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<b>Contractor</b>			
UBTL INC			
<b>Document Title</b>			
SUPPORT: LTR FROM ARCO CHEM CO TO USEPA RE DEVELOPMENTAL TOXICITY SCREENS IN FEMALE RATS ADMINISTERED 4 REFINERY STREAMS DERMALLY DURING GESTATION DAYS 0-20 W/ATTCHMTS DATED 032294			
<b>Chemical Category</b>			
HEAVY COKER GAS OIL; FULL RANGE GAS OIL; HYDROCRACKER FEED;*			

SUPP

OFFICE OF TOXIC SUBSTANCES  
CODING FORM FOR GLOBAL INDEXING

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IN(CBTS)

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

Subject: TSCA Section 8(e) Notice on Refinery Streams  
Suspected of Containing Varying Levels of  
Carbazoles that are Present in Carbon Black Oil  
(CAS# 64741-62-4; 8EHQ-1185-0576)

Dear Sir/Madam:

In accordance with the provisions of Section 8(e) of the Toxic Substances Control Act, the Atlantic Richfield Company (ARCO) is submitting information on the results of studies in experimental animals to assess the developmental toxicity of four refinery streams suspected of containing carbazoles. This information supplements previous TSCA submittals on these materials.

This is one of several follow-up studies initiated by ARCO in response to previous reports by Mobil and ARCO (8EHQ-1185-0576) of adverse effects on rat fetuses after dermal exposure to carbon black oil (CBO) a material which contains carbazoles. The objective of the ARCO studies being reported in this letter was to determine the maternal and fetal toxicity of selected refinery streams that, like CBO, may contain carbazoles.

The materials examined in this study were Heavy Coker Gas Oil, (ARCO sample F-274; CASN# 64741-31-7) Full Range Gas Oil (ARCO sample F-275; CASN# 68410-00-4), Hydrocracker Feed (ARCO sample F-276; CASN# 64741-57-7) and Light Coker Gas Oil (ARCO sample F-277; CASN# 64741-82-8). The preliminary results of earlier screening studies on three of these materials (Heavy Coker Gas Oil, Light Coker Gas Oil and Hydrocracker Feed) were submitted to EPA in letters from ARCO dated November 13, 1991 and November 23, 1993. The studies on these three materials submitted today were performed on additional samples of these materials obtained from another refinery within our system.

EPA-OTS



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### ABSTRACT

The objective of this study was to determine the developmental toxicity of F-274 following daily dermal administration to female Sprague-Dawley rats on Days 0 through 20 of gestation.

Three groups, each with 12 presumed pregnant female rats, were administered F-274 topically at doses of 1, 50 or 250 mg/kg/day once daily during Days 0 through 20 of gestation. A fourth group of 15 presumed pregnant female rats served as a sham control group. With the exception of test article application, these control animals underwent the same procedures as the 1, 50 and 250 mg/kg dose groups.

Each female was observed twice daily for viability and once daily for signs of toxicity. General Health Check observations were performed on Days 0, 4, 8, 12, 16 and 20 of gestation and on Days 0 and 4 of lactation. Body weights were recorded for each female within 48 hours of receipt, near the end of the quarantine period, on Days 0, 4, 8, 12, 16, and 20 of gestation, and on Days 0 and 4 of lactation. Food consumption was measured for Days 0 to 4, 4 to 8, 8 to 12, 12 to 16, and 16 to 20 of gestation; and Days 0 to 4 of lactation. Dermal irritation at the test site was evaluated and recorded each day prior to test article application; on presumed Gestation Day 25 for females that mated but did not deliver, and on Lactation Days 0 and 4 for females that did deliver. On Day 4 of lactation or on the day following the death of all pups for females that delivered a litter, or on Gestation Day 25 for females that did not deliver a litter, each female was sacrificed and subjected to a gross necropsy. The uterine horns of each female were examined to determine the number of implantation sites.

On Days 0 and 4 of lactation, each pup was weighed and its sex was determined. Each pup was also examined for any gross abnormalities. On Days 1 - 3 of lactation, each litter was observed for determination of the number of dead or missing pups. On Day 4 of lactation, all surviving pups were sacrificed and discarded.

Signs of maternal toxicity considered to be related to administration of F-274 included dermal irritation at doses of 1, 50 and 250 mg/kg, and decreased body weight, body weight change, food consumption and relative food consumption at doses of 50 and 250 mg/kg.

Signs of developmental toxicity considered to be related to administration of F-274 included decreased number of total and live pups delivered per litter at a dose of 50 mg/kg. At a dose of 250 mg/kg F-274 there were no litters delivered and the number of implantation sites were significantly decreased.

