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Dear Sir:

In May, 1987, Mobil submitted a TSCA Section 8(e) notification on the toxicity of clarified slurry oil (CAS 64741-62-4). Supplemental submissions to this 8(e) have been made on several other refinery streams to describe the relationship between stream composition and toxicity. This submission consists of an interim report from a developmental toxicity study done on Heavy Coker Gas Oil (CAS #64741-81-7). This report describes evidence of maternal toxicity consisting of clinical observations, decreased food consumption and net body weight gain, decreased thymus weights (absolute and relative), and increased liver weights (relative). Maternal toxicity was observed at all dose levels tested. Evidence of developmental toxicity was seen at or above 30 mg/kg and included in utero death (resorption) and decreased fetal weights. These results reflect only the biophase portion of the study. Fetal skeletal and visceral evaluations, as well as clinical chemistry results, will be submitted when they become available.

A report for the following study is enclosed:

Study #	CAS #	Study Title
64168-I	64741-81-7	Developmental Toxicity Study in Rats Exposed Dermal to Joliet Heavy Coker Gas Oil (JHCGO)

Sincerely,

C.R. Mackerer
C. R. Mackerer, Ph.D.

Enclosures

INTERIM REPORT

DEVELOPMENTAL TOXICITY STUDY IN RATS EXPOSED DERMALLY
TO JOLIET HEAVY COKER GAS OIL

STUDY NUMBER 64168-I

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SUMMARY

Joliet Heavy Coker Gas Oil (JHCGO) was applied once daily via dermal application to presumed-pregnant rats at doses of 0, 8, 30, 125, or 250 mg/kg/day. All animals were dosed on gestation days 0-19; cesarean sections were performed on gestation day 20.

Maternal toxicity was observed at all dose levels. The effects noted were decreased food consumption, decreased net body weight gain, decreased thymus weights (both absolute and relative) and increased liver weights (relative). Numerous clinical signs of maternal toxicity were also observed at 30 mg/kg and above. They included red vaginal discharge, paleness, emaciation, and moribundity (250 mg/kg). With the exception of the clinical observations, a dose-related response was demonstrated for all maternal parameters evaluated. Consequently, adverse effects observed at lower dose levels were considered biologically significant in the few cases where statistical significance was not achieved. Application of JHCGO also resulted in skin irritation which ranged from slight (8 mg/kg) to severe (250 mg/kg).

Developmental toxicity was observed at 30 mg/kg and above. In utero death (resorption) was significantly increased and fetal weights significantly decreased at 125 and 250 mg/kg. The incidence of resorption was also increased at 30 mg/kg and, although not statistically significant, is considered to be biologically significant.

In summary, these preliminary data indicate that JHCGO applied dermally to pregnant rats produces maternal toxicity at 8 mg/kg and above, and developmental toxicity at 30 mg/kg and above.

1.0 INTRODUCTION

A developmental toxicity study was conducted at Mobil's Environmental and Health Sciences Laboratory (EHSL) in which Joliet Heavy Coker Gas Oil (JHCGO) was applied to the skin of pregnant rats. JHCGO, a refinery stream produced by thermal cracking of vacuum residuum, was applied dermally since production and processing of this material can result in repeated human skin contact. The primary objectives of this study were to evaluate the effects of dermal JHCGO exposure on female rats during gestation and to determine if such exposure adversely affects fetal viability and development. Selection of dose levels was based on the results of a subchronic toxicity study using this material (Study No. 64165).

In order to obtain a more complete profile of maternal toxicity, the following procedures were also performed: maternal necropsy, weighing of the liver and thymus, serum chemistry analysis, hematology analysis, micronucleus analysis, and DNA adduct analysis (fetal liver samples were also collected for DNA adduct analysis). Serum chemistry and hematology results will not be presented in this Interim Report, but will be discussed in the Final Report. The micronucleus and DNA adduct analyses were performed under separate protocols and will be issued as separate reports by the Genetic Toxicology group (Study Nos. 64169 and 64170, respectively).

