guide for the Care and Use of Laboratory Animals (NIH, 1996).

Environmental conditions were continuously monitored, recorded, and controlled using an automatic system (Barber-Colman Network 8000 System, Loves Park, IL) during the course of the study. The animal room used for this study, Animal Research Facility Room 202, was maintained on a 12:12 hour light:dark cycle. Target conditions for temperature and relative humidity in the animal room were 69-79°F and 30-70%, respectively (NIH, 1996). The animal room was maintained at a temperature range of 70.0-73.0°F. The relative humidity range was 41.6 - 62.6%. There were no excursions outside the protocol-mandated range for temperature or for relative humidity; the environmental conditions did not affect the design, conduct or conclusions of this study.

All maternal rats were individually identified by ear tag after arrival at RTI. In addition, each sperm-positive female received a dam study number. All data generated during the course of this study were tracked by these numbers.

## Mating

For breeding, individual females were placed in the home cage of singly-housed males (i.e., one male and one female). On the following morning and each morning thereafter, the females were examined for the presence of vaginal sperm and/or copulation plug. The days on which sperm were found were designated as gd 0 (Hafez, 1970). Sperm-positive females were individually housed until scheduled sacrifice on gd 20. Sperm-negative females were retained in the same male's cage and

checked for sperm on successive mornings until insemination occurred or the treatment groups were filled, whichever came first. When all treatment groups were filled, remaining sperm-negative females were euthanized by carbon dioxide asphyxiation and discarded, or transferred to other projects, according to RTI Standard Operating Procedures, with documentation of the fate of all animals in the study records

### Study Design and Treatment

The study was conducted with two (2) treatment groups and a sham control group, each comprised of 25 sperm-positive rats. The dates of performance were as follows: the animals were paired on February 18, 1997; gd 0 dates were February 19-21, 1997, dosing dates (gd 5 through 11) were February 24 through March 4, 1997, and necropsy dates (gd 20) were March 11-13, 1997.

The doses selected by the Sponsor's Representative were 0.0, 50.0 and 250.0 mg/kg/day. The rationale for choosing these doses is as follows. A number of studies had been previously performed with other samples of this test material (variously identified as "F-179" and "F115-01") administered by cutaneous application, either "neat" (undiluted) or diluted in acetone, with the dosing site occluded or unoccluded.

Exposure of non-pregnant rat females (and males) to carbon black oil by occluded dermal application for 28 days (either "neat" [undiluted] or diluted in acetone; UBTL, 1989) or for 90 days (UBTL, 1991a), 6 hrs/day, 5 days/week, resulted in a moderate anemia at 10 and 50 mg/kg/day (males - 28 days), or at 50 mg/kg/day (females - 28 days), and at 0.500 ml/kg/day (males - 90 days) or at 0.100 and 0.500 ml/kg/day (females - 90 days), accompanied by increased liver weights and decreased thymus weights. There were apparently no spleen effects (expected with anemia). The anemia can be categorized as "mild to moderate" since the MCV (mean corpuscular volume), the size of the erythrocyte (RBC) in  $\mu^3$ , did not increase (examined in the 90-day study); with profound anemia, the MCV almost always increases as more immature (larger) RBC are released in the bone marrow to compensate for more severely reduced peripheral RBC counts. Therefore, in non-pregnant female rats, there is evidence of a mild to moderate treatment-related anemia.

Exposure of pregnant rats from -7 (one week prior to mating) through gd 20 by dermal application (with dams and litters maintained out to postnatal day 4) at 0.0, 0.05, 10 and 250 mg/kg/day or for gd 0-19 at 1, 10, 50 and 250 mg/kg/day resulted in no live litters (all litters fully resorbed) at 250 mg/kg/day, increased resorptions at 10 mg/kg/day and reduced maternal body weights, weight gain and feed consumption at 10 and 250 mg/kg/day (-7 through gd 20), or increased resorptions (reduced live litter size) and reduced maternal body weights, weight gains and feed consumption at 1 through 250 mg/kg/day, and no live litters (all litters fully resorbed) at 250 mg/kg/day. Fetal body weights were reduced at 1, 10 and 50 mg/kg/day after exposure on gd 0-19, but pup survival and weights were unaffected on postnatal days 0-4 after exposure on -7 through gd 20. No treatment-related increased incidences of fetal malformations or variations were reported (UBTL, 1991b). Therefore, resorptions and reduced fetal (but not pup) weights were the only developmental toxicity findings after gestational exposure.

Exposure to the undiluted test material daily from gd 0 through 20 at 0, 1, 10, 50 and 250 mg/kg/day resulted in increased resorptions in all groups, with no live fetuses at 250 mg/kg/day and reduced fetal body weights in surviving litters at 1 through 50 mg/kg/day (Argus, 1989). These results confirm the previous results after gestational exposure, with no pre-gestational exposure and no postnatal component.

Exposure of female rats by dermal application for two weeks prior to and during cohabitation (maximum of seven days) continuing until presumed gd 0 at 0, 0.1, 1, 10, 50 and 250 g/kg/day

resulted in no effects on estrous cyclicity, mating, fertility or any gestational parameters including pre- or postimplantation loss, and therefore no effects on resorptions or live litter size (Argus, 1992a).

