

PETROLEUM COKE:
A 21-DAY TOXICITY TEST TO DETERMINE THE EFFECTS
OF THE TEST SUBSTANCE ON SEEDLING EMERGENCE
AND GROWTH OF TERRESTRIAL PLANTS

AMENDED FINAL REPORT

WILDLIFE INTERNATIONAL, LTD. PROJECT NUMBER: 472-102

OECD PROPOSAL FOR REVISION OF GUIDELINE 208
and
U.S. EPA OPPTS NUMBERS 850.4100 AND 850.4225

AUTHORS:



STUDY INITIATION DATE: April 22, 2004

STUDY COMPLETION DATE: July 6, 2006

AMENDED REPORT DATE: April 10, 2007

SUBMITTED TO:

American Petroleum Institute
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Washington DC 20005

Wildlife International, Ltd.

8598 Commerce Drive
Easton, Maryland 21601
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GOOD LABORATORY PRACTICE COMPLIANCE STATEMENT

SPONSOR: American Petroleum Institute

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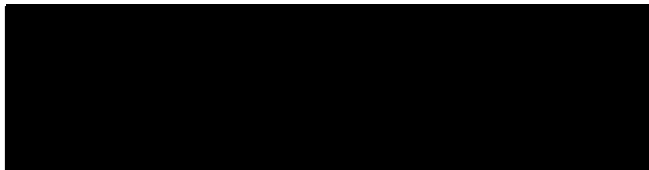
This study was conducted in compliance with Good Laboratory Practice Standards as published by the U.S. Environmental Protection Agency (40 CFR Part 792, 17 August 1989) (1); OECD Principles of Good Laboratory Practice (ENV/MC/CHEM (98)17) (2); and Japan MAFF (11 NohSan, Notification No. 6283, Agricultural Production Bureau, 1 October 1999) (3), with the following exceptions:

Periodic analyses of soil and well water for potential contaminants were performed using a certified laboratory and standard U.S. EPA analytical methods, but not under Good Laboratory Practice Standards.

The characterization of the test substance, and the stability of the substance under conditions of storage at the test site, were not determined in compliance with Good Laboratory Practice Standards.

The concentration and homogeneity of the test substance in the carrier (soil) were not determined analytically.

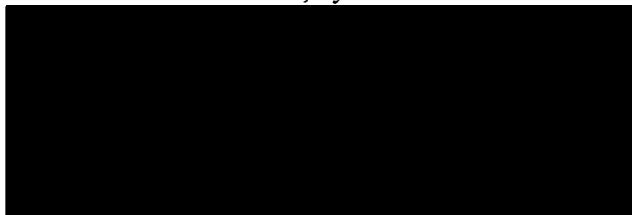
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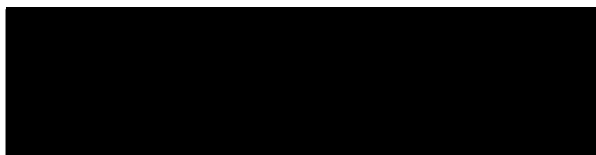
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QUALITY ASSURANCE STATEMENT

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ACTIVITY:	DATE CONDUCTED:	DATE REPORTED TO:	
		STUDY DIRECTOR:	MANAGEMENT:
Protocol	May 18, 2004	May 18, 2004	May 19, 2004
Test Substance Preparation and Planting	February 7, 2005	February 7, 2005	February 7, 2005
Dry Weight Measurements	March 1, 2005	March 1, 2005	March 2, 2005
Data and Draft Report	March 7-8, 2005	March 8, 2005	March 10, 2005
Final Report	July 5, 2006	July 5, 2006	July 6, 2006
Amended Report	March 30, 2007	March 30, 2007	April 10, 2007

All inspections were study-based unless otherwise noted.



4-10-07

Date

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AMENDED REPORT APPROVAL

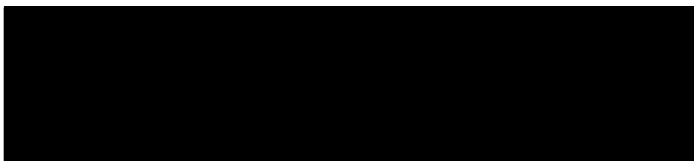
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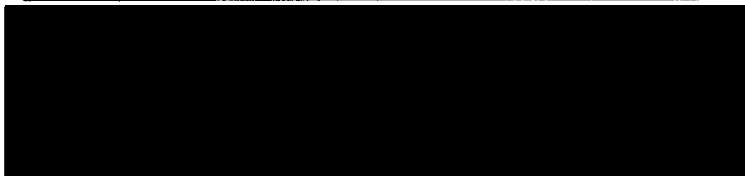
This report was reviewed by the individuals involved in the conduct and management of the study, and was found to be an accurate reflection of the methods used, data collected and results of the study.

STUDY DIRECTOR:



16 Apr 2007
Date

WILDLIFE INTERNATIONAL, LTD. MANAGEMENT:



10 April 07
Date

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[Redacted Signature]

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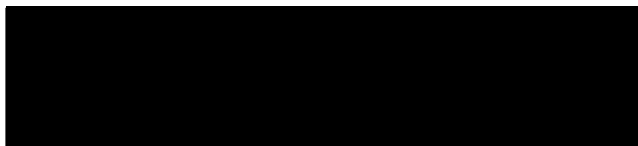
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AMENDED REPORT APPROVAL

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STUDY DIRECTOR:

[Redacted Signature]

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WILDLIFE INTERNATIONAL, LTD. MANAGEMENT:

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SUMMARY

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TITLE: Petroleum Coke: A 21-Day Toxicity Test to Determine the Effects of the Test Substance on Seedling Emergence and Growth of Terrestrial Plants

WILDLIFE INTERNATIONAL, LTD. PROJECT NO: 472-102

TEST SUBSTANCE: Petroleum Coke

GUIDELINES: OECD Guideline for Testing of Chemicals, Proposal for Revision of Guideline 208: *Terrestrial Non-Target Plant Tests*
U.S. EPA Series 850 – Ecological Effects Test Guidelines OPPTS Number 850.4100: *Terrestrial Plant Toxicity, Tier I (Seedling Emergence)*; and 850.4225: *Seedling Emergence, Tier II*

TEST DATES:	Study Initiation:	April 22, 2004
	Experimental Start (OECD):	February 7, 2005
	Experimental Start (EPA):	February 7, 2005
	Biological Termination:	February 28, 2005
	Experimental Termination:	March 1, 2005

LENGTH OF EXPOSURE: 21 days

TEST ORGANISMS: Corn (*Zea mays*), Radish (*Raphanus sativus*), Soybean (*Glycine max*)

SOURCE OF TEST ORGANISMS:

<u>Test Species / Variety Seed</u>	<u>Source</u>
Corn(<i>Zea mays</i>) / Mandan Bride	Johnny's Selected Seeds, Albion, ME, USA
Radish(<i>Raphanus sativus</i>) / Cherry Belle	Meyer Seed Co., Baltimore, MD, USA
Soybean (<i>Glycine max</i>) / Williams 82	Missouri Seed Foundation, Columbia, MO, USA

NOMINAL TEST LEVELS: 0 (Control) and 1000 mg/kg soil dry weight

RESULTS: Soil incorporated with Petroleum Coke resulted in no adverse effects on the three species tested. Therefore, the nominal test concentration of 1000 mg/kg was determined to be a NOEC for corn, radish, and soybean.

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INTRODUCTION

Wildlife International, Ltd. conducted a 21-day seedling emergence study to determine the effects of petroleum coke on the seedling emergence and growth of three species of plants for American Petroleum Institute at the Wildlife International, Ltd. greenhouse facility in Easton, Maryland. Petroleum coke is defined as the product formed by subjecting the heavy tar-like residue remaining following oil refining to high temperatures and pressures. It consists of primarily elemental carbon with considerably smaller amounts of hydrocarbons, sulfur and trace amounts of heavy metals. The in-life phase of the test was conducted from February 7 to 28, 2005. Dry weight measurements were completed on March 1, 2005. Raw data generated at Wildlife International, Ltd. and a copy of the final report are filed under Project Number 472-102 in archives located on the Wildlife International, Ltd. site.

OBJECTIVE

The objective of this study was to determine the effect of petroleum coke on the seedling emergence and growth of three species of plants during a 21-day exposure period.

EXPERIMENTAL DESIGN

The experimental design for this study consisted of a negative control and one treatment group. Each group had four replicate pots with ten seeds planted in each pot. Petroleum coke was incorporated into the test soil for each treatment group prior to the planting of seeds. The nominal test substance concentration was 1000 mg of petroleum coke per kilogram of dry soil (mg/kg), and was based on the results of a prior range-finding test (Appendix 1). A control group, which received no test substance incorporation, was maintained concurrently.

Seeds were impartially assigned to prelabelled growth pots on the day of test initiation. The replicate pots were placed in a randomized block design on a greenhouse table after planting. Observations of emergence and general assessments of seedling condition were made on Days 7, 14, and 21, while observations of height and assignment of plant condition scores were made only on Day 21. After final observations were completed, plants were clipped at soil level and the above-ground portion was dried for the determination of dry weights.

MATERIALS AND METHODS

The study was conducted according to the procedures outlined in the protocol, “Petroleum Coke: A 21-Day Toxicity Test to Determine the Effects of the Test Substance on Seedling Emergence and Growth of Terrestrial Plants” (Appendix 2). The protocol was based on procedures specified in the OECD Proposal for Revision of Guideline 208: *Terrestrial Non-Target Plant Tests* (4); and the U.S. Environmental Protection Agency Series 850 - Ecological Effects Test Guidelines OPPTS Numbers 850.4100: *Terrestrial Plant Toxicity, Tier I (Seedling Emergence)* (5) and 850.4225: *Seedling Emergence, Tier II* (6).

Test Substance

The test substance was green petroleum coke (CAS Number 64741-79-3). The test substance was received from Experimental Pathology Laboratories, Herndon, VA, for API on October 7, 2003. It was assigned Wildlife International, Ltd. identification number 6484 upon receipt and was stored under ambient conditions. The test substance was a black powder identified as 3.3 Micron Mean Petroleum Coke (aka Milled Powder).

The identity, strength, purity, composition (Appendix 5), storage stability, and method of selection, synthesis, fabrication and/or derivation (Appendices 3 and 4) of each batch of the test substance and the maintenance of these records were the responsibility of the Sponsor.

Bulk Soil

The soil used for mixing with the test substance represented a loam soil, and was composed of kaolinite clay, industrial quartz sand, and peat. Crushed limestone was added to buffer the pH of the soil, and a slow-release fertilizer was added to provide nutrients essential for plant growth. A sample of soil representative of that used in this study was sent to Agvise Laboratories, Inc., in Northwood, North Dakota, for analysis of the particle size distribution and organic matter content of the soil. The soil was determined to consist of 50% sand, 29% silt, and 21% clay, with an organic matter content of 1.7%. The soil pH was measured to be 7.4. A copy of the complete report from Agvise Laboratories, Inc. was filed in the archives at Wildlife International, Ltd. along with the raw data for this study.

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Preparation of Test and Control Soils

On February 7, the test soil for each replicate was prepared by mixing Petroleum Coke into bulk soil with a nominal soil moisture of 15%. Bulk test soil (2.2 kg wet weight, 1.87 kg dry weight) was weighed into a mixing bowl. The test substance required for each replicate pot (1.87 g) was weighed into a glass vial. A portion of the soil was removed and placed in a beaker, into which the contents of the test substance vial were added. The contents of the beaker were then stirred by hand using a glass rod. After mixing the aliquot of soil and petroleum coke, it was added to the remaining bulk soil for the replicate and mixed manually by hand with a stainless steel spoon. The negative control pre-mix and test soil were prepared in the same manner as the other test groups, but no test substance was added. The control soils were mixed first, followed by the test soils to avoid cross-contamination.

Planting of Seeds

After the soil for each replicate was prepared, it was placed in the test pot and seeds were planted. Seeds were planted in plastic pots (approximately 16 cm in diameter and 12 cm deep) on the day of test substance application. A template was used to gently compact the soil and leave ten approximately uniform holes for planting. One indiscriminately selected seed was planted in each of the holes, for a total of ten seeds in each pot. Holes were then closed by slightly depressing the soil surface.

Test Species

The common and scientific names for the three species tested, the seed source, and their approximate planting depths are listed below:

<u>Test Species / Variety: Seed</u>	<u>Source: Planting</u>	<u>Depth</u>
Corn (<i>Zea mays</i>) / Mandan Bride	Johnny's Selected Seeds, Albion, ME, USA	20 mm
Radish (<i>Raphanus sativus</i>) / Cherry Belle	Meyer Seed Co., Baltimore, MD, USA	6 mm
Soybean (<i>Glycine max</i>) / Williams 82	Missouri Seed Foundation, Columbia, MO, USA	20 mm

These species were chosen because they are economically important, and are readily cultivated test organisms that are widely used in research. Seeds were selected from a single size

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class within each species in order to reduce the potential for bias from differing seed sizes. Seeds used in this study were not treated with fungicides, insecticides or repellents prior to test initiation.

Watering of Seedlings

Water lost through transpiration and evaporation was replaced by subirrigation with well water from the greenhouse facility. Seedlings were subirrigated to minimize the potential for the leaching of the test substance through the soil. Subirrigation trays were filled to a predetermined depth to help standardize the amount of water delivered to each tray. The days on which watering occurred are listed in Appendix 6.

Environmental Conditions

The environmental conditions (temperature and relative humidity) of the test are summarized in Appendix 6. The temperature within the greenhouse was controlled with a Wadsworth MicroStep S/A Environmental Control System. Artificial lighting (high pressure sodium) was used to supplement natural sunlight in order to provide a minimum 14-hour photoperiod. The temperature and relative humidity within the greenhouse were continuously monitored during the test with the Wadsworth control system.

Pesticide and Metal Screening of Well Water and Soil

The well water and soil used for plant studies are analyzed periodically to determine concentrations of selected organic and inorganic constituents. No analyses were measured at levels that were expected to have an impact on the study. Reports for the latest analyses are stored in the archives at the Wildlife International, Ltd. site in Easton, Maryland.

Observations and Measurements

Observations on Days 7 and 14 were made to document seedling emergence. Observations on Day 21 were made to document seedling emergence and growth, and to determine changes in the general condition of seedlings following test initiation. Observations consisted of noting whether emergence had or had not occurred, and assessing the condition of each seedling. Emergence was defined as the presence of visible plant tissue at the surface of the soil. Seedling condition was described by noting the presence or absence of possible signs of phytotoxicity such as necrosis, leaf wrinkle, chlorosis, plant lodging or plant stunting. Each emerged seedling was then assigned a numerical score (see Table 1) that described the plant condition (7). Condition score is a subjective

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or qualitative assessment that determines whether damage is slight, moderate, or severe. A score of 10 does not mean that 10% of the plant is showing the effect (e.g. chlorosis), merely that the severity of the effect (e.g. chlorosis) is very slight.