2.0 METHODOLOGY

2.1 Experimental Design

Presumed-pregnant rats were distributed into five experimental groups with dose levels of 0, 8, 30, 125, and 250 mg/kg/day. JHCGO was administered daily to each female throughout gestation (days 0-19). The dosing regimen is summarized in Table 1. At the start of the dosing phase of the study,

each group contained fifteen presumed-pregnant females.

TABLE 1

Summary of Experimental Design

GROUP	DOSE LEVEL (mg/kg/day)	MATERIAL	DAYS OF ADMINISTRATION*
Group 1	0	Sham Control	0-19
Group 2	8	JHCGO	0-19
Group 3	30	JHCGO	0-19
Group 4	125	JHCGO	0-19
Group 5	250	JHCGO	0-19

* Designation as gestation day 0 followed detection of a vaginal plug (in situ or expelled) and spermatozoa in the vaginal lavage fluid.

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 abortion. A prepartum investigation on a variety of maternal and fetal parameters was undertaken to assess the influence of JHCGO on reproductive performance and development of offspring. The inclusive dates for specific study activities were as follows:

Acclimation Period: December 17, 1991 to January 6, 1992
Mating Period: January 6-17, 1992
Gestational Period: January 7 to February 6, 1992
Cesarean Section: January 27 to February 6, 1992

2.2 Animal Data

One hundred male Sprague-Dawley rats [VAF/Plus CrI:CD(SD)BR; 7 weeks old] were obtained from Charles River Breeding Laboratories, Kingston, New York (the breeder males were received in July of 1991 from the same supplier and location). The females were acclimated to the test facility for three

weeks before the breeding period was initiated. Tap water and Purina Certified Rodent Chow #5002 (meal) were provided ad libitum during the course of the study. Animals were housed in air-conditioned rooms set to maintain 20-22°C, 40-60% relative humidity, and 12-hour light-dark cycles. Each female was individually identified by a numbered metal ear tag on gestation day 0.

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 and observed daily for evidence of having engaged in breeding activity. Each morning during the period of cohabitation, the drop-pan papers under the animal cages were checked for the presence of expelled vaginal sperm plugs; additionally, each female rat was examined for the presence of in situ vaginal sperm plugs. Vaginal lavage fluid was obtained from each female which exhibited a vaginal plug in situ or on the drop-pan papers, and was examined for the presence of spermatozoa. Females that were positive for sperm plug as well as for spermatozoa were considered to be at day 0 of presumed gestation and were placed in individual housing units. The cohabitation period was continued until 75 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 among the experimental groups using a computer-generated table of random numbers for a stratified sample size of five. This procedure was continued each morning during the cohabitation period until each group contained fifteen presumed-pregnant females.

2.7 Observations During Gestation

2.7.1 Appearance and Clinical Signs

Each presumed-pregnant female was observed at least once a day throughout gestation until sacrifice for signs of pathosis, abortion, premature delivery and/or death. All unusual findings were recorded.

2.7.2 Body Weights and Food Consumption

The body weight of each female was measured to the nearest 0.1 gram on days 0, 3, 6, 10, 13, 16, and 20 of gestation. Similarly, the amount of food consumed by each animal was calculated for gestation day intervals 0-3, 3-6, 6-10, 10-13, 13-16, and 16-20. Stainless-steel feeders, identified individually by female rat number, were weighed on the first and last day of the specified interval. Feeders were weighed before noon. Since rats feed mainly at night, more definite control of the weighing time is unnecessary. When a rat spilled nonrecoverable amounts of food, the "consumption data" for that animal were excluded from data calculations for that collection interval.

2.8 Female Necropsy

Each female rat was sacrificed by over-exposure to diethyl ether on its 20th day of presumed gestation. The abdominal cavity was exposed and the reproductive organs were examined grossly for evidence of pathosis. Following removal of the uterus and ovaries, the carcass was given to the Pathology Group for macroscopic examination of the remaining organs. The thymus and liver of each pregnant female were weighed (to the nearest 0.001 gram), and the weights recorded by a member of the Pathology Group. Only the livers of pregnant females were preserved in 10% neutral buffered formalin.