An evaluation of the critical period of susceptibility for resorptions and reduced body weights was also performed (Argus, 1992b). In this study, exposure of dams by dermal application to 0 (gd 0-19, 25 rats/group), 0.05 (gd 0-19, 25 rats/group) or to 1, 50 and 250 mg/kg/day for gd 0-2, 3-5, 6-8, 9-11, 12-14, 15-17 or 18-19 (10 rats/interval, seven intervals/dose) resulted in no effects at 0.05 mg/kg/day (gd 0-19). Maternal body weights were unaffected at any dose or interval; maternal weight gain was reduced at 1, 50 and 250 mg/kg/day for all intervals. Maternal feed consumption was reduced at 1, 50 and 250 mg/kg/day for intervals encompassing gd 6-19; at 50 and 250 mg/kg/day, feed consumption was also reduced during exposure on gd 0-5. During the postdosing period, feed consumption remained reduced at 1, 50 and 250 mg/kg/day after dosing on gd 15-19. The incidences of total and early resorptions per litter were statistically significantly increased when dosing occurred on gd 6-8 and were apparently (but not statistically significantly) increased when dosing occurred on gd 9-11 (data from exposure on gd 3-5 and 12-14 were equivocal).

Therefore, the doses in the present study were chosen as follows: 250 mg/kg/day to cause maximum incidence of resorptions, and 50 mg/kg/day to cause a slight to no increase in resorptions (based on exposure on gd 6-8; Argus, 1992b). The dosing duration, gd 5 through 11, was chosen to encompass the time of effect (Argus, 1992b). In addition, assessment of maternal hematologic status, prior to, during, and subsequent to exposures was incorporated into the study design to assess if treatment-related maternal anemia was present as a possible explanation for the observed resorptions. See Table 1 for summarization of study design and doses.

Sperm-positive female rats (dams) were assigned to treatment groups by a stratified randomization method designed to provide uniform mean body weights across dose groups on gd 0 at the initiation of the study. On gd 0, maternal body weights ranged from 214.8 to 255.8 g. On gd 4, all study males were shaved on the interscapular dorsum, over an area of 3" x 3" with additional shave. The dosing area performed as necessary, with appropriate documentation, throughout the dosing period, gd 5 through 11. On gd 5 through gd 11, in the morning, the test material was gently distributed over the shaved dosing site (using a "cross hatch" application pattern) using the following equipment: a Drummond Digital Microdispenser (Drummond Scientific Co., Broomall, PA) at settings for delivery of specific  $\mu$ l (11-15  $\mu$ l) for the low dose (0.05 mg/kg/day) and a Digital Adjust Micro/Pettor SM1 (Data International, Inc., Miami, FL) at settings for delivery of specific  $\mu$ l (50-70  $\mu$ l) for the sham control and high dose (0.24 mg/kg/day), with calibration verified using the test material. Each dam was fitted with an "Elizabethan" collar (Lomir Biomedical Inc., Malone, NY) immediately after application. The test material and collar remained on each animal for six hours during each dosing day. At the end of each daily dosing interval, six hours, the collar was removed and the test material was removed by gently wiping the dosing site with one or more gauze pads wetted with acetone. Each animal was returned to a clean cage to preclude possible exposure to any test substance that could have been present on the cage during the exposure period. The dosing volume to be applied was adjusted based on each animal's most recent body weight. If the test material became a "sticky solid", it was gently warmed up to 30°C prior to administration; the Sponsor had indicated that this procedure would liquefy the test material (and the test material would not be too warm to apply to the dosing site). For females in the sham control group, the site was shaved as described above, the site was "dosed" with an empty syringe, and the animals were collared as described above. Six hours later the collar was removed and the "dosing" site was gently wiped as described above. Unoccluded cutaneous application was chosen by the Sponsor as the route of administration.

Clinical observations of all animals were made once daily on gd 0-4 (prior to dosing period) and on gd 12-20 (after dosing period) and at least twice daily, at the time of administration and at the time of removal of the test material, throughout the dosing period (gd 5 through gd 11). The

dosing site was also examined twice daily during the dosing period, at the time of application and at the time of removal of the test material, and at least once each day during the post-dosing period as described below. To the extent possible, the examining technicians were blind to dose conditions. Observations were made for (but not limited to):

- A. Any response with respect to body position, activity, coordination, or gait.
- B. Any unusual behavior such as head flicking, compulsive biting or licking, circling, etc.
- C. The presence of:
  - 1. Convulsions or tremors
  - 2. Increased salivation
  - 3. Increased lacrimation or red colored tears
  - 4. Increased or decreased urination or defecation (including diarrhea)
  - 5. Piloerection
  - 6. Mydriasis or miosis (enlarged or constricted pupils)
  - 7. Unusual respiration (fast, slow, gasping, or retching)
  - 8. Vocalization
- D. Evaluation of dosing site for:
  - 1. Drying
  - 2. Edema
  - 3. Erythema
  - 4. Eschar formation

Evaluation of skin irritation at the dosing site was made according to the criteria of Draize et al. (1944; FHSA, 1980):

Text Table A  
Draize Scoring System for Skin Irritation

<u>Erythema and eschar formation</u>		<u>Value</u>
No erythema.....		0
Very slight erythema (barely perceptible).....		1
Well-defined erythema.....		2
Moderate to severe erythema.....		3
Severe erythema (beet redness) to slight eschar formation (injuries in depth).....		4
Maximum possible.....		4
 <u>Edema formation</u>		
No edema.....		<u>Value</u> 0
Very slight edema (barely perceptible).....		1
Slight edema (edges of area well defined by definite raising).....		2
Moderate edema (raised approximately 1 mm).....		3
Severe edema (raised more than 1 mm and extending beyond area of exposure).....		4
Maximum possible.....		4

Dams were weighed on gd 0, 5, 7, 9, 11, 13, 15, 17 and 20. Maternal weight gains were calculated for gd 0-5 (pre-treatment), 5-7, 7-9, 9-11, 11-13, 13-15, 15-17, 17-20, 5-11 (treatment), 11-20 (post-treatment), and 0-20 (gestation). Maternal feed consumption was also evaluated from gd 0-5 (pre-treatment), 5-7, 7-9, 9-11, 11-13, 13-15, 15-17, 17-20, and 11-20 (post-treatment period), 5-11 (treatment period) and 0-20 (gestation period), reported as g/day and g/kg body weight/day.