UBTL Study No. 67005  
Protocol No. ATX-93-0069

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Based on the results of this study, the no-observable-effect level for maternal toxicity of F-274 is less than 1 mg/kg. If maternal toxicity is considered excluding dermal irritation effects, the no-observable-effect level for maternal toxicity is considered to be 1 mg/kg. The no-observable-effect level for signs of developmental toxicity is considered to be 1 mg/kg.

Given the design of the study and the observed results, it is not possible to determine if the effects observed were a result of an effect on the dam and the ability to produce and carry a conceptus, or a direct effect on the embryo/fetus.

### OBJECTIVE

The objective of this study was to determine the developmental toxicity of F-274 following daily dermal administration to female rats on Days 0 through 20 of gestation.

### TEST ARTICLE

#### Description

The test article, F-274, is an amber colored viscous liquid with a slightly cracked or burnt oil odor.

Information on the methods of synthesis, lot number, expiration date, and stability, as well as data on composition or other characteristics which define the test article, are the responsibility of the Sponsor.

#### Storage

The test article was stored in a refrigerator under an argon blanket.

#### Reserve Sample

A reserve sample of the test article was taken and stored in a refrigerator.

### TEST SYSTEM AND HUSBANDRY

#### Test System

Female Sprague-Dawley rats (CrI:CD®Br) were obtained from SASCO, Inc. (Madison, Wisconsin facility). Males from the same strain and source as the females were used for mating.

The females were acclimated for 12 to 14 days before initiation of mating. During acclimation, the animals were observed twice daily for viability, and general health status observations were performed weekly.

Each female was assigned a unique number that was permanently indicated on the animal with an ear tag and identified on the animal's cage card.

At initiation of treatment, the females were between 9 and 10 weeks of age and weighed between 197 and 252 g.

#### Husbandry

The animals were individually housed in stainless steel, wire mesh-bottomed cages, except during the mating period when females were

cohabited with males overnight and during lactation when females were housed with their litters. Between Days 13 and 15 of gestation, each female was placed in a nesting box and a suitable bedding material was added for nesting purposes.

The animal room environmental controls were set to maintain a ventilation rate of no less than 10 air changes per hour, a temperature range of 64 - 79°F, 40 - 70% relative humidity, and a 12-hour light/12-hour dark cycle. The animal room temperature and humidity were monitored daily.

Fresh certified rodent feed was provided ad libitum throughout acclimation and the study period. PROLAB™ R-M-H 3000 (Agway Inc.) was provided during acclimation. Beginning approximately one week before initiation of mating, the animals were fed PROLAB™ R-M-H 3200 Meal (Agway Inc.).

Water was provided ad libitum. The water is analyzed periodically for impurities according to UBTL Standard Operating Procedures.

There were no potential contaminants believed to be in the food, water, or bedding that would adversely affect the outcome of the study.

#### EXPERIMENTAL DESIGN

Females were assigned at random to four groups on the day mating was confirmed, as outlined in the following:

<u>Group Number</u>	<u>Treatment</u>	<u>Dose Level (mg/kg)</u>	<u>Number of Females</u>
1	Sham Control <sup>a</sup>	0	15
2	F-274	1	12
3	F-274	50	12
4	F-274	250	12

a Sham control animals were shared with Study Number 67006 (ATX-93-0071) With the exception of test article application, these animals underwent the same procedures as the test article treatment group animals.

#### PROCEDURES

##### Protocol

This study was conducted in accordance with Protocol No. ATX-93-0069 (Appendix A).

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### Animal Preparation

Prior to the first application of the test article, the backs of all females were closely clipped of hair using electric Oster animal clippers equipped with a size 40 blade. Clipping was repeated as necessary. Care was taken to leave the skin intact during clipping. Prior to the first application of test article, the application site was examined for evidence of dermal irritation.

### Dose Preparation

On the day of dosing, the test article aliquots were removed from the refrigerator and warmed to 22°C in a waterbath for approximately 20 minutes, prior to dosing. The test article was administered neat.

### Dose Administration

The test article was applied to previously clipped, intact (with the possible exception of scratches due to mating activity) dermal sites on the backs of the females. The 1, 50 and 250 mg/kg dose groups received the test article on presumed Days 0 through 20 of gestation. If dermal irritation was present, the test article was applied to the skin showing the least amount of irritation. Elizabethan collars were applied just prior to dosing and were removed no sooner than 6 hours later. At the time of collar removal, any excess test article noted was wiped from the site using clean Webril® pads.

The dose administered was based upon the Gestation Day 0 body weight.

