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Chemical Category	COKER LIGHT GAS OIL, VACUUM TOWER OVERHEADS; LIGHT CYCLE OIL		

SUPP

OFFICE OF TOXIC SUBSTANCES
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Dear Sir:

In May, 1987, submitted a TSCA Section 8(e) notification on the toxicity of clarified slurry oil (CAS 64741-62-4) and the relationship between subchronic and developmental toxicity and chemical composition. Supplemental submissions to this 8(e) have been made for several other refinery streams, further describing the relationship between stream composition and toxicity. This submission consists of interim reports from single dose oral developmental toxicity studies done on ten refinery streams. Preliminary data from these studies were submitted to the EPA on April 21, 1993. Final reports for similar studies on three other streams (Clarified Slurry Oil, Syntower Bottoms and Distillate Aromatic Extract) were submitted on July 24, 1990, November 30, 1990 and February 5, 1991 respectively. We believe the effects seen in these studies are due largely to polycyclic aromatic compounds in agreement with conclusions reported in our previous submissions.

The interim reports which constitute the present submission show evidence of teratogenicity for six of the ten refinery streams tested in the latest studies. The most prominent findings include digit and tail anomalies, micrognathia, and cleft palate. The streams for which one or more of these effects were observed are listed below. Final reports for these studies will be submitted when they become available.

<u>Study #</u>	<u>CAS #</u>	<u>Test Article</u>
65371-I	64741-82-8	Coker Light Gas Oil
65371-I	64741-49-7	Vacuum Tower Overheads
65371-I	64741-59-5	Light Cycle Oil
65370-I	68915-97-9	Heavy Atmospheric Gas Oil
65370-I	64741-81-7	Heavy Coker Gas Oil
65370-I	64741-81-7	Heavy Coker Gas Oil

Confidentiality is being claimed for the company name and names of company employees. All pages containing this information have been stamped "confidential." Two copies of this notification are being submitted; the confidential information has been circled in one copy and excised from the other. The latter copy is intended for the EPA's Public Files. The substantiation for this confidentiality claim is attached.

Sincerely,

Enc.

62 pgs.

SUBSTANTIATION OF CONFIDENTIALITY CLAIM

Confidentiality is being claimed for the submitter's identity and the names of company scientists which appear in the submission.

1. This confidentiality claim is being made on the submitter's behalf.
2. No time limit is specified for this claim since we cannot assign a time at which these materials will no longer be of commercial interest.
3. The reports contained in this submission have not been previously submitted to any governmental agency.
4. The reports are kept in an Archive and other company confidential files to which access is restricted to authorized personnel.
5. No one outside _____ has access to any of the reports involved in this confidentiality claim.
6. The information for which confidentiality is being claimed does not appear in any advertising or promotional material, material safety data sheet, technical data sheet, professional or trade publication, or any other media available to the public or to our competitors.
7. To our knowledge, no confidentiality determinations have been made by the EPA, other Federal agency, or court in connection with this information.
8. These claims are being made in order to retain maximum utility of the information for the corporation which incurred the costs of the studies being reported.
9. The refinery streams discussed in this submission are not patented.
10. The substances covered by this submission have been commercially available and/or manufactured by our competitors for many years. They are intermediates in the manufacture of lubricant base oils and other refinery products.
11. Reverse engineering is not an issue in this confidentiality claim.
12. Disclosure of this information would not reveal confidential processes or concentrations of substances in a mixture. The information is unrelated to the effects of the substances on human health or the environment.
13. CAS numbers are provided in the submission cover letter.
14. The subjects of this notification and the information being claimed confidential are not subject to FIFRA regulation or reporting.