The growth of emerged seedlings was evaluated by assessing the height and dry weight of living seedlings at test termination. Seedling height was measured to the nearest whole centimeter from the surface of the soil to either the tip of the tallest leaf (corn and radish) or to the tip of the apical meristem (soybean). Seedlings were then clipped at soil level, the shoots of all living seedlings within a replicate were placed in a labeled bag, and dried. The total dry weight of the replicate was determined, and the mean weight per plant was calculated by dividing the total weight by the number of seedlings weighed.

Conditions for the Validity of the Test

The following criteria used to judge the validity of the test was met:

1. There was at least 80% emergence of plants in each control group and the emerged plants exhibited normal growth for the duration of the test.

Data Analyses

Statistical analyses were used to aid in the evaluation of effects of test substance application on seedling emergence, survival, mean shoot weight, and seedling height. These variables were defined for statistical analysis as follows:

Seedling Emergence: The number of emerged seedlings per ten planted seeds in each pot.

Survival: The number of living seedlings in each pot per ten planted seeds.

Mean Shoot Weight: The average dry shoot weight of living emerged seedlings in each pot.

Height: The average height of living emerged seedlings in each pot.

Mean seedling emergence, survival, weight, and height of the control and treatment groups were compared with a t-test, using the DUNNET T option of the GLM (general linear model) procedure of SAS version 8 (8). Significance was determined at the level of 0.05 ($p < 0.05$).

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Additionally, test data were evaluated to determine the observed effects on condition and growth. The no-observed-effect-concentration (NOEC) is defined as a test substance concentration that shows no adverse effect on a variable of interest. The t-test was used to aid in establishing the NOEC by determining if the treatment group differed significantly from the control group.

RESULTS AND DISCUSSION

Test Soils

The measured soil moisture of 12% was used to calculate the concentration of petroleum coke in each replicate pot. Results of the calculations are provided in Table 2.

Biological Results

The results of the test are summarized for each species in Table 3. Complete results are presented by species in Appendices 7 through 9. There were no apparent effects on any endpoint in corn, radish, and soybean.

CONCLUSIONS

The effects of petroleum coke on the seedling emergence and growth of three species of plants were evaluated during a 21-day exposure period. Soil incorporation of petroleum coke resulted in no adverse effects on the three species tested. Therefore, the nominal test concentration of 1000 mg/kg dry soil was determined to be a NOEC for corn, radish, and soybean.

REFERENCES

- 1 **Title 40 of the Code of Federal Regulations, Part 792** . 1989. *Toxic Substances Control Act (TSCA) Good Laboratory Practice Standards*.
- 2 **OECD**. 1998. *OECD Principles of Good Laboratory Practice*. ENV/MC/CHEM (98) 17. Environmental Directorate, Paris.
- 3 **Ministry of Agriculture, Forestry and Fisheries, Japan (MAFF)**. 1999. Good Laboratory Practice (GLP) for Agricultural Chemicals. 11 NohSan, Notification No. 6283, Agricultural Production Bureau, 1 October 1999.
- 4 **OECD Guideline for the Testing of Chemicals**. July , 2000. Proposal for Revision of Guideline 208: *Terrestrial Non-Target Plant Tests*.
- 5 **U.S. Environmental Protection Agency**. 1996. Series 850- Ecological Effects Test Guidelines (*draft*), OPPTS Number 850.4100: Terrestrial Plant Toxicity, Tier I (Seedling Emergence).
- 6 **U.S. Environmental Protection Agency**. 1996. Series 850- Ecological Effects Test Guidelines (*draft*), OPPTS Number 850.4225: Seedling Emergence, Tier II.
- 7 **Frans, Robert E. and Ronald E. Talbert**. 1977. Design of Field Experiments and the Measurement and Analysis of Plant Responses. Pages 15-23 *in* B. Truelove, ed. Research Methods in Weed Science. Southern Weed Science Society, Auburn University, Alabama.
- 8 **SAS Institute, Inc.** 1999. SAS Proprietary Software Version 8, Cary, NC, SAS Institute, Inc.

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Table 1Seedling Condition Rating System ¹

Rating Category		Description
0	No Effect	No noticeable effect
10		Effect barely noticeable
20	Slight Effect	Some effect, not apparently detrimental
30		Effect more pronounced, not obviously detrimental
40		Effect moderate, plants appear able to recover
50	Moderate Effect	More lasting effect, recovery somewhat doubtful
60		Lasting effect, recovery doubtful
70		Heavy injury, loss of individual leaves
80	Severe Effect	Plant nearly destroyed, a few surviving leaves
90		Occasional surviving leaves
100	Complete Effect	Death of entire plant

¹ Rating scale adapted from: Frans, Robert E. and Ronald E. Talbert. 1977. Design of Field Experiments and the Measurement and Analysis of Plant Responses. Pages 15-23 in B. Truelove, ed. Research Methods in Weed Science. Southern Weed Science Society, Auburn University, Alabama.

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Table 2

Calculated Petroleum Coke Concentrations in Test Soils From a 21-Day
Seedling Emergence Test with Corn, Radish, and Soybean

Species	Nominal Concentration (mg/kg)	Calculated Concentration (mg/kg)			
		Replicate A	Replicate B	Replicate C	Replicate D
Corn	1000	964	964	966	963
Radish	1000	964	964	962	963
Soybean	1000	964	964	964	965

$$\frac{1869 \text{ mg}}{1.939 \text{ kg}} = 964 \text{ mg/kg}$$

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Table 3

Effects of Petroleum Coke on Seedling Emergence, Survival, Height, and Dry Weight
in a 21-Day Seedling Emergence Test with Corn, Radish, and Soybean¹

Test Concentration (mg/kg)	Number of Emerged Seedlings (% Reduction)			Seedling Survival	Seedling Height (cm)	Dry Weight (g)
	Day 7	Day 14	Day 21	(% Reduction)	(% Reduction)	(% Reduction)
Corn						
Control	9.25 ± 0.96	9.50 ± 0.58	9.50 ± 0.58	9.50 ± 0.58	53.1 ± 5.74	0.564 ± 0.0753
1000	9.00 ± 0.82 (3%)	9.00 ± 0.82 (5%)	9.00 ± 0.82 (5%)	9.00 ± 0.82 (5%)	55.8 ± 2.80 (-5%)	0.634 ± 0.0500 (-12%)
Radish						
Control	9.75 ± 0.50	9.75 ± 0.50	9.75 ± 0.50	9.25 ± 0.50	13.8 ± 0.29	0.231 ± 0.0216
1000	9.25 ± 0.96 (5%)	9.25 ± 0.96 (5%)	9.25 ± 0.96 (5%)	8.75 ± 0.96 (5%)	14.1 ± 0.79 (-2%)	0.231 ± 0.0218 (0%)
Soybean						
Control	9.50 ± 0.58	9.50 ± 0.58	9.50 ± 0.58	9.50 ± 0.58	16.9 ± 1.78	0.377 ± 0.0442
1000	9.25 ± 0.96 (3%)	9.75 ± 0.50 (-3%)	9.75 ± 0.50 (-3%)	9.75 ± 0.50 (-3%)	17.9 ± 1.57 (-6%)	0.383 ± 0.0330 (-2%)

¹ No treatment group mean is significantly different from the control mean ($p>0.05$).

Appendix 1**Exploratory Non-GLP Rangefinding Toxicity Test with Petroleum Coke****Introduction**

An exploratory non-GLP rangefinding test was conducted from December 14, 2004 to January 4, 2005 in the Wildlife International, Ltd. plant testing facility. The test was conducted using soil-incorporated test substance, and was terminated 21 days after initiation.

Methods and Materials

Test soils were prepared for each replicate (a pot containing ten planted seeds) individually. The test substance was weighed into glass vials. For each replicate, 2.2 kg of bulk soil was weighed into a tared container. A small amount (approximately 250 g) of soil was then removed and mixed with the test substance to make a pre-mixture for each replicate. The pre-mixture was then added to the remaining bulk soil and mixed manually using a stainless steel spoon. Test pots were filled with the soil and seeds were planted to initiate the test. Nominal test soil petroleum coke concentrations were 10, 100, and 1000 mg petroleum coke per kilogram soil dry weight (mg/kg). A negative control was prepared in the same manner as the other test groups, but no test substance was added.

After planting, the test pots were placed on a bench in a greenhouse with a minimum 16-hour light photoperiod, where they remained for the duration of the test. Water was added to the test pots by sub-irrigation as needed throughout the 21-day test period. The number of emerged seedlings was observed on Days 7, 14, and 21. Additionally on Day 21, the condition of each emerged seedling was assessed, the height of each living seedling was measured, and the fresh weight of all living seedlings within each replicate was determined.

Data Analysis

The number of emerged seedlings, mean number of living seedlings, mean height, and mean fresh weight was determined for each test concentration. Differences from the respective control means were calculated and expressed as percent reduction.

Appendix 1 (Continued)**Exploratory Non-GLP Rangefinding Toxicity Test with Petroleum Coke****Results**

The results of the rangefinding test are included in the attached table. Departures from the control means, including both reductions and increases, were observed among all three species. There was no dose-response apparent among radish and corn seedlings, and any reductions were not consistent between different variables such as height and weight. For soybeans, no effects on emergence or survival were found. However, there were 13% and 21% reductions from control means for fresh weight and height, respectively, at the 1000 mg/kg level. Because the reductions were observed at the highest soil concentration and involved two parameters, the data suggested a marginal effect at the highest concentration.

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Appendix 1 (Continued)

Exploratory Non-GLP Rangefinding Toxicity Test with Petroleum Coke

STUDY: Petroleum Coke: A 21-Day Toxicity Test to Determine the Effects of the Test Substance on Seedling Emergence and Growth of Terrestrial Plants
SPONSOR: American Petroleum Institute
PROJECT NO.: 472-102

Species	Emergence	Survival	Biomass (g)	Height (cm)
Treatment Group (mg/kg)	Mean $\pm$ SD (% Reduction)	Mean $\pm$ SD (% Reduction)	Mean $\pm$ SD (% Reduction)	Mean $\pm$ SD (% Reduction)
Corn				
Control	10.00 $\pm$ 0.00	10.00 $\pm$ 0.00	33.1 $\pm$ 5.77	41.0 $\pm$ 3.89
10 (15%)	8.50 $\pm$ 0.71	8.50 $\pm$ 0.71 (15%)	32.1 $\pm$ 1.00 (3%)	45.9 $\pm$ 2.57 (-12%)
100 (15%)	8.50 $\pm$ 0.71	8.50 $\pm$ 0.71 (15%)	35.2 $\pm$ 0.64 (-6%)	44.2 $\pm$ 0.77 (-8%)
1000	9.50 $\pm$ 0.71 (5%) (10%) (5%)	9.00 $\pm$ 1.41	31.4 $\pm$ 0.59	41.8 $\pm$ 4.05 (-2%)
Soybean				
Control	8.00 $\pm$ 1.41	8.00 $\pm$ 1.41	18.4 $\pm$ 2.74	15.2 $\pm$ 1.72
10 (0%)	8.00 $\pm$ 1.41	8.00 $\pm$ 1.41 (0%)	17.8 $\pm$ 1.05 (3%)	14.6 $\pm$ 0.43 (4%)
100	10.00 $\pm$ 0.00 (-25%) (-25%) (-22%)	10.00 $\pm$ 0.00	22.5 $\pm$ 2.34	14.6 $\pm$ 0.85 (4%)
1000 (-6%)	8.50 $\pm$ 2.12	8.50 $\pm$ 2.12 (-6%)	15.9 $\pm$ 1.81 (13%)	12.1 $\pm$ 0.93 (21%)
Radish				
Control	8.50 $\pm$ 2.12	8.00 $\pm$ 2.83	15.9 $\pm$ 3.18	11.5 $\pm$ 0.78
10	6.00 $\pm$ 2.83 (29%) (25%) (17%)	6.00 $\pm$ 2.83	13.2 $\pm$ 4.62	12.3 $\pm$ 0.97 (-8%)
100 (24%)	6.50 $\pm$ 0.71	6.50 $\pm$ 0.71 (19%)	14.7 $\pm$ 4.08 (7%)	10.9 $\pm$ 1.21 (5%)
1000	9.00 $\pm$ 0.00 (-6%) (-13%) (-5%)	9.00 $\pm$ 0.00	16.6 $\pm$ 2.26	10.7 $\pm$ 0.31 (7%)

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Appendix 2

Protocol, Amendment and Deviation

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PROTOCOL

PETROLEUM COKE: A 21-DAY TOXICITY TEST TO DETERMINE
THE EFFECTS OF THE TEST SUBSTANCE ON SEEDLING EMERGENCE
AND GROWTH OF TERRESTRIAL PLANTS

U.S. Environmental Protection Agency
Series 850 - Ecological Effects Test Guidelines
OPPTS Number 850.4100 and 850.4225

and

OECD Guideline for Testing of Chemicals
Proposal for Revision of Guideline 208: Terrestrial Non-Target Plant Tests

Submitted to

American Petroleum Institute
1220 L Street, N.W.
Washington, DC 20005

Wildlife International, Ltd.

8598 Commerce Drive
Easton, Maryland 21601
(410) 822-8600

March 30, 2004

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PETROLEUM COKE: A 21-DAY TOXICITY TEST TO DETERMINE
THE EFFECTS OF THE TEST SUBSTANCE ON SEEDLING EMERGENCE
AND GROWTH OF TERRESTRIAL PLANTS

SPONSOR:

American Petroleum Institute
1220 L Street, N.W.
Washington, DC 20005

SPONSOR'S REPRESENTATIVE:

[REDACTED]

SPONSOR'S TECHNICAL STUDY MONITOR:

[REDACTED]

TESTING FACILITY:

Wildlife International, Ltd.
8598 Commerce Drive
Easton, Maryland 21601

STUDY DIRECTOR:

[REDACTED]
Wildlife International Ltd.

LABORATORY MANAGEMENT:

[REDACTED]

FOR LABORATORY USE ONLY

Proposed Dates:	* - To be amended. JRP 22 Apr 04	
Experimental		Experimental
Start Date:	* _____	Termination Date: * _____
Project No.:	472-102	
Test Concentrations:	* _____	
Test Substance No.:	6484	Reference Substance No. (if applicable): * _____

PROTOCOL APPROVAL

[REDACTED]

22 Apr 2004
DATE

23 Apr 1 2004
DATE

01 April, 2004
DATE

PROTOCOL NO.: 472/033004/SEED-10/SUB472

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Wildlife International, Ltd.

