

CODING FORMS FOR SRC INDEXING

Microfiche No.		OTS0509763-16	
New Doc ID	000811604K	Old Doc ID	8EHQ-0295-0576S
Date Produced	01/09/95	Date Received	02/01/95
		TSCA Section	8E
Submitting Organization		CONFIDENTIAL	
Contractor		CONFIDENTIAL	
Document Title		SUPPORT: TERATOGENICITY STUDY IN RATS EXPOSED ORALLY TO A SINGLE DOSE OF A REFINERY STREAM WITH COVER LETTER DATED 012395 (SANITIZED)	
Chemical Category		COKER LIGHT GAS OIL; VACUUM TOWER OVERHEADS; LIGHT CYCLE OI*	

SUPP

OFFICE OF TOXIC SUBSTANCES
CODING FORM FOR GLOBAL INDEXING

REV. 7/27/82

Microfiche No. (7) *	OTS 0509763-16		1	No. of Pages	2
Doc I.D.	3	Old Doc I.D.	8EHQ-0295-05765		
Case No.(s)	5				
Date Produced (6)	6	Date Rec'd (6)	7	Conf. Code *	8
					N
Check One:	<input type="checkbox"/> Publication <input type="checkbox"/> Internally Generated <input type="checkbox"/> Externally Generated				
Pub/Journal Name	9				
					9
Author(s)	10				
Organ. Name	11				
Dept/Div	12				
P.O. Box	13	Street No./Name			14
City	15	State	16	Zip	17
					18
MID No. (7)	19	D & B NO. (11)			20
Contractor	21				
Doc Type	PE				
Doc Title	23				
Chemical Name (300 per name)	25	CAS No. (10)			24

0 0 0 0

PDCN:8886000 36 8E HQ. 0295-0576

8E HQ-66-36 FLWP

000811604 H

January 23, 1995

Certified Mail

Return Receipt Requested

U.S. Environmental Protection Agency
OPPT Document Control Officer
Attn: TSCA Document Receipts (TS-790)
401 "M" Street, S.W.
Washington, DC 20460

COMPANY SANITIZED

RECEIVED
95 FEB -1 AM 9:23

EPA Document Control No.:

8EHO-1185-0675

0576

Dear Sir:

In May, 1987, [redacted] 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. Interim (preliminary) reports for single dose developmental toxicity studies on several streams were submitted in September, 1993. At this time we are submitting a final report for certain of these studies. The test articles for which developmental effects were seen, with CAS numbers, are given below. We believe the effects seen in this study are due largely to polycyclic aromatic compounds in agreement with conclusions reported in our previous submissions.

Maternal toxicity involving decreased net body weight gain was seen for all three materials listed below. Decreased thymus weight and decreased fetal weights were reported for LCO. Malformations observed included digit anomalies (CLGO, LCO, VTO), micrognathia (VTO), and cleft palate (VTO). The current submission adds data from visceral evaluations; however, no remarkable findings were observed for any of the test materials.

<u>Study #</u>	<u>CAS #</u>	<u>Test Article</u>
65371	64741-82-8	Coker Light Gas Oil (CLGO)
65371	64741-49-7	Vacuum Tower Overheads (VTO)
65371	64741-49-5	Light Cycle Oil (LCO)

This study was carried out at

Confidentiality is being claimed for company identifiers 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 claim is attached.

Sincerely,

Enclosures

TABLE OF CONTENTS
VOLUME 2*

	Pages
6.0 APPENDICES	
6.1 Individual Clinical Observations.....	10
6.2 Individual Maternal Body Weights.....	6
6.3 Individual Maternal Body Weight Change.....	6
6.4 individual Uterine Weight and Net Maternal Body Weight Change.....	6
6.5 Individual Liver and Thymus Weights.....	6
6.6 Individual Parental Necropsy Observations.....	3
6.7 Individual Cesarean Section Data.....	6
6.8 Individual Fetal Status and Uterine Location.....	6
6.9 Individual Fetal Body Weights.....	6
7.0 Individual Fetal External Observations.....	24
7.1 Individual Fetal Soft Tissue Observations.....	14

* Volume 2 (Appendices) will not be distributed with this Final Report
but will be stored in the Document Archives.

SUMMARY

Five refinery streams - Coker Light Gas Oil (CLGO), 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.

Administration of LCO, LCCN, VTO, and CLGO 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 > CLGO > 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 CLGO, LCO, and VTO. Malformations observed included digit anomalies [CLGO ($p < 0.01$), LCO, VTO], micrognathia (VTO), and cleft palate (VTO). However, fetal visceral evaluations showed no remarkable findings for all groups. 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: CLGO > VTO > HVGO (no effects).

