

## Reproductive Toxicity

### Test Substance - Reproductive Toxicity

**Category** (64741-79-3) Coke, petroleum  
**Chemical:**  
**Test Substance:** (64741-79-3) Coke, petroleum  
**Test Substance Purity/Composition and Other Test Substance** See Petroleum Coke Category Analysis document attachment, Appendix B.  
**Comments:**  
**Category** Measured  
**Chemical Result Type:**

### Method - Reproductive Toxicity

**Route of Administration:** Inhalation  
**Type of Exposure:** Particulate  
**Species:** Rat  
**Mammalian Strain:** Sprague-Dawley  
**Gender:** Both M/F  
**Number of Animals per Dose:** 12  
**Concentration:** 0, 30, 100, 300 mg/m<sup>3</sup>  
**Year Study Performed:** 2004  
**Method/Guideline Followed:** OECD 421  
**GLP:** Yes  
**Exposure Period:** 6 - 8 Weeks  
**Frequency of Treatment:** 6 hours/day, 7days/week  
**Post-Exposure** 0

**Period:**

**Method/Guideline and Test Condition Remarks:**

This study was designed to assess possible effects on reproductive performance, in male and female Sprague Dawley CD(R) rats when Petroleum Coke was administered as a dust, via nose-only inhalation exposure. For male and female rats, this study design permitted detection of effects on gonadal function, mating behavior, conception, development of the conceptus, parturition and pup survival to post-natal Day 4.

The exposure levels were based primarily upon findings in a two-week range-finding study (03-6147) in which exposure to the test substance resulted in alveolar and/or bronchiolar epithelial hyperplasia/hypertrophy at exposure levels of 25, 75 and 200 mg/m<sup>3</sup>. Both the severity (slight to moderate) and the incidence (0 to 100%) were exposure level-responsive. Absolute lung weight was increased by approximately 12 to 16% at the highest concentration. The highest exposure concentration chosen for this reproduction screen, 300 mg/m<sup>3</sup>, was selected as a balance between the above pulmonary effects, the longer exposure period of this study and greater respiratory demands of pregnancy.

The animals were approximately 6 weeks old at receipt and approximately 8 weeks old at exposure initiation.

The weight of the animals at initiation of exposures was:

Mean Range

Male: 258 235-280

Female: 216 199-232

Individual weights of animals placed on test were within  $\pm 20\%$  of the mean weight for each sex.

Male and female Sprague-Dawley CD(R) rats (12/sex/group) were exposed once daily for 6 hours/day for 7 consecutive days to 0 (Air only), 30, 100 or 300 mg/m<sup>3</sup> of Petroleum Coke for 2 weeks prior to mating initiation. In addition, male rats were exposed during the mating and post-mating periods until euthanized for a minimum exposure of 28 days and necropsied. Female rats continued to be treated once daily (6 hours/day) during the mating period. Once mated, female rats were treated once daily (6 hours/day) during gestation (Days 0-19) until euthanized on post-natal Day 4 and

necropsied.

Exposure levels were determined using a gravimetric sampling procedure 4 times per chamber per day. Particle size distribution measurements were also made once per chamber per week

The following parameters were evaluated:

#### Viability

Observations for mortality and general condition were made at least twice daily (once in the morning and once in the afternoon).

#### Clinical observations

All animals were observed as a group at least once during each exposure. This was routinely performed near the middle of each exposure. Each animal was removed from its cage and a detailed physical observation performed prior to randomization. Male rats had a detailed physical observation performed once weekly beginning during the pre-mating period and continuing through euthanasia. Female rats had a detailed physical observation performed weekly during the pre-mating period and on Gestation Days 0, 7, 14, 20 and Lactation Days 0 (except if parturition did not complete on the same day as it initiated), 1 and 4. Female rats without evidence of mating continued to be observed weekly during the mating and post-mating period until euthanized. Examinations during non-exposure periods included observations of general condition, skin and fur, eyes, nose, oral cavity, abdomen and external genitalia, occurrence of secretions and excretions, and autonomic activity (e.g., lacrimation, piloerection, pupil size, unusual respiratory pattern). Changes in gait, posture and response to handling as well as the presence of clonic or tonic movements, stereotypy (e.g., excessive grooming, repetitive circling) or bizarre behavior (e.g., self-mutilation, walking backward) was recorded. Pertinent behavioral changes, signs of difficult or prolonged parturition and all signs of toxicity, including mortality, were recorded. These records included, as appropriate, time of onset, degree and duration.