REPORT RELEASE

TO LIAISON: _____

STUDY NUMBER: 65371-J

CRU NUMBERS: 87213, 85244, 84152, 88195, 86270

SAMPLE NAMES: BCLGO, HVGO, LCCN, LCO, VTO

STUDY TITLE: Teratogenicity Study in Rats Exposed Orally to a Single Dose of a Refinery Stream

REQUESTING DIVISION: _____

RESULTS:

Coker Light Gas Oil (BCLGO), Heavy Vacuum Gas Oil (HVGO), Light Catalytically Cracked Naphtha (LCCN), Light Cycle Oil (LCO), and Vacuum Tower Overheads (VTO) were orally administered to presumed-pregnant rats at a dose level of 2000 mg/kg on gestation day 13. Control group animals received tap water at a dose level of 2000 mg/kg on gestation day 13. All animals remaining on study were sacrificed on gestation day 20.

Based on the data available at this time, the refinery streams BCLGO, LCO, and VTO clearly demonstrate teratogenic potential when administered as a single, oral dose of 2000 mg/kg on gestation day 13. Malformations observed included digit anomalies [BCLGO ($p < 0.01$), LCO, VTO], micrognathia (VTO), and cleft palate (VTO). Body weight was also reduced in fetuses from dams exposed to LCO. Oral administration of LCCN and HVGO resulted in no adverse developmental effects. For severity of developmental effects, including teratogenicity, the materials may be ranked as: BCLGO > VTO > HVGO (no effects); LCCN and LCO are not included in the ranking due to small sample size (3 and 4 females, respectively) in those groups.

Administration of LCO, LCCN, VTO, and BCLGO resulted in maternal toxicity as indicated by clinical observations, significant ($p < 0.01$) transient weight loss following exposure, a reduction in net body weight gain, and a decrease in absolute and relative thymus weights (LCO only). HVGO produced no maternal toxicity. Reproductive parameters, including viable litter size and percent resorptions, were not affected by refinery stream administration. For severity of maternal toxicity produced, the materials (excluding LCCN and LCO) may be ranked as follows: VTO > BCLGO > HVGO (no effects).

6-21-93

Date
Study Director

Date
Supervisor

7/21/93

Date
Manager, Mammalian/Genetic Toxicology

8/1/93

Date
Manager, Environmental and
Health Sciences Laboratory

DISTRIBUTION: Archives/original All signatories

SUMMARY

Five refinery streams - Coker Light Gas Oil (BCLGO), Heavy Vacuum Gas Oil (HVGO), Light Catalytic, Cracked Naphtha (LCCN), Light Cycle Oil (LCO), and Vacuum Tower Overheads (VTO) - were orally administered to presumed-pregnant rats at a dose level of 2000 mg/kg on gestation day 13. Control group animals received tap water at a dose level of 2000 mg/kg on gestation day 13. All animals remaining on study were sacrificed on gestation day 20.

Administration of LCO, LCCN, VTO, and BCLGO resulted in maternal toxicity as indicated by clinical observations: significant ($p < 0.01$) transient weight loss following exposure, a reduction in net body weight gain, and a decrease in absolute and relative thymus weights (LCO only). Based on clinical signs of toxicity for those dams initially exposed to LCO and LCCN, it was determined that fetal viability may be compromised and the remaining dams in those groups were not dosed. HVGO produced no maternal toxicity. Reproductive parameters, including viable litter size and percent resorptions, were not affected by refinery stream administration. For severity of maternal toxicity produced, the materials may be ranked as follows: VTO > BCLGO > HVGO (no effects); LCCN and LCO are not included in the ranking due to the small sample size (3 and 4 females, respectively) in those groups.

Teratogenicity was observed in fetuses from dams exposed to BCLGO, LCO, and VTO. Malformations observed included digit anomalies [BCLGO ($p < 0.01$), LCO, VTO], micrognathia (VTO), and cleft palate (VTO). In addition, body weight reduction was observed in fetuses from dams exposed to LCO. LCCN and HVGO produced no adverse fetal effects. For severity of developmental effects, including teratogenicity, the materials (excluding LCCN and LCO) may be ranked as: BCLGO > VTO > HVGO (no effects).