2.8.1. Uterine/Ovarian Examination

The ovaries and uterus of each rat were excised and examined grossly. The number of corpora lutea (a measure of the number of eggs ovulated) per ovary of each pregnant female was counted and recorded. The ovaries of nonpregnant females were grossly examined and then discarded. All remarkable findings were recorded. The weight of the intact uterus was measured to the nearest tenth of a gram and recorded. The uterine contents of each pregnant rat were 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, examined grossly for evidence of implantation, and then discarded. The uteri and ovaries (except those given to the Genetic Toxicology group for DNA analysis) of pregnant females were discarded following data collection.

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 tenth of a gram, and grossly examined for external anomalies. The following definitions and terminology were used in describing fetal findings [1]:

- Malformation:** A permanent structural change that may adversely affect survival, development, or function. 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 construction that may not adversely affect survival or health.
- Incidental:** An incidental finding is generally an

accidental event, e.g., accidentally, tip of tail was cut off.

After gross evaluation, fetuses in each litter (except one litter in Group 2 for which all fetuses were inadvertently prepared for skeletal exam) were equally distributed into two groups, and preparation began for either soft tissue or skeletal evaluations. Results of fetal visceral and skeletal evaluations will be presented in the Final Report.

2.9 Data Analyses

Data were collected, processed, and analyzed using the Reproduction module of 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.

Thymus and liver weights were collected, processed, and analyzed using the Pathology module of the Grosse Data Acquisition/Reporting System. The data were statistically evaluated using analysis of variance and Tukey's Test.

For all statistical analyses, differences between control and treated groups were considered to be statistically significant if the probability of the difference being due to chance was less than 5% ($p < 0.05$).

3.0 RESULTS

3.1 Clinical Observations

Incidental observations reported during gestation are presented in Table 2. The red nasal exudate and chromodacryorrhea noted in control and treated groups are common in animals that are collared and/or stressed. Scratches were observed on the backs of four animals. Three of these were noted at the time of the first clipping and probably occurred during mating

activity. Scratches appeared on one female in Group 5 during the latter part of gestation. She was probably scratching in response to the irritation of the treated skin; this case of "scratches" is identified as a separate observation in Table 2. Several females developed neck lesions in spite of the protective soft rubber tubing that lined the inner surface of the cardboard collar.

JHCGO-related observations reported during gestation are also included in Table 2. Skin irritation was present in all groups exposed to JHCGO. The irritation ranged from slight at 8 mg/kg (erythema and flaking) to severe at 125 and 250 mg/kg (thickening of the skin, fissuring of the skin, and open sores). Clinical signs of maternal toxicity were evident and, in some cases, severe at 125 and 250 mg/kg. One female in the high dose group (250 mg/kg) was sacrificed moribund on gestation day 16. She had no stool, was emaciated and cool to the touch, and had severe vaginal bleeding (red vaginal discharge). Uterine examination revealed 20 implantation sites, all of which were resorbed. Another female in this group exhibited decreased activity and labored breathing on gestation day 17.

Red vaginal discharge was observed at 30 mg/kg and above; the incidence increased with increasing dose level. In all cases, the discharge could be attributed to resorption of the offspring. Several females at the 125 and 250 mg/kg dose levels became pale and their skin became cool to the touch following the onset of the red vaginal discharge.

3.2 Body Weights and Food Consumption

Mean body weights, mean body weight changes, and mean uterine and net body weights are presented in Tables 3, 4, and 5, respectively. In general, the mean body weights of all groups treated with JHCGO were significantly lower than the mean weights of the control group throughout most of gestation (Table 3). It should be noted that, on gestation day 0, there was no

significant difference among the mean body weights for the groups.

Overall mean body weight gain (gestation days 0-20) decreased with increasing dose level. Mean body weight gains were significantly reduced at 30, 125, and 250 mg/kg. At 30 mg/kg, the significance was apparent when overall body weight gain was calculated. The decrease in body weight gain was not severe at the 125 and 250 mg/kg dose levels and achieved statistical significance for nearly all intervals measured (Table 4). Although not statistically significant, body weight gain was also reduced throughout the gestational period at 8 mg/kg.

Net body weight gain was significantly reduced at 125 and 250 mg/kg (Table 5). Statistical significance was not achieved for the mean net body weight changes at 8 and 30 mg/kg, however both were reduced compared to the control mean value.