#### Body weights

Body weights of the male rats and the female rats were recorded at the time of randomization into test groups, on the day treatment was initiated and at least weekly thereafter throughout the study until euthanized. Mated female rats were weighed on

Gestation Days 0, 7, 14 and 20 and female rats that delivered litters were weighed on Lactation Days 1 and 4. Female rats without evidence of mating continued to be weighed weekly during the mating and post-mating period until euthanized. A terminal body weight was also recorded for each animal.

#### Feed consumption

Feed consumption for the male rats and the female rats were measured pretest and weekly during the pre-mating treatment period. Feed consumption was not measured during the mating period when male rats were being co-housed with female rats. Feed consumption for the male rats was measured weekly, and if not mated, for the female rats, during the post-mating period. For pregnant or confirmed mated female rats, feed consumption was measured on Gestation Days 0-7, 7-14 and 14-20 and on Lactation Days 1-4.

#### Organ weights

A total of 16 organs were taken at the scheduled necropsy, weighed wet, recorded and organ/body weight ratios and organ/brain weight ratios calculated for all animals. Prior to weighing, all organs were carefully dissected and properly trimmed to remove fat and other contiguous tissue in a uniform manner. Organs were weighed as soon as possible after dissection to avoid drying. Paired reproductive organs were weighed separately. The organs taken were:

Adrenal glands

Brain (medulla/pons, cerebrum and cerebellum)

Epididymides

Larynx

Lungs (with mainstem bronchi)

Lymph node (mediastinal)

Nasopharynx

Ovaries (with oviducts)

Pituitary

Prostate

Seminal vesicles (with coagulating gland)

Testes

Thymus

Trachea

Uterus with vagina

All macroscopic lesions

#### Macroscopic observations.

Macroscopic postmortem examinations were performed on all animals. Postmortem examinations included examination of external surface, all orifices,

cranial cavity, nasal cavity (external examination), neck and its associated tissues and organs, thoracic, abdominal and pelvic cavities and their associated tissues and organs, and external surfaces of the brain. Special attention was paid to the organs of the reproductive system. Macroscopic postmortem examinations (external only) were performed on all surviving F1 pups on Lactation Day 4.

#### Histopathological evaluations

Microscopic examinations for control and high exposure group male and female animals were performed on 16 different tissues and organs. During the microscopic examination of the testes, special emphasis was placed on the stages of spermatogenesis and the interstitial testicular cell structure. Histopathological examination of the ovary detected qualitative depletion of the primordial follicle population. Any abnormalities not noted during macroscopic postmortem examinations that were seen during histological processing were recorded.

The following protocol deviations occurred during this study:

1. Due to technician oversight, Animal Nos. 4503, 4504 and 4510 were not transferred to fetal pathology for sacrifice on their proposed Gestation Day 26 as per protocol, but were transferred on Gestation Days 28, 27 and 27, respectively.

2. For logistical reasons, non-pregnant Animal Nos. 1504 and 4509 were transferred to necropsy 24 days after completion of mating rather than the protocol suggested period of 26 days following mating.

3. Due to technician oversight, macroscopic observations for Animal No. 2011, sacrificed on 25 June 2004, were not recorded.

4. Due to technician oversight, organ weights were recorded for those female rats (Nos. 1504, 4503, 4509 and 4510) that did not deliver pups, although not required by the protocol.

Exposure method: nose-only inhalation

Group 1	0	mg/m3	12 males & 12 females
Group 1	30	mg/m3	12 males & 12 females
Group 1	100	mg/m3	12 males & 12 females
Group 1	300	mg/m3	12 males & 12 females

#### Exposure conditions:

The test substance was administered as a dust in the breathing air of the animal. After being sieved through a stainless steel sieve, the test substance was packed to 800 psi into dust feeder cups (small diameter) using a press. The cup was then mounted onto the dust feeder, which was controlled using a speed selector. House line air was delivered from a regulator and a backpressure gauge into the inlet of a gas drying unit, via 1/4" tubing to a "Y" tube, which split the airflow into the generation and dilution systems. The generation air (20.0Lpm) was directed, via 1/4" tubing, through a flow meter, regulated by a metering valve, then into a backpressure gauge connected to a dust feeder, via 1/4" tubing. The test substance was then directed into the inlet of a brass cyclone, via 1/2" tubing. The test substance laden air was then directed into the top of a cast aluminium and alloy exposure chamber equipped with polycarbonate nose-only tubes. The dilution air (5.0 Lpm) was directed, via 1/4" tubing, to a flow meter regulated by a metering valve into the dilution port at the top of the chamber.