For maternal hematologic assessment, each dam was bled without anesthesia (via tail vein) in the morning, on gd 5 (immediately prior to the first application), 7, 9, 11 (immediately prior to the

last application), 13 and 20 (immediately prior to scheduled sacrifice), 200  $\mu$ l (0.2 ml) of blood withdrawn per time. To take each maternal blood sample, individually labeled conical capped micro-centrifuge tubes were preloaded with 5  $\mu$ l of 15% aqueous EDTA. At the time of blood sampling, the EDTA was taken up into a 1 cc tuberculin syringe from the micro-centrifuge tube with a 26-gauge 1/2 inch needle (Becton-Dickinson). The tail was gently washed with liquid soap and warm water and dried, and 0.200 ml of blood was slowly removed from the lateral tail vein (initial withdrawals from the more distal portion of the tail, subsequent withdrawals from more proximal portions of the tail and from either lateral tail vein, as necessary) with the needle and syringe. The needle was removed and the blood sample expressed into the individually labeled micro-centrifuge tube for transfer to the Clinical Chemistry Group for analysis. Please note that at the times of serial blood sampling and analyses, the pregnancy status of the study females was not known. Therefore, all samples were analyzed and documented in the Clinical Pathology Report (Appendix 1). Once the study females were sacrificed on gd 20, the pregnancy status was determined. Therefore, the statistical analyses of the maternal hematology data (Report Table 5) included only pregnant females.

Hematological analyses were performed on a Serono-Baker Diagnostics System 9010 Automated Hematology Analyzer. Manual dilution methods were utilized to minimize the quantity of blood needed. The system 9010 analyzer, with adjustable thresholds for accommodation of veterinary samples, directly measured RBC, WBC, and platelet counts, mean red cell volume (MCV), mean platelet volume (MPV), and hemoglobin, and calculates hematocrit, mean corpuscular hemoglobin (MCH), mean corpuscular hemoglobin concentration (MCHC), and red cell distribution width (RDW) in a single blood sample. Commercially available, preassayed controls at normal, low, and high concentration levels were assayed concurrently with study samples. Whole blood smears were prepared and stained with Wright's stain and examined microscopically for WBC differential, nucleated red blood cells, and RBC morphology. Heinz body formation in control and high-dose animals (with slides prepared for all animals) were evaluated from smears of whole blood incubated with crystal violet. Likewise, slides prepared from whole blood incubated with new methylene blue were quantitated microscopically for reticulocyte levels. Increases in nucleated RBCs and reticulocytes are often indicative of anemia, and formation of Heinz bodies, a by-product of unstable hemoglobin, may be seen after acute toxic episodes or splenectomy. (See Appendix I for details on methodology and results.)

On gd 20, approximately one to one and a half days before expected parturition, maternal animals were sacrificed by asphyxiation with CO<sub>2</sub>, thoracic and abdominal cavities and organs examined, and their pregnancy status was confirmed by uterine examination. Uteri which presented no visible implantation sites or uteri with one-horn pregnancies were stained with ammonium sulfide (10%) in order to visualize any implantation sites which may have undergone very early resorption (Salewski, 1964). At sacrifice, the body, liver, spleen, thymus, kidney(s) and uterus of each sperm-positive female were weighed. Maternal liver, spleen, thymus, kidney(s), and any gross lesions were retained in fixative but were not examined histologically. Ovarian corpora lutea were counted, and uterine contents (i.e., number of total implantation sites, resorptions, dead fetuses, live fetuses) were recorded. Live fetuses were dissected from the uterus and immediately placed on a moist paper towel over a tray of ice, a procedure which induces anesthesia by lowering the core body temperature below 25°C (Lumb and Jones, 1973; Blair, 1979). All live fetuses were weighed, sexed and examined for external morphological abnormalities, including cleft palate. All live fetuses were euthanized, after external examination, by intraperitoneal injection of sodium pentobarbital euthanasia solution. Each fetus (initially alive or dead) appropriately identified, received a ventral abdominal incision to allow entry of fixative and was retained in buffered neutral 10% formalin for possible subsequent further examination.