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INTRODUCTION

Wildlife International, Ltd. will conduct a toxicity test with three species of plants to determine the effects of petroleum coke on seedling emergence and early growth. The test will be conducted at the Wildlife International, Ltd. plant testing facility near Easton, Maryland. Petroleum coke is defined as the product formed by subjecting the heavy tar-like residue remaining following oil refining to high temperatures and pressures. It consists of primarily elemental carbon with considerably smaller amounts of hydrocarbons, sulfur and trace amounts of heavy metals. The three species to be tested include soybean, corn and radish. The study will be performed based on procedures in the U.S. Environmental Protection Agency Series 850 - Ecological Effects Test Guidelines OPPTS Number 850.4100 (1) and 850.4225 (2) and in the OECD Guideline for Testing of Chemicals: Proposal for Revision of Guideline 208: "Terrestrial Non-target Plant Tests" (3). Raw data for all work performed at Wildlife International, Ltd. and a copy of the final report will be filed by project number in archives located on the Wildlife International, Ltd. site, or at an alternative location to be specified in the final report.

OBJECTIVE

The objective of this study is to determine the effect of the test substance on the seedling emergence and growth of three species of plants during a 21-day exposure period.

EXPERIMENTAL DESIGN

The target test concentrations will be selected by the Sponsor in consultation with Wildlife International, Ltd., and will be based upon information such as the results of exploratory range-finding toxicity data, known toxicity data, physical/chemical properties of the test substance or other relevant information. Concentrations will be expressed as mg/kg (dry weight of soil).

For each plant species tested, seeds will be planted and exposed to a series of five concentrations of the test substance and a negative control. There will be four replicates for each treatment and control group, with each replicate consisting of a growth pot containing ten seeds. The replicates will be placed on a benchtop in a greenhouse according to a randomized design. Data collected from all replicates within a treatment group will be combined for calculating EC25 and EC50 values, as well as the no-observed-effect concentration (NOEC) and lowest-observed-effect concentration (LOEC).

PROTOCOL NO.: 472/033004/SEED-10/SUB472

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Wildlife International, Ltd.

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One application of each of the various treatments will be made by soil incorporation of the test substance prior to planting seeds. The duration of the in-life portion of the test will be 21 days following planting, during which time possible phytotoxic effects of the test substance on seedling emergence and growth of emerged seedlings will be evaluated.

MATERIALS AND METHODS**Test Substance**

The test substance is green coke (CAS Number 64741-79-3) milled to approximately 2-3 micron particle size. Information on the characterization of test, control or reference substances is required by Good Laboratory Practice Standards (GLPs), 40 CFR Part 160.31. The Sponsor is responsible for providing Wildlife International, Ltd. written verification that the test substance has been characterized according to GLPs prior its use in the study. If written verification of GLP test substance characterization is not provided to Wildlife International, Ltd., it will be noted in the compliance statement of the final report.

The Sponsor is responsible for all information related to the test substance and agrees to accept any unused test substance and/or test substance containers remaining at the end of the study.

Test System

The three species of plants used in this study were chosen because they are economically important, and are readily cultivated test organisms that are widely used in research. The common and scientific names for the species and their approximate planting depths are listed below:

<u>Monocots:</u>		Planting Depth
Corn	<i>Zea mays</i>	2.0 - 2.5 cm
<u>Dicots:</u>		
Soybean	<i>Glycine max</i>	2.0 - 2.5 cm
Radish	<i>Raphanus sativus</i>	6 mm

Seeds will be selected from a single size class within each species. The seeds of most plant species are sorted according to size by the supplier prior to being obtained by Wildlife International,

PROTOCOL NO.: 472/033004/SEED-10/SUB472

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Wildlife International, Ltd.

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Ltd. However, in some cases it may be necessary to further sort seeds to form a more uniform size class that reduces the potential for bias from differing seed sizes.

Seeds used in this study will not have been treated with fungicides, insecticides or repellents prior to test initiation. Seeds will be obtained from a producer or supplier such as Meyer Seed Company, Baltimore, Maryland. Any documentation provided from the supplier concerning the identification and history of the seeds used will be included in the study data.

Environmental Conditions

The test will be conducted within a greenhouse. Environmental conditions, including temperature and light intensity, will be controlled using a Wadsworth MicroStep/SA environmental control system. Temperature and relative humidity in the study room will be continuously monitored with a Campbell Scientific data logger, and daily conditions throughout the test will be reported. A photoperiod of at least 14 hours light will be maintained during the test. Artificial lighting may be used to lengthen short-day photoperiods or to supplement natural sunlight on overcast days.

Test Substrate

Test plants will be grown in plastic pots approximately 16 cm in diameter and 12 cm in depth. An unsterilized artificial sandy-loam or sand soil substrate will be used for testing. The soil will be composed of kaolinite clay, industrial quartz sand and peat mixed in a 4:50:2 ratio (w:w:w). Crushed limestone will be added to buffer the pH of the soil, and a slow-release fertilizer will be added to provide nutrients essential for plant growth. Typically, the soil consists of approximately 50% sand, - 30% silt, and 20 % clay, with a slightly acid pH and an organic matter content less than 3% (organic carbon < 1.5%). A sample of soil used in this study will be sent to Agvise Laboratories, Inc., in Northwood, North Dakota, for analysis of the particle size distribution and organic matter content of the soil. Soil characterization will include, but may not be limited to, the determination of particle size distribution, organic matter content, and pH. Those items relevant to the conduct of the study will be discussed in the final report. The complete report from Agvise Laboratories, Inc. will be filed in the archives located at Wildlife International, Ltd. The results of the characterization will be stored in the archives located at the Wildlife International, Ltd. site, and those items relevant to the conduct of the study will be discussed in the final report.

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Neither the well water nor the artificial soil are expected to have contaminants present in quantities known to be capable of interfering with the study. Analyses will be performed at least once annually to determine the concentrations of selected organic and inorganic constituents of water and soil used in this study. Results of the analyses will be stored in the archives located on the Wildlife International Ltd. site.

Preparation of Test Treatments

Concentrations of the test substance in the soil will be prepared on a dry weight basis (e.g., mg test substance/kg dry soil). The test substance will be incorporated into the soil for each treatment level prior to planting. Green Coke will be added to approximately 3 kg of soil for each test concentration. The mixing time necessary to achieve a homogeneous mixture will be determined in a separate method verification study.

Justification for Route of Exposure

The test substance will be administered to the test organisms in soil. The route of exposure is justified because it is the maximum exposure pathway to developing seeds and seedlings.

Artificial Soil Sampling

Samples of the experimental soils will be collected on Day 0 for chemical analysis to verify/measure test concentrations of the test substance in the artificial soil. All samples will be placed in uniquely identified Nalgene® jars. Samples will be analyzed for the components of petroleum coke listed in Table 1, if possible. The soil sampling scheme is summarized below:

ESTIMATED NUMBERS OF VERIFICATION SAMPLES

Experimental Group	Day 0
Control	2
Level 1-Low Concentration	2
Level 2	2
Level 3	2
Level 4	2
Level 5-High Concentration	2
Total = 12 Samples	

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Wildlife International, Ltd.

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The above numbers of samples represent those collected from the test and do not include quality control (QC) samples such as matrix blanks and fortifications prepared and analyzed during the analytical chemistry phase of the study.

Soil Analyses

Samples of the experimental soils will be stored in a freezer until prepared and/or extracted and analyzed, unless samples will be analyzed immediately following collection. Chemical analyses of soils will be performed by Wildlife International, Ltd. The analytical method used will be based upon chromatographic methodology and/or ICP analysis for metals. The methodology used to analyze the test samples will be documented in the raw data and summarized in the final report.

Test Procedure

Growth pots will be filled with test or control soil, and ten seeds of one species will be planted per replicate. The seeds will be planted at the appropriate depth and will be approximately equally spaced. Seeds will be assigned to test and control groups and planted in growth pots uniquely identified with a minimum of the species name, project number, treatment group designation, and replicate. This method of application was chosen because contaminated soil is the most likely route of exposure to plants. After planting, the growth pots will be placed on benches in the greenhouse in a randomized configuration to minimize bias from microclimates which may exist within the greenhouse. Initial watering will be done to the soil surface after planting. Thereafter, water will be supplied to the growth pots by sub-irrigation to help ensure that sufficient water is available for seedling growth. Records of the days that watering occurs and source of water used will be kept in the study data.

The growth pots will be observed weekly after test initiation in order to determine the number of emerged seedlings. The in-life portion of the test will terminate twenty-one days after initiation or 14 days after at least 50% emergence of seedlings in the control groups, whichever is later. If any portion of the test is extended, the duration of and the reason for the extension will be documented in the data and discussed in the final report. At the termination of the in-life portion of the test, height measurements and the condition of the emerged seedlings will be recorded. The height of each living seedling within a replicate will be determined in order to calculate the mean seedling height per replicate. The exact method used to measure height may vary with species, and will be described in the raw data and included in the final report.

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Wildlife International, Ltd.

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At the in-life phase termination, the condition of seedlings will be assessed utilizing a rating system based upon Frans and Talbert (4). A numerical rating will be assigned to help characterize changes in the seedlings' morphology including necrosis, chlorosis, general development, or any other characteristic that may be deemed a response of the seedling to the treatment. Ratings may range from 0 to 100, 0 indicating normal seedling appearance, 100 indicating emerged seedlings that have died prior to test termination. Intermediate scores reflect the severity of changes in plant condition. After final observations are completed, plants will be clipped at soil level and the above-ground portion (shoots) of all living plants within each replicate will be dried to a constant weight. The mean shoot dry weight of each replicate will be calculated.

Disposition of Test Plants

After test termination, all plants will be incinerated or disposed of using other appropriate methods. The method used will be documented in the raw data.

Conditions for the Validity of the Test

The following criteria will be used to judge the validity of the test:

1. There must be at least 80% emergence of plants in each control group and the emerged plants should exhibit normal growth for the duration of the test.

Statistical Calculations

This section includes proposed statistical analyses. Additional tests or analyses may be performed when warranted at the discretion of the Study Director or by Sponsor request.

An evaluation of potential effects of the test substance on seedling emergence, the growth of emerged seedlings, as characterized by shoot weight and height, and seedling condition will be made. Statistical analyses will include the determination of effect concentrations (EC estimates), and the determination of which treatment groups differ significantly from the control group(s). All statistical analyses will be based on nominal test concentrations.

The 25 and 50% effect concentrations and their 95% confidence intervals will be determined when warranted using an appropriate technique, such as Probit analysis or linear interpolation. When

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Wildlife International, Ltd.

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possible, EC estimates will be made for mean seedling emergence, mean shoot weight and height of seedlings at test termination.

The data will be evaluated to determine the lowest-observed-effect concentration (LOEC), defined as the lowest concentration of test substance used in the study that shows an adverse effect on a variable of interest. The no-observed-effect-concentration (NOEC) will be defined as the maximum concentration which shows no adverse phytotoxic effects and below which no phytotoxic effects are manifested. Dunnett's two-tailed test will be used to determine significant differences from the control(s) at the 0.05 level of significance. Significant differences from the control, or their absence, may help establish the LOEC and NOEC.

All statistical analyses will be performed on a personal computer using commercially available statistical software programs (5). The specific statistical tests and the programs used to perform the tests will be described in the final report of the study.

RECORDS TO BE MAINTAINED

Records to be maintained for data generated by Wildlife International, Ltd. will include but not be limited to:

1. Copy of signed protocol.
2. Identification and characterization of the test substance, if provided by the Sponsor.
3. Dates of initiation and termination of the test.
4. Test soil calculation and preparation.
5. Observations.
6. The methods used to analyze test substance concentrations and the results of analytical measurements.
7. Statistical calculations, if applicable.
8. Test conditions (temperature, humidity, etc.).
9. Calibration records for application equipment.
10. Copy of final report.

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Wildlife International, Ltd.

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FINAL REPORT

A final report of the results of the study will be prepared by Wildlife International, Ltd. The report will include, but not be limited to, the following, when applicable.

1. Name and address of the facility performing the study.
2. Dates upon which the study was initiated and completed, and the definitive experimental start and termination dates.
3. A statement of compliance signed by the Study Director addressing any exceptions to Good Laboratory Practice Standards.
4. The test substance identification including name, chemical abstract number or code number, strength, purity, composition, and other information provided by the Sponsor.
5. A copy of the protocol and protocol amendments.
6. Stability and solubility of the test substance under the conditions of administration, if provided by the Sponsor.
7. A description of the methods used to conduct the test.
8. A description of the test species, including the source and scientific name.
9. The methods used to allocate seeds to test substrates and begin the test, the number of seeds and replicates per treatment, and the duration of the test.
10. A description of circumstances that may have affected the quality or integrity of the data.
11. The name of the Study Director and the names of other scientists, professionals, and supervisory personnel involved in the study.
12. A description of the transformations, calculations, and operations performed on the data, a summary and analysis of the biological data and analytical chemistry data, and a statement of the conclusions drawn from the analyses.
13. Statistical methods used to evaluate the data.
14. The signed and dated reports of each of the individual scientists or other professionals involved in the study, if applicable.
15. Analysis of soil particle size, pH and organic matter content of the soil as provided.
16. Output of statistical programs.
17. The location where raw data and final report are to be stored.
18. A statement prepared by the Quality Assurance Unit listing the dates that study inspections and audits were made and the dates of any findings reported to the Study Director and Management.

PROTOCOL NO.: 472/033004/SEED-10/SUB472

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Wildlife International, Ltd.

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19. If it is necessary to make corrections or additions to a final report after it has been accepted, such changes will be made in the form of an amendment issued by the Study Director. The amendment will clearly identify the part of the final report that is being amended and the reasons for the amendment, and will be signed by the Study Director.

CHANGING OF PROTOCOL

Planned changes to the protocol will be in the form of written amendments signed by the Study Director and approved by the Sponsor's Representative. Amendments will be considered as part of the protocol and will be attached to the final protocol. Any other changes will be in the form of written deviations signed by the Study Director and filed with the raw data. All changes to and deviation from the protocol will be indicated in the final report.

GOOD LABORATORY PRACTICES

This study will be conducted in accordance with Good Laboratory Practice Standards for EPA (40 CFR Part 160 and/or Part 792); OECD Principles of Good Laboratory Practice (ENV/MC/CHEM (98) 17); and Japan MAFF (11 NohSan, Notification No. 6283, Agricultural Production Bureau, 1 October 1999). Each study conducted by Wildlife International, Ltd. is routinely examined by the Wildlife International, Ltd. Quality Assurance Unit for compliance with Good Laboratory Practices, Standard Operating Procedures and the specified protocol. A statement of compliance with Good Laboratory Practices will be prepared for all portions of the study conducted by Wildlife International, Ltd. The Sponsor will be responsible for compliance with Good Laboratory Practices for procedures performed by other laboratories (e.g., residue analyses or pathology). Raw data for all work performed at Wildlife International, Ltd. and a copy of the final report will be filed by project number in archives located on the Wildlife International, Ltd. site or at an alternative location to be specified in the final report.

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Wildlife International, Ltd.