Samples for determination of the exposure levels were withdrawn from the breathing zone in the exposure chambers through glass fiber filters mounted open-faced in a filter holder. Samples were withdrawn at least four times per exposure from the normal sampling portal. The filter papers were weighed before and after sample collection, and the gravimetric concentration in mg/m<sup>3</sup> was calculated by dividing the weight difference by the volume of air sampled.

The mean ( $\pm$  standard deviation) exposure concentrations of Petroleum Coke were determined to be  $31.2 \pm 4.6$ ,  $99.4 \pm 13.9$  and  $300.7 \pm 34.7$  mg/m<sup>3</sup> for the three test substance exposed groups, respectively. Chamber environmental conditions averaged 20°C temperature and 44% relative humidity. The average mass median diameter was determined to be 2.287  $\mu$ m with an average geometric standard deviation of 2.848 indicating that the particles for the test substance exposed groups were highly respirable to the test animals.

#### Statistical methods used

- 1) Continuous data

The following parameters were analyzed statistically:

Body weights  
Body weight changes  
Feed consumption values  
Gestation length  
Number of implantation sites and corpora lutea  
Pre- and post-implantation loss  
F1 pup weights (each weighing interval during lactation)  
Number of pups per litter  
Number of male and female pups  
Pup weight distinguished by sex and as a composite for both sexes (litter as experimental unit)  
Absolute organ weights, organ weight to body weight ratios and organ weight to brain weight ratios

#### Method of Analysis

Mean values of all exposure groups were compared to the mean value for the control group at each time interval.

Evaluation of equality of group means were made by the appropriate statistical method. For all parameters except for organ weights, the standard one-way analysis of variance (ANOVA) using the F ratio to assess significance was used (Dunlap and Duffy, 1975; Armitage, 1971). If significant differences among the means were indicated, additional testing was performed using Dunnett's t-test to determine which means were significantly different from the control (Dunlap et al., 1981). Organ weight data was analyzed only by parametric methods. Bartlett's test (Bartlett, 1937; Sokal and Rohlf, 1995) was performed to determine if groups had equal variances. The standard one-way analysis of variance (ANOVA) using the F ratio to assess significance was used (Dunlap and Duffy, 1975). If significant differences among the means were indicated, additional tests were used to determine which means were significantly different from the control: Dunnett's t-test (Dunlap et al., 1981; Dunnett, 1955, 1964) for homogeneous data, or Cochran and Cox's modified t-test (Cochran and Cox, 1959) for non-homogeneous data. Bartlett's test for equality of variance was conducted at the 1% significance level; all other statistical tests were conducted at the 5% and 1% significance levels.

#### Exceptions

Statistical evaluations were not performed when the standard deviation for the control group was 0 and/or N (number of animals) in the control group was less than or equal to two.

2) INCIDENCE DATA

The following parameters were analyzed statistically:

- Mortality rate
- Mating indices, pregnancy rates, male fertility indices
- Litter survival indices
- Gestation indices
- Incidence of dams with no viable pups
- Mean pup survival indices (Days 0 and 4)

Incidence Data Analysis

A Fisher Exact Test with Bonferonni correction was performed to identify differences between the control and treatment groups (Siegel, 1956). All statistical tests were conducted at the 5% and 1%, two-sided risk levels.

**Test Results - Reproductive Toxicity**

**Concentration (LOAEL/LOAEC/NOAEL/NOAEC):**

LOAEL/LOAEC/NOAEL/NOAEC	Population	Value Description	Value/Lower Concentration	Upper Concentration	Units
NOAEC	Male (systemic)	>	300		mg/m <sup>3</sup>
NOAEC	Female (systemic)	>	300		mg/m <sup>3</sup>
LOAEC	Parental (F0)	=	300		mg/m <sup>3</sup>
NOAEC	Parental (F0)	=	100		mg/m <sup>3</sup>
NOAEC	Offspring (F1)	>	300		mg/m <sup>3</sup>

**Results:**

There was no effect of treatment on survival. All animals survived until the termination of the study. The test animals were generally unremarkable during the non-exposure periods. There were no exposure-related differences in absolute body weights or in body weight changes or feed consumption in the test substance exposed animals, compared to the Air Control animals.