Based on the data available at the time of this Interim Report, the refinery streams BCLGO, LCO, and VTO clearly demonstrate teratogenic potential when administered as a single, oral dose of 2000 mg/kg on gestation day 13. Oral administration of LCCN and HVGO resulted in no adverse developmental effects.

1.0 INTRODUCTION

As part of the Toxicology Testing Program, various refinery streams have been tested at _____ for their potential to produce adverse effects on the developing conceptus. To date, eleven refinery streams and two crude oils have been tested in this capacity via dermal application [1-13]. In general, these materials produced evidence of developmental toxicity in the presence of maternal toxicity.

Although the predominant signs of developmental toxicity observed included increased fetal death and reduced fetal body weights, possible evidence of teratogenicity (abnormal development) was also demonstrated by a majority of the streams [1,2,4-10,12]. Some of these data have been presented in a Project Status Report [14]. Unequivocal evidence was lacking on the ability of these streams to produce frank terata because 1) severe maternal toxicity was a confounding factor and 2) the high incidence of fetal death may have masked teratogenic outcomes.

To eliminate these confounding factors, the study design was altered to allow for a larger dose to be administered over a shorter period of time. The teratogenic potential of Clarified Slurry Oil (CSO) was subsequently confirmed by the dermal route of administration [15]. To further limit maternal exposure time and maximize the dose, CSO [16], Syntower Bottoms [17] and Distillate Aromatic Extract [18], were administered via gavage on a single day of gestation. These data have also been the subject of a Project Status Report [19]. In general, the oral dosing regimen was a more effective means by which to minimize maternal toxicity and fetal lethality, and maximize teratogenic potential. A total of eight refinery streams and two crude oils have recently been evaluated using this experimental design. Preliminary results for three of the refinery streams and the two crude oils have been summarized [20]. This report contains the results for the five remaining refinery streams - BCLGO, HVGO, LCCN, LCO, and VTO.

Recently, a correlation between end points used to measure subchronic and developmental toxicities and chemical component classes of refinery streams has been demonstrated [21]. As the concentration of specific chemical class components increased, the severity and/or incidence of select measured end points also increased. Data from the present study will also be used to expand efforts in this area and to determine if abnormal structural development is related to specific chemical class components found in refinery streams.

2.0 METHODOLOGY

2.1 *Experimental Design*

Presumed-pregnant rats were distributed among six experimental groups: one oral control and five refinery stream-exposed groups (Table 1). At the start of the dosing phase of the study, each group contained twelve presumed-pregnant females. Based on signs of overt toxicity observed in the first females exposed to the test material, Groups 4 (LCCN) and 5 (LCO) were reduced to five and four females, respectively. The remaining females in those groups who were not yet exposed to the materials were removed from study (see Section 3.1: Clinical Observations).

temperature deviation noted. The average humidity ranged from 36-53% during the mating period, with a low of 31% and a high of 65%. During the gestation period, the average humidity ranged from 47-51% with a high of 69% and a low of 37%. The temperature and humidity deviations were minor and are not considered to have affected the outcome of the study.

2.3 Mating Period

During the mating period, female rats which had not previously borne pups were placed with male rats in a ratio of 1:1. Each morning during the period of cohabitation, females were monitored for the presence of vaginal or tray plugs. If either were present, a vaginal lavage sample was obtained and examined for the presence of spermatozoa. Females that were positive for vaginal plug or spermatozoa were considered to be at day 0 of presumed gestation and were placed in individual housing units. The cohabitation period was continued until 72 presumed-pregnant female rats were obtained. Female rats which showed no evidence of breeding activity, and the male rats used for breeding, were returned to the general rat population in the facility.

2.4 Assignment to Experimental Groups

Presumed-pregnant female rats were distributed to one of Groups 1-6 using a computer-generated table of random numbers for a stratified sample of six. This procedure was continued each morning until all six groups contained 12 presumed-pregnant females.