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REFERENCES

- 1 **U.S. Environmental Protection Agency.** 1996. Series 850- Ecological Effects Test Guidelines (*draft*), OPPTS Number 850.4100: Terrestrial Plant Toxicity, Tier I (Seedling Emergence).
- 2 **U.S. Environmental Protection Agency.** 1996. Series 850- Ecological Effects Test Guidelines (*draft*), OPPTS Number 850.4225: Terrestrial Plant Toxicity, Tier II (Seedling Emergence).
- 3 **OECD.** 1998. *Guideline for Testing of Chemicals, Proposal for Revision of Guideline 208: Terrestrial Non-target Plant Tests.* Organization for Economic Cooperation Development.
- 4 **Frans, Robert E. and Ronald E. Talbert.** 1977. Design of Field Experiments and the Measurement and Analysis of Plant Responses. Pages 15-23 in B. Truelove, ed. Research Methods in Weed Science. Southern Weed Science Society, Auburn University, Alabama.
- 5 **SAS Institute, Inc.** 1999. SAS Proprietary Software Version 8, Cary, NC. SAS Institute, Inc.

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Wildlife International, Ltd.

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Table 1.

Analytes of Interest in Petroleum Coke

PAH	Metals and Sulfur
Acenaphthene	Nickel
Acenaphthylene	Vanadium
Anthracene	Iron
Benzo(a)anthracene	Copper
Benzo(a)pyrene	Selenium
Benzo(b)fluoranthene	Arsenic
Benzo(g,h,i)perylene	Sulfur
Benzo(k)fluoranthene	
Chrysene	
Dibenzo(a,e)pyrene	
Dibenz(a,h)anthracene	
Fluoranthene	
Fluorene	
Indeno(1,2,3-cd)pyrene	
Naphthalene	
Perylene	
Phenanthrene	
Pyrene	

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Wildlife International, Ltd.

Page 1 of 3

AMENDMENT TO STUDY PROTOCOL

STUDY TITLE: Petroleum Coke: A 21-Day Toxicity Test to Determine the Effects of the Test Substance on Seedling Emergence and Growth of Terrestrial Plants

PROTOCOL NO.: 472/033004/SEED-10/SUB472

AMENDMENT NO.: 1

SPONSOR: American Petroleum Institute

WIL PROJECT NO.: 472-102

EFFECTIVE DATE: February 1, 2005

AMENDMENT: On page 2, add the following information:

Proposed Experimental Start Date (EPA): February 7, 2005

Proposed Experimental Start Date (OECD): February 7, 2005

Proposed Experimental Termination Date: March 1, 2005

Test Concentrations: 0 and 1000 mg/kg

Reference Substance Number: not applicable

REASON:

This information is added to complete the protocol.

AMENDMENT: On page 3, **Experimental Design**, change the second paragraph to:

For each species tested, seeds will be planted and exposed to a single concentration of the test substance and a negative control. There will be four replicates for the treatment and the control group, with each replicate consisting of a growth pot containing ten seeds. The replicates will be placed on a benchtop in a greenhouse according to a randomized design. Data collected from all replicates within a treatment group will be combined in order to determine if the concentration tested is a no-observed-effect-concentration (NOEC).

REASON:

The test will be conducted as a limit test.

AMENDMENT: On page 5, **Test Substrate**, change the third sentence to:

The soil will be a mixture of kaolinite clay, sand and peat.

REASON:

This wording reflects current procedures.

AMENDMENT: On page 5, **Test Substrate**:

Delete the last sentence in the paragraph.

REASON:

Redundant.



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Wildlife International, Ltd.

Page 2 of 3

AMENDMENT: On page 6, **Preparation of Test Treatments**, change paragraph to:

The concentration of test substance in soil will be based on a dry weight basis (e.g., mg of test substance per kg dry soil). The test substance will be incorporated into the soil for each replicate individually before planting. Green Coke will be added to approximately 2.2 kg (wet weight) of soil for each replicate and mixed thoroughly prior to planting. The soil moisture content will be determined prior to test initiation.

REASON:

Test soils will be prepared by replicate in order to verify proper dosing for each replicate pot.

AMENDMENT:

Remove the sections entitled **Artificial Soil Sampling and Soil Analyses**, including Table 1.

REASON:

There will be no analytical confirmation of the test concentration, which will be noted accordingly in the Good Laboratory Practice Statement of the final report.

AMENDMENT: page 7, **Test Procedure**:

Delete the fourth sentence.

REASON:

Redundant.

AMENDMENT: page 8, **Statistical Calculations**, second paragraph, change the second sentence to:

Statistical analyses will be used to determine if the test concentration differs from the control group.

REASON:

Calculations of effect concentrations (EC estimates) are not required for limit tests.

AMENDMENT: page 8, **Statistical Calculations**:

Delete the third paragraph.

REASON:

Calculations of effect concentrations (EC estimates) are not required for limit tests.

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Wildlife International, Ltd.

Page 3 of 3

AMENDMENT: page 9, Statistical Calculations, change the first full paragraph to:

The data will be evaluated to determine if the concentration tested is a no-observed-effect-concentration (NOEC), which is defined as a test concentration which shows no adverse effect on a variable of interest. Dunnett's test (one-tailed) will be used to determine significant differences from the control at the 0.05 level of significance. Significant differences, or their absence, may help establish whether the test concentration is a NOEC.

REASON:

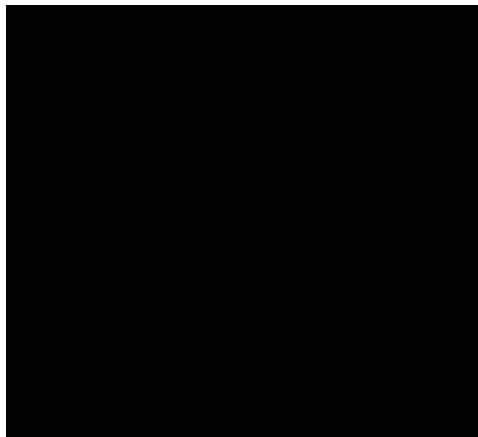
Wording is corrected to reflect statistical analysis of a limit test.

AMENDMENT: page 9, Records to be Maintained:

Delete items 6 and 9.

REASON:

No chemical analysis will be conducted on test soils, and no equipment will be used for application of the test substance.



8 Feb 05
DATE

2/7/05
DATE

2/8/2005
DATE

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Wildlife International, Ltd.

Page 1 of 1

DEVIATION FROM STUDY PROTOCOL

STUDY TITLE: Petroleum Coke: A 21-Day Toxicity Test to Determine the Effects of the Test Substance on Seedling Emergence and Growth of Terrestrial Plants

PROTOCOL NO.: 472/033004/SEED-10/SUB472

DEVIATION NO.: 1

SPONSOR: American Petroleum Institute

WIL PROJECT NO.: 472-102

DATE(S) OF DEVIATION: February 7, 2005

DEVIATION:

The moisture content of the bulk soil used for the test was determined concurrent with test initiation rather than prior to test initiation.

REASON:

Soil moisture is dynamic and should be measured as closely as possible to the test initiation in order to obtain an accurate estimate of the conditions at the start of the test. There will be no adverse impact on the test as a result of this deviation.

DEVIATION:

The soil sample sent to Agvise Laboratories for characterization was not collected from soil actually used in the test.

REASON:

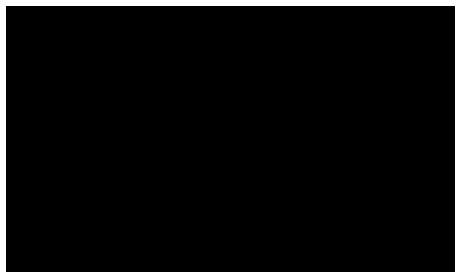
The protocol should indicate that the soil will be representative of that used in the test. The sample was collected from the same soil lot that was used in the test, but was collected approximately 3 weeks prior to test initiation.

DEVIATION:

Protocol states that initial watering of test pots following application would be made to soil surface. Plants were watered via subirrigation instead.

REASON:

Biologist oversight. The emergence rate of seedlings in the study exceeded the minimum requirements. Therefore, there is no adverse impact on the study as a result of this deviation.



11 Mar 05
DATE

15 Mar 05
DATE

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Appendix 3

Test Article Selection

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THE PACE CONSULTANTS INC.
Post Office Box 53473 Houston, Texas 77052 853/351-7800 Fax 853/351-7887
A Member of Jacobs Engineering Group

February 22, 2001



American Petroleum Institute
1220 L Street, NW
Washington, D.C. 20005-4070

Attached is Pace's report covering Task 1 and 2 entitled "U.S. Delayed Coker Petroleum Coke Quality Survey 1998-1999."

We would be pleased to answer any questions concerning this work for API. Please contact me at 832/351-7811 or email [REDACTED]

For PACE



Attachment

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U.S. DELAYED COKER PETROLEUM COKE QUALITY SURVEY 1998-1999

INTRODUCTION

In 1998 the United States Environmental Protection Agency (EPA) challenged chemical producers and importers to provide voluntarily basic toxicity information on their high production volume (HPV) chemicals, defined as those chemicals which are produced in or imported to the U.S. in amounts greater than 1 million pounds per year. The goal of the HPV Challenge Program is to ensure that the American public has access to basic information about the hazards associated with chemicals manufactured and used in the greatest quantities in the United States. It is designed to generate the complete hazard screening data for HPV commercial chemicals.

The American Petroleum Institute (API) serves as administrator of the Petroleum HPV Testing Group, a consortium made up of 72 member companies from API, the National Petrochemical & Refiners Association (NPRA), the Gas Producers Association (GPA) and the Asphalt Institute. These companies represent 92% of the nation's refinery capacity. The Petroleum HPV Testing Group has sponsored 396 substances produced and used by the nation's petroleum industry to meet the EPA's HPV challenge.

Pace was retained by the API HPV Testing Group to assist in identifying potential sources of U.S. petroleum coke samples that could be used in the HPV testing program. As the first step in this process, Pace undertook a review of its quarterly petroleum coke production data to help characterize current U.S. petroleum coke production qualities. Pace has now completed the review of its 1998 and 1999 quarterly petroleum coke production data for all U.S.-based delayed cokers. The results of this review are discussed below.

METHODOLOGY

Pace's petroleum coke production database was used to determine quality characteristics of petroleum coke produced by U.S. refineries. Pace has conducted a survey of U.S. petroleum coker production on a quarterly basis since the second quarter of 1983. Refineries provide the bulk of the data, but some data are also gathered from other market participants. These data are maintained in a database from which the 1998 and 1999 quarterly data were extracted for this study. It was decided that data analysis would concentrate on delayed cokers (excluding needle cokers) since for 1999 our delayed coker data set includes 92+% of all the petroleum coke produced in the United States. Accordingly, fluid and Flexicokers¹ were removed from the data set.

Needle cokers were removed from the delayed coker database because needle cokers represent a special subset of delayed coking production. Needle coke differences include:

¹ Flexicoke is a proprietary coking process developed by Exxon. It involves partially gasifying fluid coke.

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1. Needle coke quality is much higher than other delayed coke
2. Needle coke is produced using different feedstock & coking operational procedures because it is a product, not a by-product like other delayed cokes
3. The quantity of needle coke produced is very small
4. Needle coke is handled very carefully due to its high price (typically > \$350/metric ton)

SUMMARY AND DATA ANALYSIS

These data were analyzed to determine the ton-weighted average petroleum coke qualities of sulfur (wt%), nickel (ppm), vanadium (ppm), and volatile material (wt%). All data are presented on a dry basis. The results are presented in Table 1 below.

TABLE 1

U.S. DELAYED PETROLEUM COKE QUALITY SUMMARY TON-WEIGHTED QUARTERLY AVERAGES								
Quarter	Sulfur, Wt%		Nickel, ppm		Vanadium, ppm		Vol. Mat., Wt%	
	1998	1999	1998	1999	1998	1999	1998	1999
1Q	4.15	4.11	286	275	758	801	10.9	10.5
2Q	4.22	4.22	277	283	811	821	10.8	11.0
3Q	4.21	4.21	277	282	811	857	10.9	10.9
4Q	4.21	4.22	282	276	854	852	10.7	10.9
Ton-Wt Avg	4.20	4.19	280	279	809	833	10.8	10.8

Ton-weighted average qualities for each quarter were calculated in the following manner:

$$\frac{\sum (\text{quality value})_{\text{delayed coker}} * (\text{quarterly production})_{\text{delayed coker}}}{\text{Total quarterly production}}$$

Where:

quality value = sulfur, vanadium, nickel or volatile content of petroleum coke produced by each delayed coker

quarterly production = petroleum coke produced by that delayed coker

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Pace next reviewed the data to determine a ton-weighted frequency distribution for each of the qualities listed. The results of this analysis are presented in Table 2 and in Figures 1 through 4.

TABLE 2

U.S. DELAYED PETROLEUM COKE QUALITY SUMMARY BY PRODUCTION QUARTILE								
Cumulative Production	Sulfur, Wt%		Nickel, ppm		Vanadium, ppm		Vol, Wt%	
	1998	1999	1998	1999	1998	1999	1998	1999
min.	0.90	0.50	50	5	45	45	7.0	4.0
25%	3.20	3.10	180	185	400	445	10.0	10.0
50%	4.45	4.60	250	250	650	675	10.7	10.7
75%	5.34	5.30	360	400	1205	1200	12.0	12.0
100%	6.90	6.30	568	568	1900	2000	14.0	14.0

Quality quartiles for each year were calculated in the following manner:

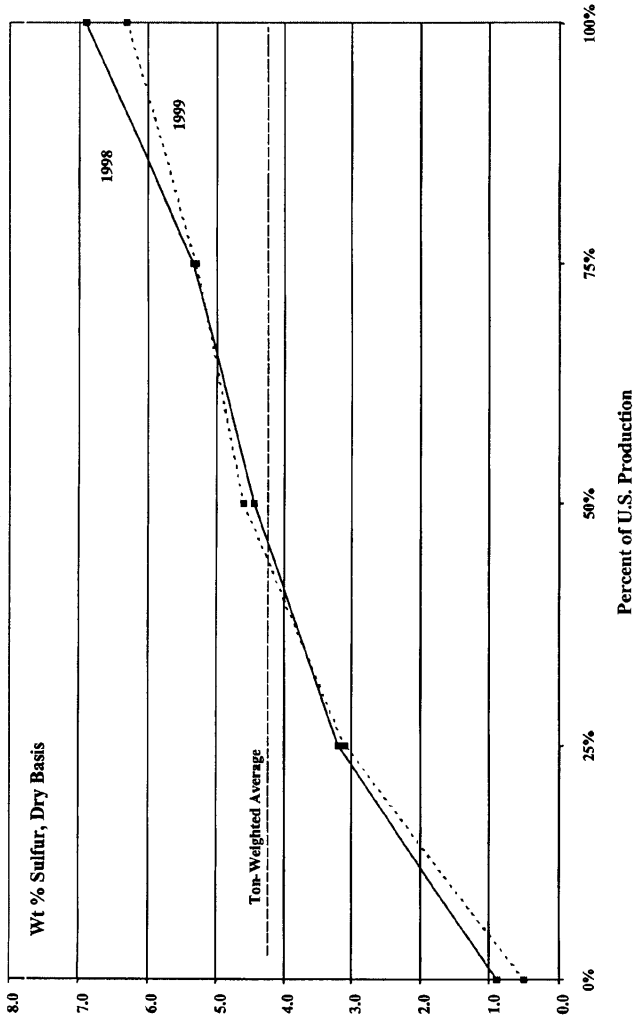
Annual data were sorted according to each specific quality value (e.g., sulfur, vanadium, nickel, and volatile content) and the cumulative production of petroleum coke by delayed coker was calculated. Quartiles were then calculated for the annual production total, and the quality value at the cumulative total that equaled each quartile was used to determine the quality for that quartile.