Mean daily food consumption values during gestation are presented in Table 6. Food consumption was significantly decreased in all groups treated with JHCGO. The number of intervals during which food consumption was significantly reduced, as well as the amount of reduction, increased with increasing dose level.

3.3 Observations at Cesarean Section

3.3.1 Maternal Necropsy Findings

Mean maternal liver and thymus weights are shown in Table 7. The mean absolute liver weight for the high dose group (250 mg/kg) was significantly reduced. Under normal conditions, liver weight increases during pregnancy. When animals resorb their litters, as was the case for animals exposed to 250 mg/kg, the liver returns to the smaller size which is normal for a non-pregnant female. Calculation of relative weights (Table 7) shows that the mean relative liver weights were significantly increased at 125 and 250

mg/kg. Absolute thymus weights were significantly reduced at 30 mg/kg and above. Relative thymus weights decreased with increasing dose level, but statistical significance was achieved only at 125 and 250 mg/kg.

3.3.2 Reproductive/Developmental Evaluations

A summary of the reproductive data is presented in Table 8. Viable litter size was significantly reduced at 125 and 250 mg/kg. Both mean number and percent resorptions were significantly increased at these same dose levels as was the number of dams with resorptions. Overall, resorption increased with increasing dose level. The increase at 30 mg/kg is considered to be biologically significant since approximately one-half of the females in this group had between 14 and 39 percent fetal resorption (the mean for the control group was 4.9 percent resorption). The biological significance of the increase in percent resorption for the 8 mg/kg group is uncertain.

The statistical significance achieved at 30 mg/kg for the number of male and female fetuses is not considered to be biologically significant and can be attributed to the unusually high number of males and low number of females in the control group.

Fetal body weights, a parameter of body growth and development, were significantly decreased for all viable fetuses at the 125 and 250 mg/kg dose levels (Table 9). In addition, there were isolated incidences of variations and malformations at 8, 30, and 125 mg/kg (Table 10). Kinked tail was noted in two fetuses; one in the 8 mg/kg dose group and one in the 125 mg/kg dose group. One fetus (30 mg/kg) had gastroschisis (protrusion of the intestines through a fissure in the abdominal wall). These scattered findings did not appear to be related to test material administration.

4.0 DISCUSSION AND CONCLUSIONS

Daily application of JHCGO on the skin of pregnant rats during gestation resulted in maternal toxicity at all dose levels. Evidence of maternal toxicity included numerous clinical signs, decreased food consumption and net body weight gain, decreased thymus weights, and increased liver weights. Although statistical significance was not always achieved at the lower dose levels, (e.g., relative liver and thymus weights at 8 and 30 mg/kg), a clear dose-response relationship exists for all maternal parameters evaluated thus far. Application of JHCGO to the skin also resulted in skin irritation which ranged from slight (8 mg/kg) to severe (250 mg/kg).

Fetal development was adversely affected by JHCGO administration. The number and percent resorptions were significantly increased and live litter size significantly decreased at 125 and 250 mg/kg. The increase in in utero death (resorption) at 30 mg/kg is considered to be biologically significant. The percent resorption was also increased at the lowest dose level (8 mg/kg), however, the increase was due to two females with 24 and 25 percent resorption, respectively. These two females may have been more sensitive to the effects of JHCGO administration.

External evaluations of fetuses revealed a significant decrease in fetal weights at 125 and 250 mg/kg as well as apparently isolated incidences of malformation and variation at 8, 30, and 125 mg/kg. With regard to the incidence of malformation/variation, the total number of affected fetuses were two, one, and two, respectively; there is no evidence of a dose response. Due to this low incidence of seemingly unrelated observations and the lack of a dose response, the observed external anomalies cannot be positively correlated with JHCGO exposure. Fetal skeletal and visceral evaluations (not yet completed) may provide more conclusive data regarding

the teratogenic potential of JHCGO.

In conclusion, the data available at the time of this interim report suggest that daily dermal administration of JHCGO throughout gestation produces maternal toxicity in the rat at levels greater than or equal to 8 mg/kg and developmental toxicity at levels greater than or equal to 30 mg/kg.