2.5 Materials Administered

Oral Control (Group 1): Tap Water

Obtained from the tap in Vivarium Room 319. Water analysis provided in study records.

Density: 1.00 g/ml (assumed)

Test Material: Coker Light Gas Oil

CAS Number: 64741-82-8

CRU Number: 87213

Density: 0.88 g/ml

Expiration Date: 12-01-93

Stability: This material is believed to be stable at room temperature.

Test Material: Heavy Vacuum Gas Oil

CAS Number: 64741-57-7

CRU Number: 85244

Density: 0.92 g/ml

Expiration Date: 12-01-93

Stability: This material is believed to be stable at room temperature.

Test Material: Light Catalytically Cracked Naphtha

CAS Number: 64741-55-5

CRU Number: 84152

Density: 0.76 g/ml

Expiration Date: 12-01-93

Stability: This material is believed to be stable when refrigerated.

Test Material: Light Cycle Oil

CAS Number: 64741-59-9

CRU Number: 86195

Density: 0.98 g/ml

Expiration Date: 12-01-93

Stability: This material is believed to be stable at room temperature.

Test Material: Vacuum Tower Overheads

CAS Number: 64741-49-7

CRU Number: 86270

Density: 0.91 g/ml

Expiration Date: 12-01-93

Stability: This material is believed to be stable at room temperature.

2.6 Test Material/Tap Water Administration

Each presumed-pregnant female on study received a single oral administration of tap water (Group 1) or refinery stream (Groups 2-6) on gestation day 13. The amount of material (tap water or refinery stream) administered to each animal was calculated using the most recently recorded body weight for that animal, the dose level of its experimental group, and the density of the material. Each material was measured using a 1.00 ml syringe (with gradations of 0.01 ml) and administered from the syringe via a 16 gauge (Group 3 only) or an 18 gauge stainless steel intubation needle.

2.7 Observations and Body Weights During Gestation

Each presumed-pregnant female was observed at least once a day throughout gestation until sacrifice for normality of appearance/behavior/excretory function and any biological discharges. All unusual findings were noted.

The body weight of each presumed-pregnant female was measured and recorded to the nearest 0.1 gram on days 0, 6, 13, 14, and 20 of gestation.

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2.8 Female Necropsy

Each female rat remaining on study was sacrificed by over-exposure to diethyl ether on its 20th day of presumed gestation. The thoracic and abdominal cavities were exposed and the organs examined grossly for evidence of pathosis. After removal of the uterus and ovaries, the carcass was given to a member of the Pathology group for measurement and recording of liver and thymus weights to the nearest 0.001 gram. No tissues were saved.

2.8.1 Uterine/Ovarian Examination

The ovaries and uterus of each rat were excised and examined grossly. The number of corpora lutea per ovary of each pregnant female was counted and recorded; the ovaries were then discarded. The ovaries of non-pregnant females were grossly examined and then discarded. All remarkable findings were recorded. The weight of each intact gravid uterus was measured to the nearest 0.1 gram and recorded. The uterine contents were then exposed and the number and location of all implantations (early/late resorptions and live/dead fetuses) were recorded. The uterus of each female rat that appeared non-gravid was pressed between two glass slides and examined grossly to confirm that no implantation sites were present.

2.8.2 Fetal Evaluations

Each live fetus was stripped of its surrounding extra-embryonic membranes, and its umbilical cord was clamped flush with the abdominal wall. The cord was then severed distal to the clamp. Each fetus was gendered, weighed to the nearest 0.1 gram, and grossly examined for external anomalies. The following definitions and terminology were used in describing fetal findings [22]:

Malformation: permanent structural deviation which generally is incompatible with, or severely detrimental to, normal postnatal survival or development. Additionally, absence of a structure which should have been present, as well as deviations in tail development, are also classified as malformations.