### Statistics

The unit of comparison was the pregnant female or the litter. Quantitative continuous data (e.g., maternal body weights, maternal hematologic parameters, fetal body weights, feed consumption, etc.) were compared among the two treatment groups and the one sham control group by the use of Bartlett's test for homogeneity of variances. If Bartlett's test indicated lack of homogeneity of variances (i.e.,  $p < 0.001$ ), then nonparametric statistical tests were employed for the continuous variables (Winer, 1962; see below). If Bartlett's test indicated homogeneous variances (i.e.,  $p > 0.001$ ), then parametric statistical tests were employed for the continuous variables. Parametric statistical procedures to be applied to selected measures from this developmental toxicity study were as follows. Appropriate General Linear Models (GLM) procedures (SAS Institute Inc., 1989a, 1989b, 1990a, 1990b, 1990c) were used for the Analyses of Variance (ANOVA). Prior to GLM analysis, an arcsine-square root transformation was performed on all litter-derived percentage data (Snedecor and Cochran, 1967) to allow use of parametric methods. For these litter-derived percentage data, the ANOVA was weighted according to litter size. GLM analysis was used to determine the significance of the dosage-response relationship (Test for Linear Trend), and to determine whether significant dosage effects had occurred for selected measures (ANOVA). When a significant ( $p < 0.05$ ) main effect for dosage occurred, Dunnett's Multiple Comparison Test (Dunnett, 1955; 1964) was used to compare each treated group to the control group for that measure. A one-tailed test (i.e., Dunnett's Test) was used for all pairwise differences from the sham control group except that a two-tailed test was used for maternal body and organ weight parameters, maternal feed consumption, fetal body weight, and percent males per litter. Nonparametric tests to be used on continuous data which did not have homogenous variances included the Kruskal-Wallis Test to determine if significant differences were present among the groups, followed by the Mann-Whitney U test for pairwise differences from the vehicle control group, if the Kruskal-Wallis test was significant (Siegel, 1956). Jonckheere's test for  $k$  independent samples (Jonckheere, 1954) was used to identify significant dose-response trends for nonparametric continuous data. Nominal scale measures were analyzed by Chi-Square Test for Independence for differences among treatment groups, and by the Cochran-Armitage Test for Linear Trend on Proportions (Cochran, 1954; Armitage, 1955; Agresti, 1990). When Chi-Square revealed significant ( $p < 0.05$ ) differences among groups, then a two-tailed Fisher's Exact Probability Test, with appropriate adjustments for multiple comparisons, was used for pairwise differences between each treated group and the control group (Snedecor and Cochran, 1967). A test for statistical outliers (SAS; 1990b) was performed on maternal body weights and feed consumption (in g/day). If examination of pertinent study data did not provide a plausible biologically-sound reason for inclusion of the data flagged as "outlier," the data were excluded from summarization and analysis and were designated as outliers. If feed consumption data in g/day were negative for a given dam and period, they were designated "unrealistic" and excluded from summarization and analysis. If feed consumption data for a given observational interval (e.g., gd 5-7, 7-9 or 9-11 during the treatment period) were designated outliers or unrealistic, then summarized data encompassing this period (e.g., treatment period, gd 5-11) also did not include this value.

### Personnel

The evaluation of carbon black oil for developmental toxicity in CD® (Sprague-Dawley) rats was conducted at Research Triangle Institute (RTI), Research Triangle Park, NC, under contract to ARCO Corporate Health and Safety, Los Angeles, CA; Dr. Mark Saperstein, ARCO, was the Sponsor's Representative. The RTI personnel indicated below contributed to the completion of this study.

Dr. R. W. Tyl served as Study Director. Developmental toxicology personnel included Ms. M. C. Marr (Laboratory Supervisor), Ms. C. B. Myers (Data Specialist), Ms. F. S. Gerling (Study Team), Ms. V. I. Wilson, Ms. L. B. Pelletier, Ms. M-S. Perry and Ms. B. T. McTaggart. Maternal blood sampling was performed by Ms. M. P. Gower. Bulk chemical handling was provided by Mr. M. M.

Veselica (Supervisor, Materials Handling Facility), Mr. D. L. Hubbard, Mr. R. A. Price, and Mr. T. D. Burnette. Maternal hematologic assessments were performed by Ms. A. F. Gilliam, MLT-ASCP, Ms. M. P. Gower, and Dr. B. F. Thomas. Animal care was provided by Dr. D. B. Feldman, DVM, ACLAM, Animal Research Facility (ARF) Veterinarian, and Mr. F. N. Ali, MBA, LATG, ILAM, ARF Supervisor. Quality Assurance personnel were Ms. S. M. Taulbee, M.S.P.H. (Manager), Ms. C. D. Keller, Ms. P. D. Hall, Mr. S. T. Sherrill, and Ms. M. E. Parker.

The final report was prepared by Dr. R. W. Tyl with assistance from Ms. C. B. Myers and Ms. F. S. Gerling on data compilation and statistical analyses and from Ms. M. C. Marr. The individual scientist reports were prepared and signed by the author(s).

The protocol and one amendment detailing the design and conduct of this study are presented in Appendix IV. The protocol was signed by the Study Director on December 20, 1996.

#### Historical Control Dataset

An historical control summary dataset for developmental toxicity studies with the CD<sub>01</sub> (Sprague-Dawley) rat in this laboratory is presented in Appendix III.

#### Storage of Records

All original data sheets for the present study are stored in the RTI archives, under the control of the RTI Quality Assurance Officer, along with all biological samples collected during the course of the study which remain the responsibility of RTI. Work sheets and computer printouts which were generated in the statistical analysis of data are stored in the RTI Archives. Copies of this report are filed with the RTI Archives as well as with ARCO, Los Angeles, CA.

#### Compliance

The study was performed, to the extent possible, in compliance with the Toxic Substances Control Act (TSCA) testing guidelines (U.S. EPA, 1985) and the OPPTS draft guidelines (U.S. EPA, 1996). All records, data and reports will be maintained in storage as specified in the TSCA GLPs (U.S. EPA, 1989) or for as long as the quality of the preparation affords evaluation, whichever is less.

The toxicology laboratories at RTI are operated in compliance with TSCA Good Laboratory Practice Standards (GLPs) (U.S. EPA, 1989). The RTI Animal Research Facility is accredited by the American Association for Accreditation of Laboratory Animal Care (AAALAC). This study was conducted in compliance with the TSCA GLP regulations and AAALAC accreditation standards.