### Mating

An in-house group of male rats (of the same strain and source as the females) were used for breeding purposes. Each female was cohabited with one male nightly and was examined daily for positive evidence of mating (presence of sperm in a vaginal smear or a copulatory plug). On the day a female showed evidence of mating (considered to be Day 0 of gestation), cohabitation with the male ceased. The mating procedure was continued until the required number of presumed pregnant females were assigned to each group.

### Clinical Observations

During quarantine, the animals were observed twice daily (a.m. and p.m.) for viability, and a general health status observation was performed weekly. After initiation of treatment, each female was observed twice daily (a.m. and p.m.) for viability and once daily (p.m.) for signs of toxicity. General Health Check observations were performed on Days 0,

4, 8, 12, 16 and 20 of gestation and on Days 0 and 4 of lactation. Dermal irritation at the test site was evaluated and recorded each day prior to test article application; on presumed Gestation Day 25 for females that mated but did not deliver, and on Lactation Days 0 and 4 for females that did deliver.

#### Body Weights

Body weights were recorded for each female within 48 hours of receipt, near the end of the quarantine period; on Days 0, 4, 8, 12, 16, and 20 of gestation; and on Days 0 and 4 of lactation.

#### Food Consumption

Food consumption was measured over the following intervals: Days 0-4, 4-8, 8-12, 12-16, and 16-20 of gestation; and Days 0-4 of lactation.

#### Litter Observations

On Days 0 (day of parturition) and 4 of lactation, each pup was weighed and its sex was determined. Each pup was also examined for any gross abnormalities. On Days 1 - 3 of lactation, each litter was observed for determination of the number of dead or missing pups. On Day 4 of lactation, all surviving pups were sacrificed and discarded.

#### Post Mortem Observations

Each female that mated was sacrificed with carbon dioxide and underwent a gross necropsy. Females that delivered a litter were necropsied on Day 4 of lactation. Those females that delivered a litter and all of the pups died were necropsied the day following the death of all their pups and those that did not deliver a litter, were necropsied on presumed Gestation Day 25.

The necropsy included a gross examination of the external body surfaces, orifices, and the cervical, thoracic and abdominal viscera. The number of implantation sites within the uterine horns was recorded. Uteri that appeared non-gravid were placed in 10% ammonium sulfide in order to reveal any implantation sites. If no implantation sites were observed, the animal was considered to be non-pregnant.

Dead pups were removed and examined externally. If there were no external abnormalities, the pups were discarded. On Day 4 of lactation, all surviving pups were sacrificed with an intraperitoneal injection of Beuthanasia-D or sodium pentobarbital.

### Statistical Analysis

Statistical analysis was performed on an IBM compatible PC using SAS, version 6.04.

Statistical evaluation of maternal body weight, body weight change, absolute feed consumption and relative feed consumption was assessed by a standard one-way ANOVA using a t-test/Least Significant Difference approach. Normal probability plots of the residuals were used to determine whether or not departure from the assumptions of normality and homogeneous variance were sufficient to invalidate the usual ANOVA analysis. If the usual analysis was invalid, a "weighted" General Linear Model (GLM) analysis was used, where the "weights" were proportional to the reciprocal of the variance. If the usual analysis was valid, the data were analyzed with a non-weighted GLM.

Statistical evaluation of the female reproductive data and litter data was assessed as follows:

For number of implantation sites, gestation length, total number of pups alive on Day 0, total number of pups (dead and alive) on Day 0 and total number of pups exhibiting abnormalities; nonparametric analysis by Wilcoxin Scores (Rank Sums) was used to assess significance. If significant differences among the means were indicated, the Kruskal Wallis test was used to determine which treatment groups differed from control.

All proportion data (dead pups at Day 0, male pups at Day 0 and 4 and survival of pups at Day 4) were adjusted using an arcsin transformation. Statistical evaluation of the transformed data was assessed by a standard one-way ANOVA using a t-test/Least Significant Difference approach.

Average live pup weight at Days 0 and 4 was analyzed by the "weighted" GLM, with litter size used as the "weights" and as a covariate in the model. The assumption was made that these weights were proportional to the reciprocal of the variance.

For all proportion and mean pup weight data, values were first derived within the litter, and group mean values were derived as a mean of the individual litter mean values.

All statistical tests were conducted at both the 5% and 1% levels of significance.

### STUDY DATES

Study Initiation Date:	November 19, 1993
Animals received:	November 10 & 12, 1993
Test article use/Dosing initiated:	November 25, 1993
Test article use/Dosing completed:	December 17, 1993
Animal termination:	December 20-23, 1993
Study Completion Date:	Refer to the signature page for the date that the final report was signed by the Study Director.