TRENDS

Comparing the ton-weighted averages to the 50% production quartile (i.e., the median) reveals the following trends:

- The weighted average nickel and vanadium content of U.S. delayed petroleum coke is higher than the median. This is a direct result of the increasing amount of heavy crudes, particularly Mexican and Venezuelan crudes, processed by U.S. refineries. Because these crudes produce petroleum cokes with nickel and vanadium contents that are significantly above the median, they skew the weighted average away from the median.
- Ton-weighted sulfur content is slightly below the median because some cokers produce petroleum cokes that are well below the median sulfur content (i.e., anode-grade coke which is calcined and primarily used to make anodes for the aluminum smelting industry).

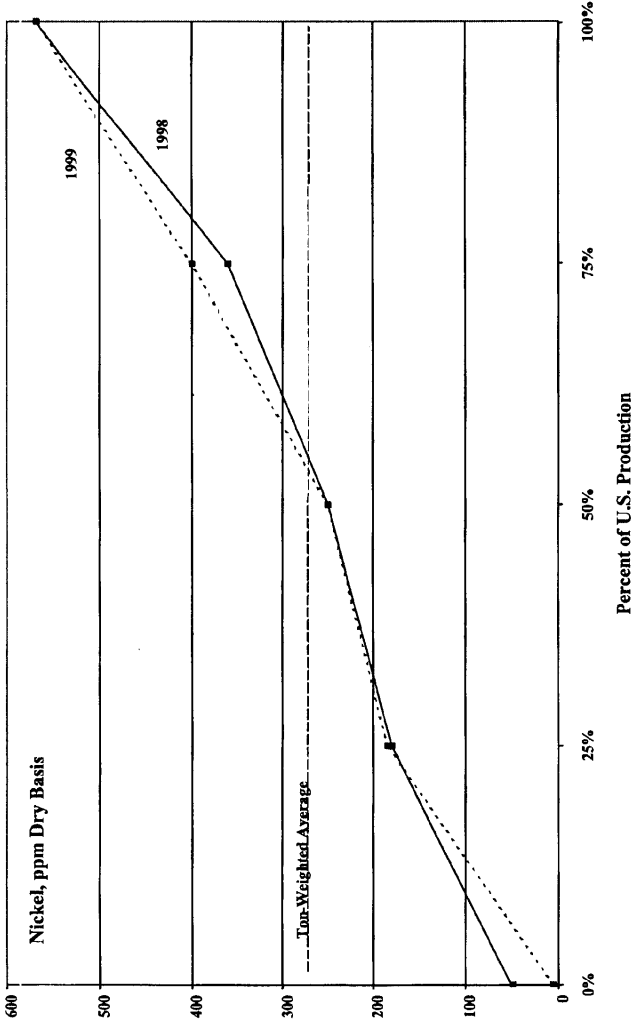
FIGURE 1
U.S. DELAYED PETROLEUM COKE
SULFUR CONTENT



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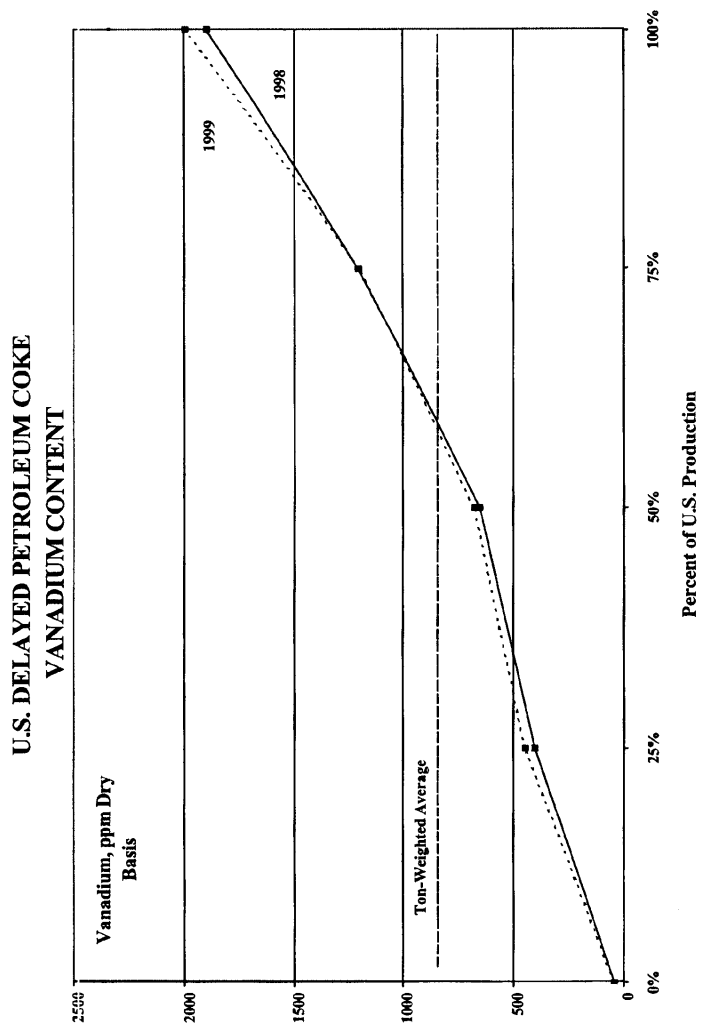
FIGURE 2

U.S. DELAYED PETROLEUM COKE
NICKEL CONTENT



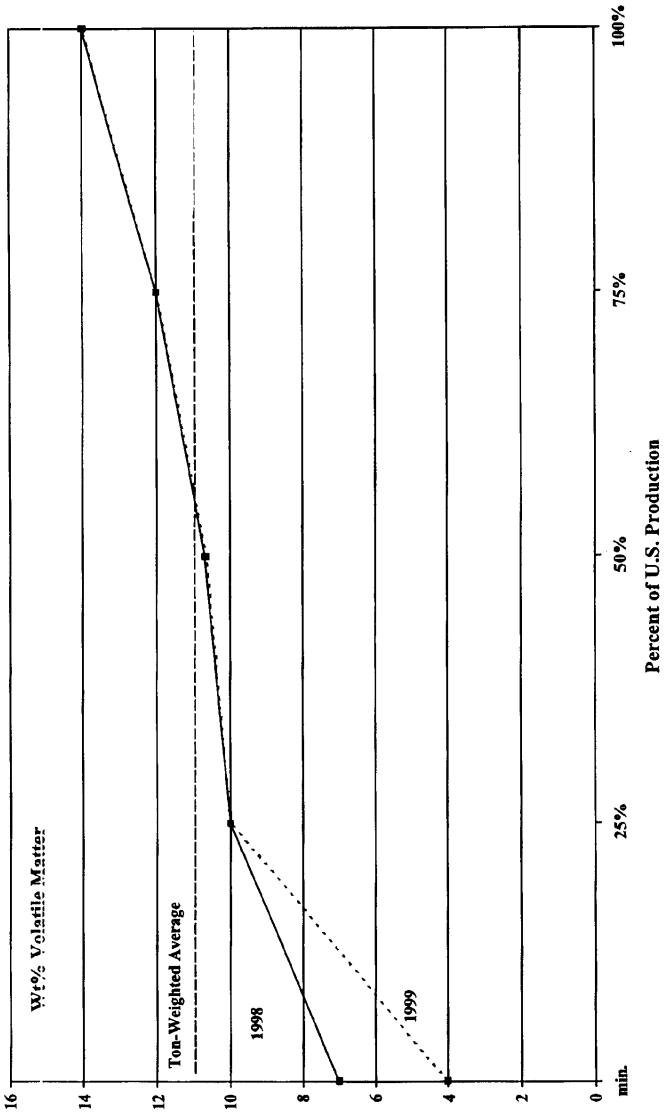
THE PACE CONSULTANTS INC.

FIGURE 3



THE PACE CONSULTANTS INC.

FIGURE 4
U.S. DELAYED PETROLEUM COKE VOLATILE MATTER CONTENT



THE PACE CONSULTANTS INC.

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- The sulfur content at the upper and lower ends of the quality spectrum was better in 1999 than in 1998. We believe the lower sulfur content in 1999 was a result of crude production cut-backs by OPEC (Organization of Petroleum Exporting Countries) and other crude oil producers. These producers preferentially reduced the production of their lower quality crude oils in order to minimize the production reductions of their higher quality (i.e. higher priced) crude oils. We see 1999 as an aberration in the general trend of increasing sulfur content in U.S. petroleum cokes.
- We expect the metals content and sulfur content of U.S. petroleum coke will deteriorate beginning in 2001 as new U.S. cokers scheduled to begin operations in the 2000-2002 time frame start up.
- The average volatile matter content is essentially equal to the median.

RECOMMENDATIONS

Pace identified candidate refineries for sampling based on the quality data from the third quarter of 2000, which is the most recent quarter for which data are available. It should be noted that these data may vary slightly from the 1998-1999 averages as increasing amounts of heavy crude are processed. Based on these data, Pace recommends the following candidates for sampling in support of the Petroleum HPV Testing Program:

PETROLEUM HPV TESTING PROGRAM DELAYED PETROLEUM COKE SAMPLE CANDIDATES						
	Candidate A		Candidate B		Candidate C	
	Value	Percentile	Value	Percentile	Value	Percentile
Sulfur, Wt%	6.00	93	5.75	86	5.50	80
Nickel, ppm	500	90	300	58	250	50
Vanadium, ppm	1,500	84	1,200	75	1,000	65
Volatiles, Wt%	10.00	25	12.00	75	13.00	88

PETROLEUM HPV TESTING PROGRAM DELAYED PETROLEUM COKE SAMPLE CANDIDATES					
	Candidate D			Candidate E	
	Value	Percentile		Value	Percentile
Sulfur, Wt%	4.20	43		5.50	80
Nickel, ppm	250	50		350	67
Vanadium, ppm	1,500	84		1,100	70
Volatiles, Wt%	15.00	100		10.00	25

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Our analysis indicates that some compromises will have to be made in obtaining a sample for the HPV program since no refinery's petroleum coke is in the upper 75th percentile in all four quality parameters we have evaluated. Additionally, we have spent some time and effort trying to find petroleum cokes which are sampled with automatic sampling equipment that has been bias tested and is operated by an independent laboratory. Unfortunately, we have found that the locations with the best sampling systems have petroleum cokes of generally better quality. Therefore, we do not believe that we will be able to find a "perfect" candidate petroleum coke.

While the sampling at the candidate refineries may not be ideal, the sampling and analysis data have been used for commercial transactions. Substantial quantities of petroleum coke from each of the candidate refineries have been sold in the petroleum coke market. Commercial transactions have relied on the laboratory results for determining quality bonus and penalties and conformance with contract quality specifications. Thus, the samples taken for the HPV study would conform to generally accepted industry sampling practice.

The sampling plan would be to have the sample analyzed for the quality parameters used in this screening analysis (i.e. sulfur, vanadium, nickel, volatile matter) as well as four other commonly tested quality parameters—gross calorific value (Btu/lb), moisture (%), ash (%), and Hardgrove Grindability Index (HGI)—to verify that the sample obtained is similar to the anticipated quality characteristics. This plan would assure that the sample submitted for detailed HPV testing conforms to our quality expectations.

We may not be able to receive authorization from a refinery to use a sample of their petroleum coke for the HPV test. Our present plan would be to approach Refineries B and C regarding obtaining a sample. In the event that these two refineries choose not to participate, then the choice would be either refinery A or E, which have high sulfur and metals but low volatile content or refinery D, which has high vanadium and volatile matter but low sulfur content. (note: each of the five candidate refineries has a different corporate owner).

Pace requests that the HPV Committee confirm Pace's recommended plan to approach refineries B and C regarding obtaining an HPV sample. It is not necessary for the HPV committee to decide now on the preferred refinery to contact in the event that refineries B and C do not wish to participate in the program. However, we would suggest that the committee begin to think about this issue so that decisions can be made expeditiously in the event that refineries B and C choose not participate.

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Appendix 4

AVEKA, Inc. Particle Processing Report

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AVEKA, Inc.

PARTICLE PROCESSING & CUSTOM RESEARCH

Date: May 29, 2003

Make Order #: 5369

Company Name: API

Contact Person: [REDACTED]

Material: Green Petroleum Coke

Objective: Task 1: Hammermill, Ball-mill and Classify Petroleum Coke to a mean particle size less than 3.6 microns. Task 2: Crush and Classify petroleum coke to a mean particle size of 2 mm.

Equipment: Homoloid JT Hammermill (SN # JT-694) with 0.0093 screen
5 Gallon Ball-mill with 0.25 inch alumina media
Majac A-12 classifier
Horiba LA-910 Laser Light Scattering Particle Sizer
Marcy 4"x 6" Jaw Crusher
Gilson Sonic Sieve

Receipt: Approximately 80 lbs. of material was received 3-19-03 from Federal Express. Confirmation of receipt (EPL Project Identification 1203-001) was returned upon delivery.

Storage: Petroleum coke was stored at room temperature in sealed polyethylene bags when the material was not being processed.

Processing Procedure:

The green petroleum coke showed high moisture content upon inspection. The high moisture content was indicated by condensation on the inside of the received petroleum coke bags. After consulting with Deborah Herron and Jacobs Consultancy, the material was dried according to ASTM D 3302-00 (Standard Test Method for Total Moisture in Coal).

Task 1

All processes were run at room temperature. The dried petroleum coke was then run through a Homoloid JT Hammermill (SN # JT-694) equipped with a 0.0093 screen.

The resulting hammermilled powder was loaded into 5-gallon ball mills loaded with 0.25 inch ceramic (alumina) media. The loading level in the ball mill was 27 lbs. of media with 5.5 lbs. of petroleum coke.

651-730-1729

2045 Wooddale Drive, Woodbury, MN 55125

FAX 651-730-1826

AMENDED

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AVEKA, Inc.

PARTICLE PROCESSING & CUSTOM RESEARCH

The mills were rotated at 36 rpm for 17.25 hours. The resulting powder had a mean particle size of 9.56 microns (Atch 1) when tested with the Horiba LA-910 in water.