## RESULTS

### Dosing Formulations

As described in the Materials and Methods, the test material was administered "neat" (undiluted) to the low and high dose groups in a dosing volume of ml/kg/day to administer the target mg/kg/day as presented in Table 1. The sham control group did not receive any administered material. Since the test material was not diluted, there were no analyses for homogeneity, stability or dose level verification by the performing laboratory. Any analyses of the bulk chemical (e.g., for purity or stability) are the responsibility of the Sponsor.

### Maternal Toxicity

Pregnancy rates were 100.0, 84.0 and 100.0% at 0, 50 and 250 mg/kg/day, respectively, with four (4) females not pregnant at scheduled sacrifice at 50 mg/kg/day. No dams died, aborted, delivered early or were removed from study. One dam at 250 mg/kg/day carried a fully resorbed litter at scheduled sacrifice; all remaining pregnant females had one or more live fetuses at term. The numbers of litters (fetuses) evaluated were 25 (332) at 0 mg/kg/day, 21 (281) at 50 mg/kg/day and 24 (233) at 250 mg/kg/day. All subsequent summary tables include only confirmed pregnant females. Maternal body weights were equivalent across groups on gd 0 and 5 (prior to the onset of test article administration). Mean maternal body weights were statistically significantly reduced at 250 mg/kg/day on gd 7, 9, 11, 13, 15, 17 and 20 (in-life and at sacrifice). There were no effects of treatment on maternal body weights at 50 mg/kg/day. Maternal body weight change was unaffected across groups for gd 0-5 (prior to onset of dosing). Maternal body weight change was statistically significantly reduced at 250 mg/kg/day for gd 5-7 (first interval of dosing period), 9-11 (last interval of the dosing period), gd 17-20 (last interval of the gestational period, in the post-dosing period), gd 5-11 (dosing period) and gd 0-20 (gestational period). Maternal gestational weight change (weight change gd 0-20 minus the gravid uterine weight) was statistically equivalent across groups, although there was a significant dose-related downward trend ( $p < 0.05$ ). There were no effects of treatment on maternal body weight changes at 50 mg/kg/day. Gravid uterine weight was statistically significantly reduced at 250 mg/kg/day, most likely due to the increased resorptions and therefore smaller litter sizes at this dose. Maternal absolute liver weight was equivalent across the groups, while maternal liver weight relative to terminal body weight was significantly increased at 250.0 mg/kg/day. Maternal spleen weight, absolute and relative, was significantly increased at 250 mg/kg/day. Maternal thymus weight, absolute and relative, was unaffected across groups. Maternal absolute kidney weights (2) were equivalent across groups, but relative kidney weight was significantly increased at 250 mg/kg/day (Table 2).

Maternal clinical observations and necropsy findings are presented in Table 3. Treatment-related clinical observations included the following. Chromodacryorrhea (secretion from the Harderian glands from hemoglobin breakdown, so-called "bloody tears," from nonspecific stress) was observed in all groups, initially dose related on gd 5, but subsequently not in a dose-related incidence, and one dam at 250 mg/kg/day presented with a swollen face on gd 5, presumably from struggling to remove the collar. Clinical weight loss, defined as greater than or equal to 5.0 g in a weigh period, was observed in three dams each at 0 and 50 mg/kg/day and in 10 dams at 250 mg/kg/day on gd 7, in one dam at 50 mg/kg/day on gd 9 and in one control dam on gd 13. Signs of skin irritation at the dosing site (based on Draize scoring) were observed beginning on gd 8 at 250 mg/kg/day through gd 11 (end of dosing period), and only on gd 11 in the dam at 50 mg/kg/day. The findings at the site of application at 250 mg/kg/day included very slight through well-defined erythema, and very slight through moderate edema at 250 mg/kg/day with more severe findings in the afternoon evaluation when the test material was removed. At 50 mg/kg/day, the only Draize score was very slight edema in one dam on the afternoon of gd 11. In addition, two dams at 250 mg/kg/day exhibited hard round raised sites near but not on the application site on gd 8 through 11

and into the post-dosing period with rapid resolution, with only one red slightly raised area (one dam) or one red unraised area (one dam) on gd 13, and only one dam with one small slightly red area on gd 14. The Animal Research Facility Veterinarian provided a tentative diagnosis of "hives" for these findings. The skin at the dosing site at 250 mg/kg/day progressed from very dry (one dam on gd 11), to rough and dry (one dam on gd 12), to dry, scaly and "wrinkled" (one dam on gd 13 and 14), to dry and scaly (one dam on gd 15 and 16), to scaly (one dam on gd 17, 18 and 19). The dry skin was most likely due to the use of acetone to remove the test material at the end of each exposure day. Alopecia (regional hair loss) was observed at 50 and 250 mg/kg/day beginning on gd 7 at 250 mg/kg/day and on gd 8 at 50 mg/kg/day. The incidence increased later in the pregnancy (beginning on gd 17), confounded by the typical alopecia observed close to term in rodents (and other mammals). Necropsy findings at scheduled sacrifice included hydronephrosis in one dam and slight hydronephrosis in one dam at 250 mg/kg/day; neither finding is considered related to treatment (Table 3).