### LOCATION OF RAW DATA

The raw data generated as a result of this study, the original protocol, protocol amendments, and the original final report will be maintained in the archives at UBTL, Inc., 520 Wakara Way, Salt Lake City, Utah, 84108.

### RESULTS

#### Mortality

There was no mortality observed during the study.

#### Clinical Observations

Summaries of female clinical observations during the gestation and lactation periods are presented in Tables 1 and 2, respectively. Individual female clinical observations are presented in Tables B1 and B2 in Appendix C.

Dermal irritation related to administration of the test article was noted for females dosed at 1 mg/kg beginning Gestation Day 3 and continuing through Gestation Day 15. Slight to moderate (primarily slight) erythema was observed at the test site. Slight dry skin was also observed at the test site.

Dermal irritation related to administration of the test article was noted for animals dosed at 50 mg/kg beginning as early as Gestation Day 2 and continuing throughout the duration of the study. Slight to moderate (primarily slight) erythema, edema, eschar and dry skin were observed at the test site.

Dermal irritation related to administration of the test article was noted for females dosed at 250 mg/kg beginning Gestation Day 2 and continuing throughout the duration of the study. Slight to severe erythema

(primarily slight and moderate) and eschar (primarily slight) were observed at the test site. Slight to moderate edema and dry skin were also observed at the test site. Vaginal discharge was observed in one female on Gestation Days 19 and 20.

Scratches on the back were observed in several rats within each of the treated and control groups. The scratches were observed within the first eight days of gestation and are considered to be a result of aggressive behavior exhibited during mating.

Shaving irritation was noted in one female of each of the dose groups and sham control group. Collar irritation was also noted in one female of the sham control group. Slight skin irritation remote from the treatment site was also noted in one female in each of the 1, 50 and 250 mg/kg groups. These observations are considered to be unrelated to treatment with F-274.

#### Body Weights and Body Weight Changes

Mean body weights and body weight changes for females are presented in Tables 3 and 4, respectively. Individual female body weights and body weight changes are presented in Tables B3 and B4, respectively, in Appendix B.

Body weights of pregnant females in the 1 mg/kg dose group were not significantly different than those of the control females throughout the duration of the study. Body weight changes for pregnant females in the 1 mg/kg dose group were significantly lower than those of the control females on Gestation Days 0 to 4 ( $p < 0.05$ ).

Body weights of pregnant females in the 50 mg/kg dose group were significantly lower than those of the control females on Gestation Days 16 ( $p < 0.05$ ) and 20 ( $p < 0.01$ ). Body weight changes for pregnant females in the 50 mg/kg dose group were significantly lower than those of control females on Gestation Days 0 to 4 ( $p < 0.05$ ), 4 to 8 ( $p < 0.01$ ), 12 to 16 ( $p < 0.05$ ) and 16 to 20 ( $p < 0.05$ ).

Body weights of pregnant females in the 250 mg/kg dose group were significantly lower ( $p < 0.01$ ) than those of the control females on Gestation Days 4, 8, 12, 16 and 20. Body weight changes for pregnant females in the 250 mg/kg dose group were significantly lower than those of control females between Gestation days 0 to 4 ( $p < 0.01$ ), 4 to 8 ( $p < 0.01$ ), 8 to 12 ( $p < 0.05$ ), 12 to 16 ( $p < 0.01$ ) and 16 to 20 ( $p < 0.01$ ).

The effects on body weight and body weight change observed at the 50 and 250 mg/kg dose levels are considered to be treatment related. A dose dependent correlation between dose and decreased body weight as

well as body weight change was observed at these dose levels. The decrease in body weight change in the 1 mg/kg dose group between Gestation Days 0 to 4 is not considered to be treatment related since this was not observed throughout the rest of the study and a dose related response was not observed.

#### Food Consumption

Mean absolute (g/animal/day) and relative food consumption (g/kg body weight/day) data for females are presented in Tables 5 and 6, respectively. Individual female absolute and relative food consumption data are presented in Tables B5 and B6, respectively, in Appendix B.

Absolute and relative food consumption of pregnant females in the 1 mg/kg dose group were not significantly different than those of the control females throughout the duration of the study.

Absolute food consumption of pregnant females in the 50 mg/kg dose group was significantly lower than those of the control females during Gestation Days 4 to 8 ( $p < 0.01$ ), 8 to 12 ( $p < 0.01$ ), 12 to 16 ( $p < 0.05$ ), 16 to 20 ( $p < 0.05$ ) and Lactation Days 0 to 4 ( $p < 0.01$ ). Relative food consumption of pregnant females in the 50 mg/kg dose group was significantly lower than those of control females during Gestation Days 4 to 8 ( $p < 0.01$ ), and Lactation Days 0 to 4 ( $p < 0.01$ ).