Based on these data, the refinery streams CLGO, 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. The 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 - DLGO, 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).

All animals were monitored throughout the study until sacrifice for 1) changes in appearance, behavior, and excretory function, and 2) signs of ill-health, mortality, and/or abortion. A prepartum investigation on a variety of fetal and maternal parameters for each of the groups was undertaken to assess the influence of the refinery streams on reproductive performance and fetal development. The inclusive dates for specific study activities were as follows:

Acclimation Period: January 5 - January 18, 1993
 Mating Period: January 18 - January 23, 1993
 Gestation Period: January 19 - February 12, 1993
 Dosing Period: February 1 - February 5, 1993
 Cesarean Section: February 8 - February 12, 1993
 Fetal Visceral Exams: November 17, 1993 - December 5, 1994

TABLE 1
Summary of Experimental Design

<u>GROUP</u>	<u>DOSE LEVEL</u> (mg/kg)	<u>MATERIAL</u>
Group 1	2000	Tap Water Control
Group 2	2000	Coker Light Gas Oil
Group 3	2000	Heavy Vacuum Gas Oil
Group 4	2000	Light Catalytically Cracked Naphtha
Group 5	2000	Light Cycle Oil
Group 6	2000	Vacuum Tower Overheads

2.2 *Animal Data*

Ninety virus-free female Sprague-Dawley rats [VAF/Plus CrI:CD(SD)BR; approximately 8 weeks old] were obtained from Charles River Breeding Laboratories, Kingston, New York. The male rats used as breeders were already in-house and had been received on December 12, 1992, from the same Charles River facility. The females were acclimated to the test facility for two weeks before the breeding period was initiated. Each female was individually identified by a numbered metal ear tag on gestation day 0. Tap water and Purina Certified Rodent Chow #5002 were provided *ad libitum* during the course of the study. Animals were maintained in air-conditioned rooms set to maintain 68-72°F, 40-60% relative humidity, and 12 hour light-dark cycles. During the entire study, the average temperature ranged from 69-71°F. On one day during the gestation period, the temperature reached a high of 73°F; this was the only 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.

0014

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: 64741-51-7

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 Naptha

CAS Number: 64741-55-5

CRU Number: 34152

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: 0.93 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.

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.

0 0 1 5

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 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 were not scheduled or performed, however, the fixed tissues have been saved and will be stored in the Tissue Archives of

2.9 Data Analyses and Storage

Raw data were collected, processed, and analyzed using the Grosse Data Acquisition/Reporting System. 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% ($p < 0.05$).

The study Support Data book, Gestation and Cesarean Section Direct Data Printout books, Visceral Examination book, Grosse System printouts of all individual animal data, and all released reports will be maintained in the Document Archives of . Fetal visceral and skeletal tissues will be stored in the Tissue Archives of

0018

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 (CLGO, 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 (CLGO, LCCN, LCO), animal cold to the touch (CLGO, LCO), and no stool (CLGO). 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 non-viscous, 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 (CLGO), 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. Individual clinical observations are presented in Appendix 6.1.

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. Individual body weights, body weight change, and uterine weight and net maternal body weight change are presented in Appendices 6.2, 6.3 and 6.4 respectively.

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. Individual liver and thymus weights are presented in Appendix 6.5. No other findings attributable to administration of the refinery streams were noted at the time of necropsy. The individual parental necropsy observations are presented in Appendix 6.6.

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 pre-implantation 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. The individual Cesarean section data is presented in Appendix 6.6. The individual fetal status and uterine location are presented in Appendix 6.7.

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. Individual fetal body weights are presented in Appendix 6.8.

A statistically significant increase ($p < 0.01$) in malformation of the hindpaw digits was observed in fetuses from a dam exposed to CLGO. 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. Summary of fetal external evaluations are presented in Table 9 and individual external evaluations are presented in Appendix 6.9. The fetal visceral evaluations showed no remarkable findings for all groups. The summary of visceral evaluations is presented in Table 10 and individual findings are presented in Appendix 7.0.

4.0 DISCUSSION AND CONCLUSIONS

In general, a single oral administration of LCO, LCCN, VTO, and CLGO 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 CLGO 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 CLGO. 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 CLGO 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 > CLGO > HVGO (no effects). For developmental effects, including teratogenic potential, the materials may be ranked as: CLGO > VTO > HVGO (no 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.