Maternal feed consumption is presented in Table 4 as g/day and as g/kg body weight/day. When the data were expressed as g/day, maternal feed consumption was statistically significantly reduced at 250 mg/kg/day for the following intervals: gd 0-5 (prior to onset of dosing), 5-7, 7-9, 9-11, 5-11 (dosing period) and gd 0-20 (gestation period). At 50 mg/kg/day, maternal feed consumption in g/day was statistically significantly reduced for gd 0-5 (prior to onset of dosing), 5-7 (first interval in dosing period), 5-11 and gd 0-20. When the data were expressed as g/kg/day, maternal feed consumption was statistically significantly reduced at 250 mg/kg/day for gd 5-7, 7-9, 9-11, and 5-11. At 50 mg/kg/day, maternal feed consumption in g/kg/day was statistically significantly reduced for gd 0-5, 5-7 and 5-11 (Table 4). Maternal individual animal data are presented in Appendix II.

Maternal hematologic data are presented in Table 5. Blood was taken and evaluated on gd 5 (immediately prior to the onset of dosing), 7, 9, 11 (immediate prior to the last dose), and on gd 13 and 20 (in the post-dosing period). On gd 5 (prior to the onset of dosing), red blood cell distribution width (a measure of the variability in size, but not shape, of the erythrocyte) was significantly increased at "50" and "250" mg/kg/day, and mean platelet volume was significantly increased at "250 mg/kg/day," due to biologic variation since these effects occurred prior to the onset of dosing. On gd 7, hemoglobin concentration and red cell distribution width were significantly increased at 250 mg/kg/day, mean platelet volume was significantly increased at 50 and 250 mg/kg/day. On gd 9, mean corpuscular volume (a measure of erythrocyte size) was significantly reduced at 250 mg/kg/day. On gd 11, mean corpuscular volume and percent reticulocytes (immature red blood cells) were significantly decreased at 250 mg/kg/day. On gd 13, mean corpuscular volume was significantly reduced at 250 mg/kg/day, and mean corpuscular hemoglobin concentration (a measure of the amount of hemoglobin per erythrocyte) was significantly increased at 50 and 250 mg/kg/day; the percent monocytes (part of the white blood cell differential examination) was significantly reduced at 250 mg/kg/day. On gd 20, red blood cell count and hemoglobin concentration were significantly reduced at 50 mg/kg/day, hematocrit (packed red blood cell volume) was significantly reduced at 50 and 250 mg/kg/day, red blood cell distribution width was significantly increased at 50 and 250 mg/kg/day, and percent reticulocytes was significantly increased at 250 mg/kg/day. No Heinz body inclusions were found in any erythrocytes in this study (see Appendix 1 for the Clinical Pathology Report).

#### Developmental Toxicity

Results of the uterine examination are presented in Table 6. There was a statistically significant reduction in the number of ovarian corpora lutea per dam at 250 mg/kg/day. The number of corpora lutea is a measure of the number of collapsed ovarian follicles after the eggs have been released, which further differentiate into endocrine structures which produce estrogen and progesterone to sustain the uterine lining during early pregnancy. Since dosing began on gd 5 prior to completion of implantation in the rat, this parameter may represent very early peri-implantation loss.

There were no significant differences among groups for implantation sites per litter, preimplantation loss (calculated as the difference between the number of corpora lutea and the number of implantation sites), or of the incidence of late fetal deaths (*i.e.*, dead fetuses) per litter. The incidence of resorptions was significantly increased at 250 mg/kg/day, as was the incidence of nonlive implants (due to the resorptions) and adversely affected implants (nonlive plus malformed) per litter. The number of live fetuses per litter was significantly reduced at 250 mg/kg/day (again due to the increased resorptions at this dose). Sex ratio (% males per litter) was unaffected. Mean fetal body weight per litter was statistically equivalent across groups, but the parameter for both sexes combined and for female fetuses (but not male fetuses) alone exhibited a significant ( $p < 0.05$ ) test for linear trend due to the values at 250 mg/kg/day; *i.e.*, fetal body weight per litter for both sexes combined at 250 mg/kg/day was 95.25% of the control values; female fetal body weight per litter at 250 mg/kg/day was 95.72% of the control rate (and the value for male fetuses at 250 mg/kg/day was 96.20% of the control value). The values for fetal body weights per litter at 50 mg/kg/day (both sexes combined or separately) was slightly greater than the values at 0 mg/kg/day; there was clearly no effect at this dose. Individual uterine findings are presented in Appendix II.

The summary and statistical analyses of fetal external malformations and variations are presented in Table 7 and the findings by defect type are presented in Table 8. There were no differences among groups in the incidence of pooled external fetal malformations or variations in this study (Table 7). Only two fetuses (both at 250 mg/kg/day) exhibited any external structural alterations. One fetus at 250 mg/kg/day (dam no. 46, fetus no. 4, male with the lowest fetal body weight in the study, 1.691 g) exhibited the only external malformations observed, specifically anasarca (whole body edema), cleft palate and micromelia (short limbs). One fetus at 250 mg/kg/day (dam no. 58, fetus no. 12, male) exhibited the only external variations observed, specifically hematomas (subepidermal ecchymoses, *i.e.*, "bruises") on the head and forelimb. Individual embryo/fetal findings by dam are presented in Appendix II.