Absolute and relative food consumption of pregnant females in the 250 mg/kg dose group were significantly lower ( $p < 0.01$ ) than those of the control females during Gestation Days 0 to 4, 4 to 8, 8 to 12, 12 to 16 and 16 to 20.

The effects on absolute and relative food consumption observed in the 50 and 250 mg/kg dose groups are considered to be treatment related since they are consistently observed throughout the treatment period. In addition, there appears to be a correlation between dose and decreases in absolute and relative food consumption at these doses.

#### Gross Pathology Data

A summary of female necropsy observations is presented in Table 7. Individual female necropsy observations are presented in Table C1, in Appendix C.

Slight dermal irritation related to administration of test article was noted in one female of the 50 mg/kg dose group and in 10 females of the 250 mg/kg dose group.

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Multiple red foci were noted in the thymus of one female in the sham control and one female in the 50 mg/kg dose group. These findings are considered to be incidental in nature and not treatment related. Early resorption sites were noted in the uteri of two females in the 250 mg/kg dose group; with a red fluid filling the uterus of one of these females. The uterus of a third female in the 250 mg/kg dose group was filled with a clear fluid. An atrophied thymus, pale lungs, masses on each uterine horn, enlarged heart, spleen and liver were noted in another female in the 250 mg/kg dose group.

Early resorption sites in the uterus are considered to be treatment related since they were observed only in the high dose group. The multiple lesions observed in one animal of the 250 mg/kg dose group are not considered to be treatment related since no evidence of similar findings was observed in other females of this or the lower dose groups.

#### Litter Data

Summaries of delivery and litter data, pup observations, and pup body weights are presented in Tables 8, 9, and 10, respectively. Individual data are presented in Tables D1, D2, and D3/D4, respectively, in Appendix D.

The gestation length in the 50 mg/kg dose group was statistically longer ( $p < 0.05$ ) than that of the sham treated controls. Total pups per litter and live pups per litter in the 50 mg/kg dose group were significantly less ( $p < 0.05$ ) than in the sham control group. No females in the 250 mg/kg dose group delivered litters. The number of implantation sites in females of the 250 mg/kg dose group were significantly less ( $p < 0.01$ ) than in the sham control group. There were no statistically significant differences observed in any of the other parameters evaluated when the F-274 treated groups were compared to the sham control group.

Average pup body weights for the 1 and 50 mg/kg dose groups were not significantly different than that of controls.

The following pup observations of hematoma, tip of tail black, eschar, missing tail, red anal region, pale in color and lethargy occurred sporadically and are considered to be incidental in nature.

#### SUMMARY AND CONCLUSIONS

Signs of maternal toxicity considered to be related to administration of F-274 included dermal irritation at doses of 1, 50 and 250 mg/kg, and decreased body weight, body weight change, food consumption and relative food consumption at doses of 50 and 250 mg/kg.

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Signs of developmental toxicity considered to be related to administration of F-274 included decreased number of total and live pups delivered per litter at a dose of 50 mg/kg. At a dose of 250 mg/kg F-274 there were no litters delivered and the number of implantation sites were significantly decreased.

Based on the results of this study, the no-observable-effect level for maternal toxicity of F-274 is less than 1 mg/kg. If maternal toxicity is considered excluding dermal irritation effects, the no-observable-effect level for maternal toxicity is considered to be 1 mg/kg. The no-observable-effect level for signs of developmental toxicity is 1 mg/kg.

Given the design of the study and the observed results, it is not possible to determine if the effects observed were a result of an effect on the dam and the ability to produce and carry a conceptus, or a direct effect on the embryo/fetus.

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**ABBREVIATION AND DEFINITION KEY**

Adj Mean            Adjusted mean = The "adjusted mean" is the actual mean calculated using each of the data values in a given dose group. The "mean" is calculated by determining mean values for each litter and subsequently calculating the mean of the litter means for a given dose group.

F                    Female

g                    Grams

kg                   Kilograms

M                    Male

mg                   Milligrams

ml                   Milliliters

N                    Number of data points

Std Dev            Standard deviation

w/v                 Weight to volume

-                    Not applicable, no value

Proportion dead       =  $\frac{\text{Number of dead pups}}{\text{Total number of pups delivered}}$   
(Day 0)

Proportion surviving   =  $\frac{\text{Number of live pups on Day 4}}{\text{Number of live pups delivered Day 0}}$   
to Day 4

Proportion males       =  $\frac{\text{Number of live male pups}}{\text{Total number of live pups}}$   
(Days 0 and 4)