The oversized petroleum coke material was removed using a Majac A-12 Classifier. The Majac was run at 1800 RPM and 8.5 cfm. The resulting particle size of the petroleum coke was a 3.3 micron mean (Atch. 2) when tested with the Horiba LA-910 in water. The Horiba LA-910 test method for the petroleum coke samples is outlined in Atch. 3.

The final yield of product was 10.5 kg of powder.

Task 2

All processes were run at room temperature. An 18" Sweco Screener was set-up with a 7 mesh (2.8 mm) top-screen and a 14 mesh (1.4 mm) bottom-screen. Petroleum coke was fed through the screener and 2-mm material was collected from between the top and bottom screen. Oversized petroleum coke was jaw crushed with a Marcy 4"x 6" Jaw Crusher and rescreened. A Gilson Sonic Sieve particle size analysis (Atch. 4) was run on the screened petroleum coke and the results showed 99.4 % of the material between 1.4 mm – 2.8 mm. Final yield was 3.3 kg of 2 mm Petroleum Coke.

Shipping

All samples were shipped UPS Ground. The following is a summary of the sample disposition.

<u>Sample/Amount</u>	<u>Address</u>	<u>Person</u>
200 grams of 2-3 micron particle size sample	ChevronTexaco Energy Research and Technology Corp. 100 Chevron Way Richmond, CA 94802 Tel: 510-242-7037	Richard Dutta

AVEKA, Inc.

PARTICLE PROCESSING & CUSTOM RESEARCH

<u>200 grams</u> of 2 mm particle sample	ChevronTexaco Energy Research and Technology Corp. 100 Chevron Way Richmond, CA 94802 Tel: 510-242-7037	Richard Dutta
<u>10.5 kg</u> of 2-3 micron particle size sample	EPL Archives, Inc. 45610 Terminal Drive Sterling, Virginia 20166 703/435-8780 ext 201	Sam Busey
<u>Remainder (slightly less than 3 kg)</u> of 2 mm particle size sample	EPL Archives, Inc. 45610 Terminal Drive Sterling, Virginia 20166 703/435-8780 ext 201	Sam Busey
<u>Leftover petroleum coke material</u> , i.e., that material not used in samples	EPL Archives, Inc. 45610 Terminal Drive Sterling, Virginia 20166 703/435-8780 ext 201	Sam Busey

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Attach 1

HORIBA LA-910**PARTICLE SIZE DISTRIBUTION DATA TABLE Standard 04/23/03**

File Name: 5369001.DAT Sample Name: Ballmilled 17.25 Hours

ID No: **/04/23-350

Dist. Form: STANDARD R.R. Index: co.rj□

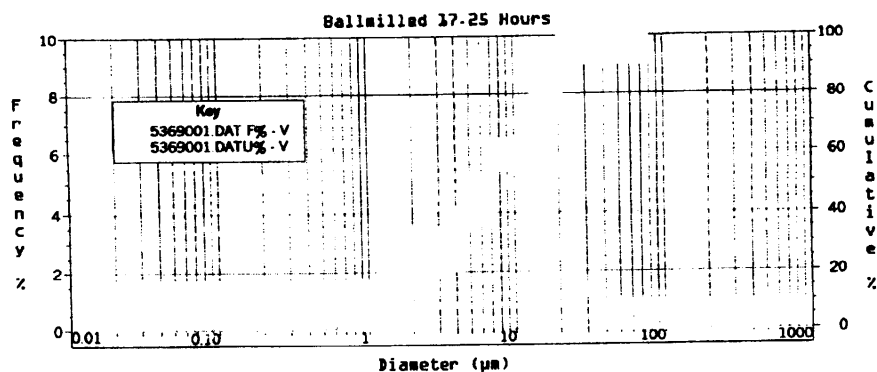
Dist. Mode: VOLUME Laser: 65.128 % Lamp: 61.185 %

Circulation: 2 Agitation: 7 U.Sonic ** (min)

Material: Petroleum Coke Source: American Petroleum

Lot No: MO5369

Test No: 5369001



				SIZE (μm)	FREQ%	UNDR%	No.	SIZE (μm)	FREQ%	UNDR%	
(1)	0.020	0.0	0.0	(28)	0.766	0.5	0.8	(55)	29.907	1.5	95.4
(2)	0.022	0.0	0.0	(29)	0.877	0.8	1.6	(56)	34.255	1.2	96.6
(3)	0.026	0.0	0.0	(30)	1.005	1.2	2.8	(57)	39.234	0.9	97.5
(4)	0.029	0.0	0.0	(31)	1.151	1.6	4.4	(58)	44.938	0.7	98.2
(5)	0.034	0.0	0.0	(32)	1.318	2.0	6.3	(59)	51.471	0.5	98.8
(6)	0.039	0.0	0.0	(33)	1.510	2.3	8.7	(60)	58.953	0.4	99.2
(7)	0.044	0.0	0.0	(34)	1.729	2.6	11.3	(61)	67.523	0.3	99.5
(8)	0.051	0.0	0.0	(35)	1.981	2.9	14.2	(62)	77.340	0.2	99.7
(9)	0.058	0.0	0.0	(36)	2.269	3.0	17.2	(63)	88.582	0.2	99.9
(10)	0.067	0.0	0.0	(37)	2.599	3.2	20.4	(64)	101.460	0.1	100.0
(11)	0.076	0.0	0.0	(38)	2.976	3.5	23.9	(65)	116.210	0.0	100.0
(12)	0.087	0.0	0.0	(39)	3.409	3.7	27.7	(66)	133.103	0.0	100.0
(13)	0.100	0.0	0.0	(40)	3.905	4.0	31.6	(67)	152.453	0.0	100.0
(14)	0.115	0.0	0.0	(41)	4.472	4.3	35.9	(68)	174.616	0.0	100.0
(15)	0.131	0.0	0.0	(42)	5.122	4.6	40.6	(69)	200.000	0.0	100.0
(16)	0.150	0.0	0.0	(43)	5.867	5.1	45.6	(70)	229.075	0.0	100.0
(17)	0.172	0.0	0.0	(44)	6.720	5.5	51.2	(71)	262.376	0.0	100.0
(18)	0.197	0.0	0.0	(45)	7.697	5.9	57.0	(72)	300.518	0.0	100.0
(19)	0.226	0.0	0.0	(46)	8.816	6.0	63.0	(73)	344.205	0.0	100.0
(20)	0.259	0.0	0.0	(47)	10.097	5.8	68.8	(74)	394.244	0.0	100.0
(21)	0.296	0.0	0.0	(48)	11.565	5.4	74.2	(75)	451.556	0.0	100.0
(22)	0.339	0.0	0.0	(49)	13.246	4.8	79.1	(76)	517.200	0.0	100.0
(23)	0.389	0.0	0.0	(50)	15.172	4.2	83.3	(77)	592.387	0.0	100.0
(24)	0.445	0.0	0.0	(51)	17.377	3.5	86.8	(78)	678.504	0.0	100.0
(25)	0.510	0.0	0.0	(52)	19.904	2.9	89.7	(79)	777.141	0.0	100.0
(26)	0.584	0.1	0.1	(53)	22.797	2.3	92.0	(80)	890.116	0.0	100.0
(27)	0.669	0.2	0.3	(54)	26.111	1.9	93.9	(81)	1019.510	0.0	100.0

Median : 6.533 (μm) Mean: 9.561 (μm) Mode: 8.237 (μm)

Std. Dev.: 10.531 (μm) Span: 10.623

Coef. Var: 110.14% Spec. Area: 15308 (cm²/cm³)

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Attch. 2

HORIBA LA-910**PARTICLE SIZE DISTRIBUTION DATA TABLE** Standard 05/15/03

File Name: 5369011.DAT Sample Name: Fines

ID No: **/04/30-568

Dist. Form: STANDARD R.R. Index: co.rj□

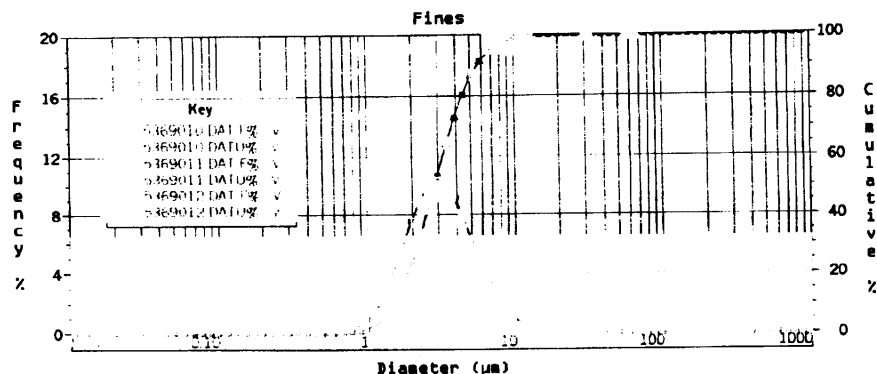
Dist. Mode: VOLUME Laser: 85.118 % Lamp: 86.338 %

Circulation: 3 Agitation: 7 U.Sonic OFF (min)

Material: Petroleum Coke Source: American Petroleum

Lot No: MO5369

Test No: 5369004



No.	SIZE (μm)	FREQ%	UNDR%	No.	SIZE (μm)	FREQ%	UNDR%	No.	SIZE (μm)	FREQ%	UNDR%
(1)	0.020	0.0	0.0	(28)	0.766	0.2	0.2	(55)	29.907	0.0	100.0
(2)	0.022	0.0	0.0	(29)	0.877	0.5	0.7	(56)	34.255	0.0	100.0
(3)	0.026	0.0	0.0	(30)	1.005	1.0	1.7	(57)	39.234	0.0	100.0
(4)	0.029	0.0	0.0	(31)	1.151	1.7	3.5	(58)	44.938	0.0	100.0
(5)	0.034	0.0	0.0	(32)	1.318	2.8	6.3	(59)	51.471	0.0	100.0
(6)	0.039	0.0	0.0	(33)	1.510	4.3	10.6	(60)	58.953	0.0	100.0
(7)	0.044	0.0	0.0	(34)	1.729	5.9	16.5	(61)	67.523	0.0	100.0
(8)	0.051	0.0	0.0	(35)	1.981	7.6	24.1	(62)	77.340	0.0	100.0
(9)	0.058	0.0	0.0	(36)	2.269	9.0	33.0	(63)	88.582	0.0	100.0
(10)	0.067	0.0	0.0	(37)	2.599	10.1	43.1	(64)	101.460	0.0	100.0
(11)	0.076	0.0	0.0	(38)	2.976	10.6	53.7	(65)	116.210	0.0	100.0
(12)	0.087	0.0	0.0	(39)	3.409	10.2	63.8	(66)	133.103	0.0	100.0
(13)	0.100	0.0	0.0	(40)	3.905	9.0	72.9	(67)	152.453	0.0	100.0
(14)	0.115	0.0	0.0	(41)	4.472	7.6	80.4	(68)	174.616	0.0	100.0
(15)	0.131	0.0	0.0	(42)	5.122	6.0	86.5	(69)	200.000	0.0	100.0
(16)	0.150	0.0	0.0	(43)	5.867	4.6	91.1	(70)	229.075	0.0	100.0
(17)	0.172	0.0	0.0	(44)	6.720	3.4	94.5	(71)	262.376	0.0	100.0
(18)	0.197	0.0	0.0	(45)	7.697	2.3	96.8	(72)	300.518	0.0	100.0
(19)	0.226	0.0	0.0	(46)	8.816	1.5	98.3	(73)	344.205	0.0	100.0
(20)	0.259	0.0	0.0	(47)	10.097	0.9	99.1	(74)	394.244	0.0	100.0
(21)	0.296	0.0	0.0	(48)	11.565	0.5	99.6	(75)	451.556	0.0	100.0
(22)	0.339	0.0	0.0	(49)	13.246	0.2	99.9	(76)	517.200	0.0	100.0
(23)	0.389	0.0	0.0	(50)	15.172	0.1	100.0	(77)	592.387	0.0	100.0
(24)	0.445	0.0	0.0	(51)	17.377	0.0	100.0	(78)	678.504	0.0	100.0
(25)	0.510	0.0	0.0	(52)	19.904	0.0	100.0	(79)	777.141	0.0	100.0
(26)	0.584	0.0	0.0	(53)	22.797	0.0	100.0	(80)	890.116	0.0	100.0
(27)	0.669	0.0	0.0	(54)	26.111	0.0	100.0	(81)	1019.510	0.0	100.0

Median : 2.840 (μm) Mean: 3.289 (μm) Mode: 2.781 (μm)

Std. Dev.: 1.844 (μm) Span: 2.780

Coef. Var: 56.08% Spec. Area: 23790 (cm²/cm³)

Attch. 3

TEST METHOD FOR API PETROLEUM COKE

Sample Preparation

May 15, 2003

Mix 0.15 – 0.2 grams of petroleum coke with 5-6 grams distilled water. Add TX-100 surfactant to aid dispersion. Mix thoroughly until no large concentrations of sample are evident.

LA-910 Preparation

Fill the test chamber to capacity with 140 ml distilled water. Add 3-4 drops of TX-100 surfactant from a 10% concentrate source, resulting in approximately a .1% diluted total. Select the relative refractive index appropriate for this material (1.61-3.02i). Circulate the solvent using a pump speed of 2-3, subtract the background. Add the sample drop by drop until the laser transmission falls into the acceptable range (70 – 95)% transmittance. Activate the sonicator to aid dispersion, cease sonication when sample is completely dispersed.

Sample Test

Measure the sample three times. Save each measurement. Overlay the three measurements on a graph. If they appear stable, the test is complete. If not, investigate. A steady increase in the laser transmission rate indicates more particles are present from pass to pass. That indicates the sample was not completely dispersed yet. A steady decrease in the laser transmission rate indicates the sample is agglomerating, settling, or dissolving.

Report

Using the Display module, graph the three test runs over one another. A stable test will appear as one line, an unstable condition will clearly show all three runs, indicating instability. If stable, select a run (typically the middle run) and print the complete data table along with the graph.