## DISCUSSION

### Maternal Toxicity

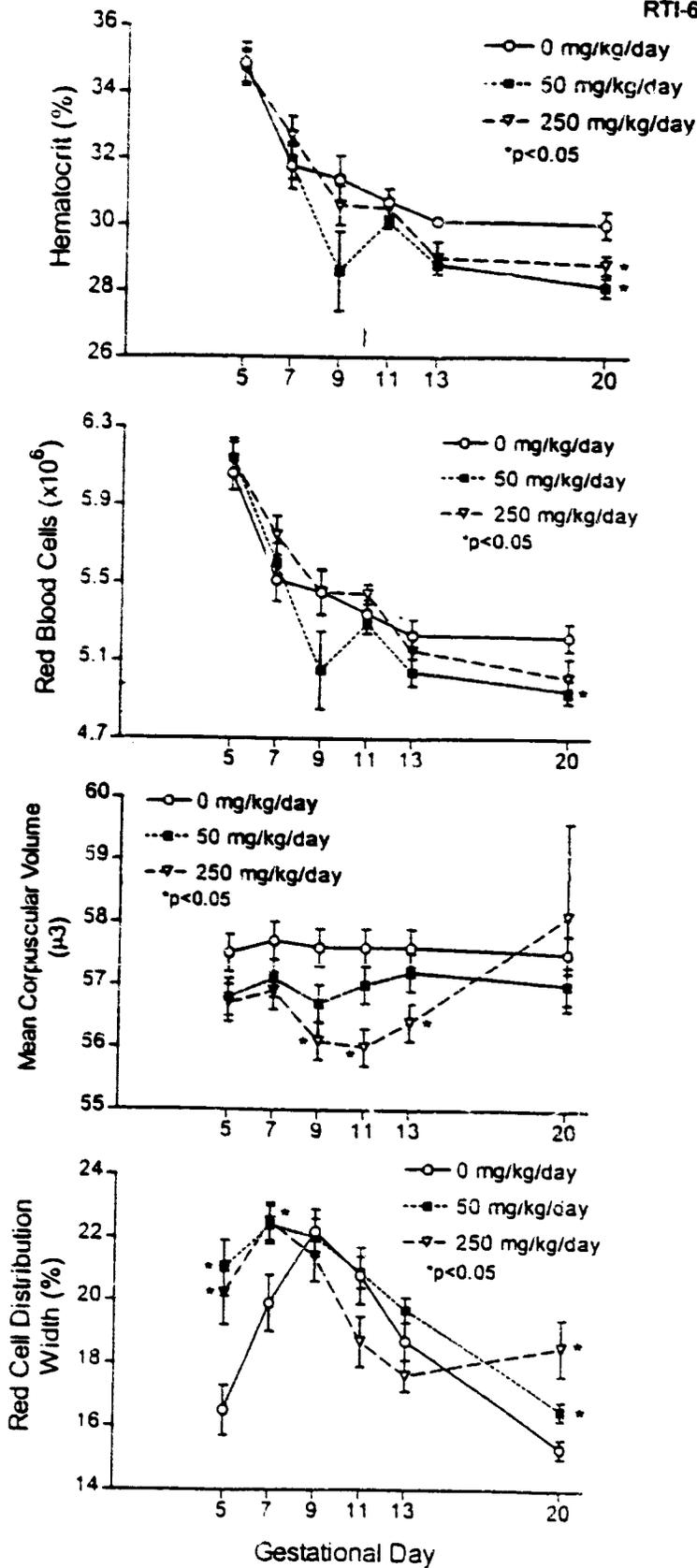
The present study has shown that carbon black oil, administered by unoccluded cutaneous application for six hours per day during early organogenesis, gd 5 through 11, resulted in maternal toxicity at 250 mg/kg/day including reduced body weights, weight gain and feed consumption, increased maternal absolute and relative spleen weight, and increased maternal relative liver and kidney weights, as well as clinical signs of toxicity, including transient effects (edema and erythema) at the dosing site. Maternal effects at 50 mg/kg/day were limited to reduced feed consumption and slight edema at the dosing site in one dam on gd 11. The changes in relative organ weights at 250 mg/kg/day most likely were due to the slightly reduced maternal body weights at this dose and, in the case of the liver, to induction of hepatic metabolizing enzymes and concomitant increase in liver mass and not necessarily to toxicity *per se* (Conney, 1967). The increased absolute and relative spleen weights at 250 mg/kg/day may be due to effects on the hematopoietic system (see below) whereby affected cells are sequestered in the spleen and the spleen increases output of hematopoietic cells (extramedullary hematopoiesis). However, the maternal hematologic data do not present a consistent picture of treatment-related anemia within or across days of evaluation. For example, red cell distribution width was increased at 250 mg/kg/day on gd 7 and 20, and at 50 mg/kg/day on gd 20 but on no other days, mean corpuscular volume was decreased at 250 mg/kg/day on gd 9, 11 and 13, but one would expect an increase in MCV if immature (larger) erythrocytes were released into the peripheral circulation. Percent reticulocytes (again a measure of immature cells in the circulation) was decreased on gd 11 and increased on gd 20 at 250 mg/kg/day. The hematocrit was slightly reduced (but significantly) at 50 and 250 mg/kg/day on gd 20, but at no other time. Other hematologic parameters were either unaffected or exhibited no dose-response pattern. Figure 1 graphically presents the data for the parameters which are most likely to show changes when anemia is present: hematocrit, number of red blood cells, mean corpuscular volume and red blood cell distribution width. These data do not support the interpretation that carbon black oil induces an anemia which may cause the early resorptions or that carbon black oil induces an anemia at all. The mild to moderate anemia observed in the 28-day study (UBTL, 1989), more pronounced in males than in females, at 50 mg/kg/day, and in the 90-day study (UBTL, 1991a) at 0.100 and 0.500 mg/kg/day, did not appear to occur in these dams after a relatively brief (seven day) exposure period. Thymic atrophy, also observed in the 28-day and 90-day studies, did not occur in the dams in the present study, based on no changes in absolute or relative maternal thymus weights at term. Although there were no consistent changes in hematologic parameters across groups within timepoints for this study, there was a clear indication of acquisition of a mild anemia within groups across time (*i.e.*, throughout the pregnancy) which is consistent with the anemia of pregnancy, due to an increase in blood volume without a compensatory increase in cellular elements.