Variation: A variation is a divergence beyond the usual range of structural constitution. It has an indeterminate effect on health and generally has no effect on survival.

Incidental: An incidental finding is generally an accidental event, e.g., accidentally, the tip of a tail was cut off.

After gross evaluation, fetuses in each litter were equally distributed into two groups; one being designated for soft tissue (visceral) examination and the other for skeletal examination. Fetuses

assigned to the soft tissue group were fixed in Bouin's solution and will be examined for soft tissue abnormalities using the Wilson technique of free-hand sectioning by razor blade. Fetuses assigned to the skeletal group were fixed in 95% ethanol. Skeletal staining and examination are not scheduled, however, these tissues will be saved in the event that skeletal examination becomes warranted. Results of the fetal visceral evaluations are not contained in this Interim Report but will be presented in the Final Report.

2.9 Data Analyses and Storage

Raw data were collected, processed, and analyzed using the Grosse Data Acquisition/Reporting System. Archiving of data and fetal tissues (skeletons and viscera) will be addressed in the Final Report. Maternal biophase data, cesarean section data, and fetal data were evaluated statistically by analysis of variance followed by group comparisons using Fisher's Exact or Dunnett's Test. Liver and thymus weight data were statistically evaluated by analysis of variance followed by group comparisons using Tukey's Test. Differences between control and treated groups were considered statistically significant if the probability of the difference being due to chance was less than 5% ($p < 0.05$).

3.0 RESULTS

With the exception of clinical observations, only data generated for pregnant animals are presented. Similarly, only the data for pregnant animals were used in calculation of means and standard deviations. For experimental Groups 4 (LCCN) and 5 (LCO), only the data for three and four pregnant females, respectively, are presented. Initial observations of the first females to receive the materials indicated that administration of LCCN and LCO orally resulted in extreme discomfort for the animals. Clinical signs revealed moderate to severe toxicity and, although the females did not die, it was determined that fetal viability may be compromised. Subsequently, the remaining females in each of these groups were not dosed, but were removed from study.

Because of the reduced number (N) of animals in the LCCN- and LCO-exposed groups, the designation of statistical significance, or lack thereof, of their results relative to the control group may not accurately reflect the significance that would have been achieved had N been greater. In view of this, and based on our experience in refinery stream related developmental toxicity, the biological significance of an effect will be considered in the absence of statistical significance, as warranted.

3.1 Clinical Observations

Incidental and refinery stream-related observations reported during gestation are presented in Table 2. In general, signs of toxicity considered to be related to refinery stream administration

were perineal staining and decreased stool (both seen in all treated groups except HVGO), and red vaginal discharge (BCLGO, LCO, VTO). Red vaginal discharge is generally indicative of fetal resorption, however, in this study, such a relationship was not confirmed. Additional toxic signs which occurred in only a few animals included: soft stool (BCLGO, LCCN, LCO), animal cold to the touch (BCLGO, LCO), and no stool (BCLGO). Although not distinguished in Table 2, two types of oral discharge and staining were noted. The discharge (1 female) and staining (1 female) seen in Group 6 (VTO) were slight and appeared to be from the test material itself as is sometimes seen when nonviscous, oil-like materials (like VTO or generic engine oils) are orally administered; this finding is considered incidental. The discharge and/or staining noted in Groups 2 (BCLGO), 4 (LCCN), and 5 (LCO) was more severe (greater quantity of discharge and large staining area), did not look like test material, and was red in color indicating toxicity. Signs of toxicity and/or stress related particularly to LCCN administration were vocalization, circling, head tilting, salivation, and rales; the first three being noted during and immediately following dosing. Signs of toxicity related particularly to administration of LCO were prostrate and hunched body positions, piloerection, and decreased activity.

The remaining findings in Table 2 are considered incidental. Chromodacryorrhea and red nasal exudate are common signs of stress in rats. They may be caused by any number of factors and are both seen routinely in control animals. One female in Group 4 was missing a digit from her left forepaw. Although not noted until gestation day 3, it was apparent that the digit had been missing prior to her assignment to the study.