Author: T.J. Roberts
Lab Manager
Avcka, Inc.
(651) 714-4293 ext 208

- 57 -

Attch. 4

5/29/03

Sieve Analysis

Sample ID: American Petroleum Institute
2mm Pet Coke

US Standard Mesh Size	Mesh Opening (Microns)	Sieve Weight (Grams)	Sieve Weight + Sample (g)	Weight of Sample (g)	% Sample Above Sieve	% Sample Below Sieve
7	2800	50.951	50.975	0.024	0.31	99.69
8	2360	50.741	52.146	1.405	18.18	81.81
10	2000	48.772	51.024	2.252	29.14	70.86
12	1700	47.324	50.173	2.849	36.86	63.14
14	1400	48.450	49.624	1.174	15.19	84.81
catch	0	220.018	220.043	0.025	0.32	99.68
Totals:				7.729	100.00	

Notes/Comments:

MO5388a.xls

AMENDED

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Appendix 5

Laboratory Characterization of 3.3 Micron Particle Size Petroleum Coke

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ANALYTICAL RESULTS

Prepared for:

Chevron Products Company
940 Hensley St. Bldg. 210

Richmond CA 94801
510-242-8191

Prepared by:

Lancaster Laboratories
2425 New Holland Pike
Lancaster, PA 17605-2425

SAMPLE GROUP

The sample group for this submittal is 857532. Samples arrived at the laboratory on Friday, June 27, 2003.
The PO# for this group is 99011184 and the release number is [REDACTED]

Client Description

Pet Coke 2mm Solid Sample
Pet Coke Micronized Solid Sample

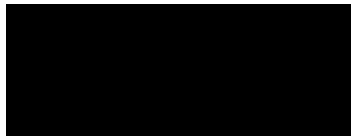
Lancaster Labs Number

4073301
4073302

1 COPY TO Lancaster Laboratories
1 COPY TO Chevron CRTG

Questions? Contact your Client Services Representative
Alicia M O'Connor at (717) 656-2300.

Respectfully Submitted,



MEMBER
LABORATORY

Lancaster Laboratories, Inc.
2425 New Holland Pike
PO Box 12425
Lancaster, PA 17605-2425

A4200
3.66

AMENDED

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Analysis Report

Page 1 of 2

Lancaster Laboratories Sample No. SW 4073302

Collected: 06/26/2003 00:00

Account Number: 10863

Submitted: 06/27/2003 10:40

Chevron Products Company

Reported: 07/09/2003 at 11:42

940 Hensley St. Bldg. 210

Discard: 08/09/2003

Pet Coke Micronized Solid Sample

Richmond CA 94801

Cost Center# ENG-4066

HPV Petroleum Cake

MICPC

CAS No.	Analysis Name	CAS Number	As Received Result	As Received Method Detection Limit	Units	Dilution Factor
07804	PAHs in Soil by GC/MS					
01191	Acenaphthene	83-32-9	N.D.	1,000.	ug/kg	10
01195	Pyrene	129-00-0	8,600.	J 1,000.	ug/kg	10
02751	1-Methylnaphthalene	90-12-0	10,000.	1,000.	ug/kg	10
03761	Naphthalene	91-20-3	11,000.	1,000.	ug/kg	10
03765	Acenaphthylene	208-96-8	N.D.	1,000.	ug/kg	10
03768	Fluorene	86-73-7	1,500.	J 1,000.	ug/kg	10
03775	Phenanthrene	85-01-8	7,800.	J 1,000.	ug/kg	10
03776	Anthracene	120-12-7	3,300.	J 1,000.	ug/kg	10
03778	Fluoranthene	206-44-0	1,400.	J 1,000.	ug/kg	10
03781	Benzo(a)anthracene	56-55-3	7,100.	J 1,000.	ug/kg	10
03782	Chrysene	218-01-9	9,400.	J 1,000.	ug/kg	10
03786	Benzo(b)fluoranthene	205-99-2	3,800.	J 1,000.	ug/kg	10
03787	Benzo(k)fluoranthene	207-08-9	N.D.	1,000.	ug/kg	10
03788	Benzo(a)pyrene	50-32-8	11,000.	1,000.	ug/kg	10
03789	Indeno(1,2,3-cd)pyrene	193-39-5	3,500.	J 1,000.	ug/kg	10
03790	Dibenz(a,h)anthracene	53-70-3	4,100.	J 1,000.	ug/kg	10
03791	Benzo(g,h,i)perylene	191-24-2	8,700.	J 1,000.	ug/kg	10
04694	2-Methylnaphthalene	91-57-6	26,000.	1,000.	ug/kg	10

Due to sample matrix interferences observed during the extraction, the normal reporting limits could not be obtained.

Due to the sample matrix an initial dilution was necessary to perform the analysis. Therefore, the reporting limits for the GC/MS semivolatile compounds were raised.

State of California Lab Certification No. 2116

Laboratory Chronicle

CAT No.	Analysis Name	Method	Trial#	Analysis Date and Time	Analyst	Dilution Factor
07804	PAHs in Soil by GC/MS	SW-846 8270C	1	07/02/2003 18:34	Susan L Scheuring	10

MEMBER

Lancaster Laboratories, Inc.
2425 New Holland Pike
PO Box 12425
Richmond CA 94801

AMENDED

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Page 2 of 2

Lancaster Laboratories Sample No. SW 4073302

Collected: 06/26/2003 00:00

Account Number: 10863

Submitted: 06/27/2003 10:40

Chevron Products Company

Reported: 07/09/2003 at 11:42

940 Hensley St. Bldg. 210

Discard: 08/09/2003

Pet Coke Micronized Solid Sample

Richmond CA 94801

Cost Center# ENG-4066

HPV Petroleum Cake

MICPC

07806 BRA Soil Extraction

SW-846 35508

1 06/30/2003 20:00 Sally L Appleyard

1

MEMBER
EVENT

Lancaster Laboratories, Inc.
2425 New Holland Pike
PO Box 12425
Lancaster PA 17604-7425

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Page 1 of 2

Quality Control Summary

Client Name: Chevron Products Company
Reported: 07/09/03 at 11:42 AM

Group Number: 857532

Laboratory Compliance Quality Control

<u>Analysis Name</u>	<u>Blank Result</u>	<u>Blank MDL</u>	<u>Report Units</u>	<u>LCS</u> <u>UREC</u>	<u>LCSO</u> <u>UREC</u>	<u>LCS/LCSO</u> <u>Limits</u>	<u>RPD</u>	<u>RPD Max</u>
Batch number: 03181SLA026	Sample number(s): 4073301-4073302							
Acenaphthene	N.D.	33.	ug/kg	91		76-109		
Pyrene	N.D.	33.	ug/kg	89		71-110		
1-Methylnaphthalene	N.D.	33.	ug/kg	87		76-101		
Naphthalene	N.D.	33.	ug/kg	87		73-103		
Acenaphthylene	N.D.	33.	ug/kg	94		73-106		
Fluorene	N.D.	33.	ug/kg	93		66-115		
Phenanthrene	N.D.	33.	ug/kg	88		70-107		
Anthracene	N.D.	33.	ug/kg	86		71-107		
Fluoranthene	N.D.	33.	ug/kg	90		69-107		
Benzo(a)anthracene	N.D.	33.	ug/kg	93		74-107		
Chrysene	N.D.	33.	ug/kg	89		72-109		
Benzo(b)fluoranthene	N.D.	33.	ug/kg	95		71-113		
Benzo(k)fluoranthene	N.D.	33.	ug/kg	97		75-112		
Benzo(a)pyrene	N.D.	33.	ug/kg	94		79-111		
Indeno(1,2,3-cd)pyrene	N.D.	33.	ug/kg	88		74-113		
Dibenz(a,h)anthracene	N.D.	33.	ug/kg	95		81-118		
Benzo(g,h,i)perylene	N.D.	33.	ug/kg	92		74-114		
2-Methylnaphthalene	N.D.	33.	ug/kg	90		70-102		

Sample Matrix Quality Control

<u>Analysis Name</u>	<u>MS</u> <u>UREC</u>	<u>MSD</u> <u>UREC</u>	<u>MS/MSD</u> <u>Limits</u>	<u>RPD</u>	<u>MXG</u> <u>MAX</u>	<u>DUF</u> <u>COND</u>	<u>DUF</u> <u>COND</u>	<u>DUP</u> <u>RPD</u>	<u>DUP</u> <u>MAX</u>
Batch number: 03181SLA026	Sample number(s): 4073301-4073302								
Acenaphthene	107	93	48-132	14	30				
Pyrene	82	68	28-144	12	30				
1-Methylnaphthalene	75	67*	72-100	5	30				
Naphthalene	77	61	38-132	9	30				
Acenaphthylene	108	91	46-128	18	30				
Fluorene	88	75	39-137	14	30				
Phenanthrene	88	74	29-143	13	30				
Anthracene	101	85	35-138	17	30				
Fluoranthene	81	72	19-145	11	30				
Benzo(a)anthracene	89	75	26-144	14	30				
Chrysene	101	80	23-150	9	30				
Benzo(b)fluoranthene	90	74	32-140	16	30				
Benzo(k)fluoranthene	103	88	36-143	16	30				
Benzo(a)pyrene	90	72	23-134	13	30				
Indeno(1,2,3-cd)pyrene	92	78	13-135	15	30				
Dibenz(a,h)anthracene	110	88	19-163	19	30				
Benzo(g,h,i)perylene	99	83	17-152	13	30				
2-Methylnaphthalene	38	19*	32-133	6	30				

* - Outside of specification

- (1) The result for one or both determinations was less than five times the LOQ.
(2) The background result was more than four times the spike added.

MEMBER

Lancaster Laboratories, Inc.
2425 New Holland Pike
PO Box 12425
Lawrenceville, GA 30046-2425

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Analysis Report



Page 2 of 2

Quality Control Summary

Client Name: Chevron Products Company
 Reported: 07/09/03 at 11:42 AM

Group Number: 857532

Sample Matrix Quality Control

	MS	MSD	MS/MSD	RPD	BKG	DUP	DUP	Dup
<u>Analysis Name</u>	<u>REC</u>	<u>REC</u>	<u>Limits</u>	<u>RPD</u>	<u>MAX</u>	<u>Conc</u>	<u>Conc</u>	<u>RPD</u>

Surrogate Quality Control

Analysis Name: PAHs in Soil by GC/MS
 Batch number: 031818LA026

	Nitrobenzene-d5	2-Fluorobiphenyl	Terphenyl-d14
4073301	101	108	92
4073302	101	99	84
Blank	87	85	83
LCS	94	92	93
MS	105	107	88
MSD	90	90	78
Limits:	47-128	55-123	39-128

*- Outside of specification

- (1) The result for one or both determinations was less than five times the LOQ.
- (2) The background result was more than four times the spike added.

MEMBER

Lancaster Laboratories, Inc.
 2425 New Holland Pike
 PO Box 12425
 Lancaster, PA 17602-0425

AMENDED

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From:
Sent:
To:
Cc:
Subject:

Found your results.

3030999 PETROLEUM COKE 2NM YCJ58009 REGULAR SERVICE *micronized*
REPORTED 06/13/2003 Marked-up: 06/12/2003 by [REDACTED] at 50-1118
[REDACTED] (474/0) Prj Id: GLOBETECH

Test code Test Name/Element/Result Test Status Analyst Status date Test Cost

30258 MICROWAVE DIGST/ICP PLUS REPORTED [REDACTED] 06/13/2003 \$200.00
AL 300.200 PPM AS <29.61 PPM B <29.61 PPM
BA <29.61 PPM BE <14.805 PPM BI <29.61 PPM
CA 121.600 PPM CD <14.805 PPM CO <14.805 PPM
CR <14.805 PPM CU <17.766 PPM FE 247.000 PPM
K <44.414 PPM LI <14.805 PPM MG 60.850 PPM
MN <29.61 PPM MO <29.61 PPM NA 114.600 PPM
NI 351.700 PPM P 30.300 PPM PB <29.61 PPM
S 58060.000 PPM SB <74.024 PPM SE <29.61 PPM
SI 554.600 PPM SN <44.414 PPM TI <14.805 PPM
V 1805.000 PPM ZN <14.805 PPM

3030251 PETROLEUM COKE YCJ58009 REGULAR SERVICE *2nm*
REPORTED 06/09/2003 Marked-up: 06/09/2003 by [REDACTED] at 50-1118
[REDACTED] (474/0) Prj Id: [REDACTED]

Test code Test Name/Element/Result Test Status Analyst Status date Test Cost

30258 MICROWAVE DIGST/ICP PLUS REPORTED [REDACTED] 06/09/2003 \$200.00
AL 321.000 PPM AS <19.279 PPM B <19.279 PPM
BA <19.279 PPM BE <9.639 PPM BI <19.279 PPM
CA 178.000 PPM CD <9.639 PPM CO <9.639 PPM
CR <9.639 PPM CU <11.567 PPM FE 310.000 PPM
K <28.918 PPM LI <9.639 PPM MG 77.370 PPM
MN <19.279 PPM MO <19.279 PPM NA 133.000 PPM
NI 367.100 PPM P <19.279 PPM PB <19.279 PPM
S 73920 PPM SB <48.197 PPM SE <19.279 PPM
SI 743.200 PPM SN <28.918 PPM TI 12.910 PPM
V 1938.000 PPM ZN 12.010 PPM

-----Original Message-----
From:

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Appendix 6

Environmental Conditions

WILDLIFE INTERNATIONAL LTD.

Project Number: 472-102

Page 1 of 1

03-01-2005

Environmental Conditions Report - GEM Room/Location: 3

Date	Temperature °C			% Relative Humidity			moles Photosynthetically Active Radiation
	Min	Max	Mean	Min	Max	Mean	
2/7/05	17.49	27.89	21.70	15.21	44.39	30.68	20.3
2/8/05	16.53	27.26	21.54	22.28	46.17	34.43	19.6
2/9/05	17.72	25.87	21.55	28.02	63.20	42.08	12.5
2/10/05	17.72	26.67	20.70	18.61	63.07	37.23	15.0
2/11/05	17.23	25.74	20.75	13.53	34.85	23.91	18.2
2/12/05	17.06	26.37	21.11	19.70	43.80	32.28	16.0
2/13/05	17.10	27.26	21.14	15.94	41.72	31.70	16.4
2/14/05	17.76	23.76	20.81	30.26	75.10	51.14	13.8
2/15/05	17.33	27.03	21.60	27.59	70.70	50.07	17.7
2/16/05	17.79	27.23	21.80	34.72	62.18	48.34	19.1
2/17/05	17.16	26.11	20.51	19.74	46.20	33.92	14.8
2/18/05	16.60	27.26	20.28	13.86	33.33	25.02	13.9
2/19/05	15.28	26.63	20.73	12.21	39.97	26.14	15.1
2/20/05	17.13	25.94	21.08	18.28	55.15	34.99	14.8
2/21/05	17.36	26.17	21.51	33.86	68.55	49.10	17.3
2/22/05	17.23	26.53	20.73	33.50	61.22	47.80	13.6
2/23/05	17.43	26.96	21.06	19.93	51.29	38.96	17.1
2/24/05	17.26	22.90	20.06	33.37	47.49	39.56	13.6
2/25/05	16.70	26.53	20.71	19.87	49.80	35.17	15.9
2/26/05	17.19	26.27	21.10	23.56	46.34	34.90	15.9
2/27/05	17.06	26.47	21.04	15.18	40.66	29.83	15.8
2/28/05	17.13	23.17	20.10	26.04	55.18	38.25	13.1
n= 22	Temperature °C			% Relative Humidity			moles Photosynthetically Active Radiation
	Min:	15.28		Min:	12.21		Min: 12.5
	Max:	27.89		Max:	75.10		Max: 20.3
	Mean:	20.98		Mean:	37.07		Mean: 15.9
	s.d.:	0.50		s.d.:	8.17		s.d.: 2.2

Watering Record:

07 Feb 05 – All species irrigated.
 11 Feb 05 – All species irrigated.
 15 Feb 05 – All species irrigated.
 18 Feb 05 – All species irrigated.
 23 Feb 05 – All species irrigated.
 25 Feb 05 – All species irrigated.
 26 Feb 05 – All species irrigated.
 27 Feb 05 – All species irrigated.