Mating and fertility indices were unaffected by the exposures for the lower two exposure groups. In the 300 mg/m<sup>3</sup> group, four pairings either did not result

in pregnancy (3/12) or did not result in delivery of viable fetuses (1/12). Of the three females that were not pregnant, one was acyclic (one control female was also acyclic). Both of the other non-pregnant dams mated but neither had corpora lutea or implantations. Consequently, the overall fertility index for the 300 mg/m<sup>3</sup> group was slightly decreased when compared with control values ([81.8% [9/11] versus 100% [11/11]), however the difference was not statistically significant. A fourth dam was pregnant, but had only two corpora lutea, two implantation sites, and no viable fetuses. The gestation index at 300 mg/m<sup>3</sup> (88.9% [8/9]) was reduced but not statistically different from the controls (100% [11/11]). However the fertility and gestation indices observed in the 300 mg/m<sup>3</sup> group were outside the testing facility's historical control data minimum (fertility index of 87.5% and gestation index of 95.2%). There were no exposure-related inter-group differences for delivery parameters, including the duration of gestation and the proportion with live litters and/or with stillborn pups. Parturition data for the female rats treated with the test substance were comparable to the air control group. The pups were unremarkable during the early postnatal period until termination at postnatal day 4. There were no meaningful differences in pup body weights or weight gains, up to postnatal day 4, in the pups feeding from dams exposed to test substance during gestation compared to the pups feeding from air control dams. The decreased fertility and gestation indices in the 300 mg/m<sup>3</sup> coupled with the single female with a low number of implantation sites and no viable fetuses were considered exposure-related.

There were exposure-related increases (up to 37% in males and 58% in females) in lung weights (absolute and relative to body weight or brain weight) in the test substance exposed animals (all groups) compared to the Air Control animals. Lungs from all test substance-treated rats were slightly to severely discolored black. Inhalation of 300 mg/m<sup>3</sup> Petroleum Coke was associated with the presence of pigment deposits, probably representing test substance, in the lungs, mediastinal lymph nodes and nasal olfactory epithelium of most male and female rats; and in the lumens of the nasal turbinates and pharynx of only male rats. Test substance-related changes characterized by proliferative and/or inflammatory responses were observed in the lungs. In the mediastinal lymph nodes draining the lungs, hyperplasia of the paracortical T lymphocyte

population accompanied the deposition of pigment. In the larynx, minimal squamous metaplasia of the respiratory epithelium occurred and was considered to be an adaptive response to inhalation of particulates. There were no exposure-related differences in the incidence of macroscopic postmortem evaluations in the pups from test substance exposed animals as compared to the pups from Air Control animals.

**Results**

**Remarks:**

**Conclusion:**

In conclusion, exposure of male and female rats to target concentrations of 30, 100 or 300 mg/m<sup>3</sup> of petroleum coke by nose-only inhalation for 4-6 weeks resulted in discolored lungs, increased lung weight, and proliferative and/or inflammatory responses in the lungs and mediastinal lymph nodes, in all test substance exposed groups. Decreased fertility and gestation indices coupled with the single female with a low number of implantation sites and no viable fetuses were observed in the 300 mg/m<sup>3</sup> exposure group. There were no effects observed on offspring survival and weight development up to postnatal day 4. Therefore, the no-observed-adverse-effect level (NOAEL) for adult males and females for systemic toxicity was 300 mg/m<sup>3</sup>. A NOAEL for the portal of entry was not established for females; the female lowest-observed-adverse-effect level (LOAEL), based on lung weight increases, was 30 mg/m<sup>3</sup>. The portal of entry NOAEL for males, based on increased lung weights, was 100 mg/m<sup>3</sup>. Based on the lower fertility and gestational indices, and low number of implantation sites with no viable fetuses for one female in the 300 mg/m<sup>3</sup> exposure group, the reproductive NOAEL was 100 mg/m<sup>3</sup>. The developmental NOAEL was 300 mg/m<sup>3</sup>.

**Reliability/Data Quality - Reproductive Toxicity**

**Reliability:**

1 - Valid Without Restrictions

**Reliability  
Remarks:**

**Key Study  
Sponsor  
Indicator:**

Key

**Reference - Reproductive Toxicity**

**Reference:**

Huntingdon Life Sciences Ltd. 2004  
Petroleum Coke: Developmental Toxicity Screening  
Study In Rats Via Nose-Only Inhalation Exposures.

Project ID 034246