3.2 *Body Weights*

Maternal mean body weight, mean body weight change, and mean net body weight change from gestation days 13-20 are presented in Tables 3-5, respectively. Mean body weight change and net body weight gain were adversely affected for all refinery streams except HVGO. The animals lost a significant ($p < 0.01$) amount of weight following exposure to the test materials, but the effect was transient and weight gain resumed throughout the rest of the study (Table 4). The effects on net body weight gain for these groups (Table 5) are not statistically significant, but are considered to be biologically significant.

3.3 *Observations at Cesarean Section*

3.3.1 *Necropsy Findings*

Absolute and relative organ weights are presented in Tables 6A and 6B, respectively. Liver weights were not adversely affected by exposure to any of the refinery streams. Both absolute and relative thymus weights were reduced in those females exposed to LCO. The reduction is considered to be biologically significant. No other findings attributable to administration of the refinery streams were noted at the time of necropsy.

3.3.2 Reproductive and Developmental Evaluations

A summary of the reproductive data is presented in Table 7. No adverse effects on reproductive performance were observed. The high preimplantation loss recorded for Group 4 (LCCN) and Group 6 (VTO) is due to one and two females, respectively, who had less than 10 implantation sites. This is not considered to be related to exposure to the test materials since implantation preceded test material administration.

Mean fetal body weights, a parameter of body growth and development, are presented in Table 8. A decrease in fetal weights was observed in fetuses from dams exposed to LCO.

A statistically significant increase ($p < 0.01$) in malformation of the hindpaw digits was observed in fetuses from a dam exposed to BCLGO (Table 9). One fetus from a dam exposed to LCO exhibited digit anomalies on both hind- and forepaws. Two fetuses from dams exposed to VTO collectively exhibited micrognathia, cleft palate, and digit anomalies. Although the findings for the LCO and VTO groups were not statistically significant, they are considered to be biologically significant.

4.0 DISCUSSION AND CONCLUSIONS

In general, a single oral administration of LCO, LCCN, VTO, and BCLGO resulted in varying degrees of maternal toxicity. Due to the obvious discomfort and morbidity observed for the first females exposed to LCO and LCCN, the remaining females in those groups were not dosed, but were removed from study. It is interesting to note that, despite the poor condition of the females and the extreme red vaginal discharge that was observed, fetal viability did not appear to be effected. Maternal toxicity was moderate for those dams exposed to VTO and BCLGO as evidenced by clinical observations, transient weight loss, and a biologically significant decrease in net body weight gain. HVGO appeared to be relatively nontoxic to the dams under the dosing regimen of this study.

Developmental effects included decreased fetal weights and fetal external anomalies. Fetuses from dams exposed to LCO weighed less than control fetuses. The fetal incidence of malformation was significantly increased in fetuses from females exposed to BCLGO. Although not statistically significant, the malformations seen in fetuses from dams exposed to LCO and VTO have been seen in other fetuses from dams exposed to refinery streams [2,5,6,8,20] and are considered biologically significant.

In conclusion, administration of BCLGO and VTO via a single oral dose on gestation day 13 resulted in maternal toxicity and teratogenicity. Administration of HVGO under the same

conditions produced no adverse effects in either the dam or the offspring. In terms of severity of maternal toxicity, the materials may be ranked as follows: VTO>BCLGO>HVGO(no effects). For developmental effects, including teratogenic potential, the materials may be ranked as: BCLGO>VTO>HVGO(no effects). Completion of fetal visceral evaluations will provide additional data for ranking each material for teratogenic effects. Although administration of both LCO and LCCN resulted in maternal toxicity and there was evidence of teratogenicity in fetuses from dams exposed to LCO, it is not feasible to include these materials in the ranking due to the small sample size.