### Developmental Toxicity

The results of the present study confirm the findings of previous studies (*e.g.*, UBTL 1991b; Argus, 1989; Argus 1992b) that cutaneous application of carbon black oil during gestation results in increased resorptions at 250 mg/kg/day.

In the present study, the vast majority of resorptions observed were early (resorption sites or sites observed after staining) which is consistent with the historical control data in this laboratory for this rat strain (Appendix III); *i.e.*, 87.5, 100.0 and 93.5% of the total resorptions were early ones at 0, 50 and 250 mg/kg/day, respectively (Text Table B). The increases in incidence at 250 mg/kg/day were due to one fully resorbed litter (dam no. 22 with 14 early resorptions only) and increases in the number of resorptions per litter. In fact, six dams (nos. 15, 28, 33, 45, 52 and 59) at this dose had

Figure 1. Selected Maternal Hematologic Parameters



Text Table B. Incidence and Distribution of Resorptions<sup>a</sup>

Parameter	Carbon Black Oil (mg/kg/day, cutaneous)		
	0	50	250
No. litters	25	21	25
No. litters fully resorbed	0	0	1
No. total resorptions	32	18	107
No. total resorptions per litter	1.28	0.86	4.28
Range per litter	0-4	0-4	0-14
No. early resorptions	28	18	100
Percent of total resorptions	98.50	100.0	93.46
No. early resorptions per litter	1.12	0.86	4.00
Range per litter	0-3	0-4	0-14
No. mid resorptions	3	0	7
Percent of total resorptions	9.38	0.0	6.54
No. mid resorptions per litter	0.12	0.0	0.28
Range per litter	0-1	0	0-2
Total late (full) resorptions	1	0	0
Percent of total resorptions	3.12	0.0	0.0

<sup>a</sup> Data are summarized from Summary Table 6 and individual animal data from Appendix II, Table A-5.

more resorptions than live fetuses per litter, a situation that did not occur at 0 or 50 mg/kg/day. One possibility that was evaluated was that the conceptuses lived longer at the low dose and died earlier at the higher dose (so that one would anticipate more advanced resorptions or dead fetuses) at the low dose. However, this was not the case. Mid resorptions (characterized as placental remnant through some fetal tissue) were present at 0 mg/kg/day (three total, 9.4% of the total resorptions in this group) and 250 mg/kg/day (seven total, 6.5% of the total resorptions in this group) with no mid resorptions at 50 mg/kg/day, and there were no dead fetuses in this study (Table 6). There is, therefore, no evidence of a dose-related shift in the timing of conceptus demise. In addition, the numbers of ovarian corpora lutea are significantly reduced (88.59% of the control value) and the numbers of implantations are reduced (93.15% of the control value) at 250 mg/kg/day, which may imply peri-implantation loss (since the rat is not through implanting by gd 5, the start of the exposure period). This peri-implantation loss is not captured by the "preimplantation loss" parameter since both components of the calculation, corpora lutea and implantation sites, are reduced. In litters with resorptions, especially early ones, the number of corpora lutea at term are not always equal to or

greater than the number of total implantation sites (the usual situation) since some corpora lutea involute and regress when part of the litter is lost; please note that this loss of corpora lutea is not necessarily one-to-one with the loss in conceptuses.

Significant fetal body weight reduction at 250 mg/kg/day, observed after exposure throughout gestation (UBTL, 1991b; Argus, 1989) was not observed in the present study although fetal body weights per litter were reduced to 95-96% of the control values in the present study with only seven days of exposure early in the pregnancy; the likelihood is that the results of the embryonic exposure were at least partially compensated for in the post-dosing fetal period when body weights are increasing rapidly. In this performing laboratory, treated group means for fetal body weights per litter which are 95% or less of the concurrent control group value are typically statistically significantly different, so the present data are consistent with the potential for reduction in fetal body weights at term after early gestational exposure.

The previous developmental toxicity studies with this test material support the contention that the consequence of exposure (*i.e.*, resorptions) is rapid in onset and limited to the early organogenesis period, since exposure during the two-week mating period until gd 0 resulted in no treatment-related resorptions (Argus, 1992a) and that no significantly increased resorptions were present after exposures beginning on gd 9 (Argus, 1992b). The present data also support vulnerability of the conceptus only during the peri-implantation period since even with a seven-day exposure period (gd 5 through 11), only resorptions, almost exclusively early resorptions, occurred, with little or no effect on subsequent development of the survivors. The rapid onset and short duration of the effects also mitigates against maternal anemia as the causative agent, since it takes time to develop and to resolve: the present study confirms that a consistent or persistent maternal anemia was not induced from exposure. The rat appears to tolerate even a profound anemia without loss of conceptuses (*e.g.*, Tyl *et al.*, 1996).

#### CONCLUSIONS

Unoccluded cutaneous exposure to carbon black oil at 0, 50 and 250 mg/kg/day on gd 5 through 11 in CD® rats results in maternal toxicity at 50 and 250 mg/kg/day and in developmental toxicity (specifically resorptions) at 250 mg/kg/day, in the absence of treatment-related maternal anemia.

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