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Appendix 7

Test Results, Corn

Emergence

Day 7

Treatment Group	Number of Emerged Seedlings in Replicate:				<i>n</i>	Mean St	d. Dev.
	A	B	C	D			
Control	9	10	8	10	4	9.25	0.96
1000 mg/kg	9	10	8		4	9.00	0.82

Day 14

Treatment Group	Number of Emerged Seedlings in Replicate:				<i>n</i>	Mean St	d. Dev.
	A	B	C	D			
Control	9	10	9	10	4	9.50	0.58
1000 mg/kg	9	10	8		4	9.00	0.82

Day 21

Treatment Group	Number of Emerged Seedlings in Replicate:				<i>n</i>	Mean St	d. Dev.
	A	B	C	D			
Control	9	10	9	10	4	9.50	0.58
1000 mg/kg	9	10	8		4	9.00	0.82

Survival

Day 21

Treatment Group	Number of Surviving Seedlings in Replicate:	A	B	C	D	<i>n</i>	Mean St	d. Dev.
Control	9		10	9	10	4	9.50	0.58
1000 mg/kg	9		10	8	9	4	9.00	0.82

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Appendix 7 (Continued)

Test Results, Corn

Seedling Height on Day 21

Treatment Group	Replicate	Height (cm) for Plant Number ¹ :										n	Mean	Std. Dev.
		1	2	3	4	5	6	7	8	9	10			
Control	A	.	49	49	46	48	45	44	52	58	52	9	49.2	4.32
	B	55	58	56	59	57	56	56	58	41	52	10	54.8	5.22
	C	.	64	65	57	66	58	60	45	62	67	9	60.4	6.77
	D	47	44	40	50	44	50	53	48	53	50	10	47.9	4.20
1000 mg/kg	A	.	42	59	63	60	54	58	58	54	49	9	55.2	6.42
	B	54	67	49	56	40	50	53	45	54	52	10	52.0	7.12
	C	.	.	55	62	62	51	59	58	62	55	8	58.0	4.07
	D	.	56	61	60	60	62	56	56	56	53	9	57.8	3.03

¹ The “.” symbol indicates that the seedling either did not emerge or died prior to measurement.

Mean Seedling Height on Day 21

Treatment Group	Mean Height (cm) for Replicate:				n	Mean	Std. Dev.
	A	B	C	D			
Control	49.2	54.8	60	44.7	9	53.1	5.74
1000 mg/kg	55.2	52.0	58	50.5	7	55.8	2.80

Mean Seedling Dry Weight on Day 21

Treatment Group	Mean Weight (g) per Plant of Replicate:				n	Mean	Std. Dev.
	A	B	C	D			
Control	0.479	0.	592	0.	653	0.	532
1000 mg/kg	0.616	0.	571	0.	678	0.	670

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Appendix 7 (Continued)

Test Results, Corn

Seedling Condition on Day 21

Treatment Group	Replicate	Condition (score.sign) ¹ for Plant Number:										<i>n</i>	Mean	Std. Dev.
		1	2	3	4	5	6	7	8	9	10			
Control	A	ne	0.-	0.-	0.-	0.-	0.-	0.-	0.-	0.-	0.-	9	0	0.0
	B	0.-	0.-	0.-	0.-	0.-	0.-	0.-	0.-	0.-	0.-	10	0	0.0
	C	ne	0.-	0.-	0.-	0.-	0.-	0.-	0.-	0.-	0.-	9	0	0.0
	D	0.-	0.-	0.-	0.-	0.-	0.-	0.-	0.-	0.-	0.-	10	0	0.0
1000 mg/kg	A	ne	0.-	0.-	0.-	0.-	0.-	0.-	0.-	0.-	0.-	9	0	0.0
	B	0.-	0.-	0.-	0.-	0.-	0.-	0.-	0.-	0.-	0.-	10	0	0.0
	C	ne	ne	0.-	0.-	0.-	0.-	0.-	0.-	0.-	0.-	8	0	0.0
	D	ne	0.-	0.-	0.-	0.-	0.-	0.-	0.-	0.-	0.-	9	0	0.0

¹ The “ne” indicates that the seedling did not emerge. A score of 0 indicates a normal seedling, while a score of 100 indicates a dead seedling. Intermediate scores are assigned to indicate the relative severity of observed signs of toxicity.

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Appendix 8

Test Results, Radish

Emergence

Day 7

Treatment Group	Number of Emerged Seedlings in Replicate:				<i>n</i>	Mean St	d. Dev.
	A	B	C	D			
Control	10	10	10	9	4	9.75	0.50
1000 mg/kg	9	8	10	10	4	9.25	0.96

Day 14

Treatment Group	Number of Emerged Seedlings in Replicate:				<i>n</i>	Mean St	d. Dev.
	A	B	C	D			
Control	10	10	10	9	4	9.75	0.50
1000 mg/kg	9	8	10	10	4	9.25	0.96

Day 21

Treatment Group	Number of Emerged Seedlings in Replicate:				<i>n</i>	Mean St	d. Dev.
	A	B	C	D			
Control	10	10	10	9	4	9.75	0.50
1000 mg/kg	9	8	10	10	4	9.25	0.96

Survival

Day 21

Treatment		Number of Surviving Seedlings in Replicate:				<i>n</i>	Mean	St d. Dev.
Group	A	B	C	D				
Control	9	10	9	9	4	9.25	0.50	
1000 mg/kg	9	8	8	10	4	8.75	0.96	

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Appendix 8 (Continued)

Test Results, Radish

Seedling Height on Day 21

Treatment Group	Replicate	Height (cm) for Plant Number ¹ :										n	Mean	Std. Dev.
		1	2	3	4	5	6	7	8	9	10			
Control	A	.	15	14	14	15	14	12	14	13	11	9	13.6	1.33
	B	13	12	15	15	13	16	11	19	13	12	10	13.9	2.38
	C	.	12	13	13	13	19	11	12	18	12	9	13.7	2.83
	D	.	15	16	14	17	13	16	11	11	15	9	14.2	2.17
1000 mg/kg	A	.	13	13	15	14	14	13	13	15	10	9	13.3	1.50
	B	.	.	16	16	14	14	11	14	15	19	8	14.9	2.30
	C	.	.	14	12	16	15	17	15	16	13	8	14.8	1.67
	D	11	11	14	18	13	15	13	11	16	14	10	13.6	2.32

¹ The “.” symbol indicates that the seedling either did not emerge or died prior to measurement.

Mean Seedling Height on Day 21

Treatment Group	Mean Height (cm) for Replicate:				n	Mean	Std. Dev.
	A	B	C	D			
Control	13.6	13.9	13	14	4	13.8	0.29
1000 mg/kg	13.3	14.9	14	13	4	14.1	0.79

Mean Seedling Dry Weight on Day 21

Treatment Group	Mean Weight (g) per Plant of Replicate:				n	Mean	Std. Dev.
	A	B	C	D			
Control	0.21	0.19	0.23	0.25	4	0.231	0.0216
1000 mg/kg	0.22	0.26	0.21	0.22	4	0.231	0.0218

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Appendix 8 (Continued)

Test Results, Radish

Seedling Condition on Day 21

Treatment Group	Replicate	Condition (score.sign) ¹ for Plant Number:										<i>n</i>	Mean	Std. Dev.
		1	2	3	4	5	6	7	8	9	10			
Control	A	100.-	0.-	0.-	0.-	0.-	0.-	0.-	0.-	0.-	0.-	10	10	31.6
	B	0.-	0.-	0.-	0.-	0.-	0.-	0.-	0.-	0.-	0.-	10	0	0.0
	C	100.-	0.-	0.-	0.-	0.-	0.-	0.-	0.-	0.-	0.-	10	10	31.6
	D	ne	0.-	0.-	0.-	0.-	0.-	0.-	0.-	0.-	0.-	9	0	0.0
1000 mg/kg	A	ne	0.-	0.-	0.-	0.-	0.-	0.-	0.-	0.-	0.-	9	0	0.0
	B	ne	ne	0.-	0.-	0.-	0.-	0.-	0.-	0.-	0.-	8	0	0.0
	C	100.-	100.-	0.-	0.-	0.-	0.-	0.-	0.-	0.-	0.-	10	20	42.2
	D	0.-	0.-	0.-	0.-	0.-	0.-	0.-	0.-	0.-	0.-	10	0	0.0

¹ The “ne” indicates that the seedling did not emerge. A score of 0 indicates a normal seedling, while a score of 100 indicates a dead seedling. Intermediate scores are assigned to indicate the relative severity of observed signs of toxicity.

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Appendix 9

Test Results, Soybean

Emergence

Day 7

Treatment Group	Number of Emerged Seedlings in Replicate:				<i>n</i>	Mean St	d. Dev.
	A	B	C	D			
Control	9	10	9	10	4	9.50	0.58
1000 mg/kg	10	9	8	10	4	9.25	0.96

Day 14

Treatment Group	Number of Emerged Seedlings in Replicate:				<i>n</i>	Mean St	d. Dev.
	A	B	C	D			
Control	9	10	9	10	4	9.50	0.58
1000 mg/kg	10	9	10	10	4	9.75	0.50

Day 21

Treatment Group	Number of Emerged Seedlings in Replicate:				<i>n</i>	Mean St	d. Dev.
	A	B	C	D			
Control	9	10	9	10	4	9.50	0.58
1000 mg/kg	10	9	10	10	4	9.75	0.50

Survival

Day 21

Treatment Group	Number of Surviving Seedlings in Replicate:				<i>n</i>	Mean St	d. Dev.
	A	B	C	D			
Control	9	10	9	10	4	9.50	0.58
1000 mg/kg	10	9	10	10	4	9.75	0.50

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Appendix 9 (Continued)

Test Results, Soybean

Seedling Height on Day 21

Treatment Group	Replicate	Height (cm) for Plant Number ¹ :										n	Mean	Std. Dev.
		1	2	3	4	5	6	7	8	9	10			
Control	A	.	15	16	15	18	16	20	14	16	8	9	15.3	3.28
	B	16	20	19	17	14	16	19	17	18	18	10	17.4	1.78
	C	.	21	18	21	18	19	22	20	17	17	9	19.2	1.86
	D	9	15	17	17	17	18	16	15	18	15	10	15.7	2.63
1000 mg/kg	A	15	19	19	13	16	17	18	18	13	18	10	16.6	2.27
	B	.	18	21	22	21	18	21	20	19	18	9	19.8	1.56
	C	19	20	19	17	15	4	18	19	19	16	10	16.6	4.70
	D	13	18	20	19	16	19	19	23	20	19	10	18.6	2.63

¹ The “.” symbol indicates that the seedling either did not emerge or died prior to measurement.

Mean Seedling Height on Day 21

Treatment Group	Mean Height (cm) for Replicate:				n	Mean	Std. Dev.
	A	B	C	D			
Control	15.3	17.4	19.2	15.7	4	16.9	1.78
1000 mg/kg	16.6	19.8	16.6	18.6	4	17.9	1.57

Mean Seedling Dry Weight on Day 21

Treatment Group	Mean Weight (g) per Plant of Replicate:				n	Mean	Std. Dev.
	A	B	C	D			
Control	0.348	0.364	0.442	0.353	4	0.377	0.0442
1000 mg/kg	0.366	0.416	0.345	0.405	4	0.383	0.0330

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Appendix 9 (Continued)

Test Results, Soybean

Seedling Condition on Day 21

Treatment Group	Replicate	Condition (score.sign) ¹ for Plant Number:								n	Mean	Std. Dev.	
		1	2	3	4	5	6	7	8				9
Control	A	ne	0.-	0.-	0.-	0.-	0.-	0.-	0.-	0.-	9	0	0.0
	B	0.-	0.-	0.-	0.-	0.-	0.-	0.-	0.-	0.-	10	0	0.0
	C	ne	0.-	0.-	0.-	0.-	0.-	0.-	0.-	0.-	9	0	0.0
	D	10.LC	0.-	0.-	0.-	0.-	0.-	0.-	0.-	0.-	10	1	3.2
1000 mg/kg	A	0.-	0.-	0.-	0.-	0.-	0.-	0.-	0.-	0.-	10	0	0.0
	B	ne	0.-	0.-	0.-	0.-	0.-	0.-	0.-	0.-	9	0	0.0
	C	0.-	0.-	0.-	0.-	0.-	0.-	0.-	0.-	0.-	10	0	0.0
	D	0.-	0.-	0.-	0.-	0.-	0.-	0.-	0.-	0.-	10	0	0.0

¹ The “ne” indicates that the seedling did not emerge. A score of 0 indicates a normal seedling, while a score of 100 indicates a dead seedling. I are assigned to indicate the relative severity of observed signs of toxicity.

LC – Leaf Curl

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Appendix 10

Personnel Involved in the Study

The following key Wildlife International, Ltd. personnel were involved in the conduct or management of this study:

1. [REDACTED]
2. [REDACTED]
3. [REDACTED]
4. [REDACTED]

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Appendix 11

Report Amendment

- | | | |
|----|--------------------------------------|--|
| 1. | Original Report:
Amended Report: | Title Page
The amended report date was added. The total number of pages was changed from 64 to 77. |
| | Reason: | To indicate that the report was amended and note change in pagination. |
| 2. | Original Report:
Amended Report: | Page 2
The amended report date was added and new signatures and dates were added. |
| | Reason: | To show the amended report date and to provide new signatures and dates for the amended report. |
| 3. | Original Report:
Amended Report: | Page 3
The audit dates for the amended report were added and a new signature and date were added. |
| | Reason: | To show the amended report audit dates and to provide a new signature and date for the amended report. |
| 4. | Original Report:
Amended Report: | Page 4
New signatures and dates were added. |
| | Reason: | To provide new signatures and dates for the amended report. |
| 5. | Original Report:
Amended Report : | Page 6
The Table of Contents was updated to show the addition of Appendix 3, to renumber all appendices from Appendix 3 through the end of the report and to add the Report Amendment appendix (Appendix 11). |
| | Reason: | The Sponsor requested that Appendix 3 be added to the final report. |
| 6. | Original Report:
Amended Report : | Page 9
Appendix 3 was referenced in the Test Substance Section of the report and the appendix references were renumbered due to addition of Appendix 3. |
| | Reason: | The Sponsor requested that Appendix 3 be added to the final report. |
| 7. | Original Report:
Amended Report : | Pages 11 and 13
The appendix references were renumbered due to addition of Appendix 3. |
| | Reason: | The Sponsor requested that Appendix 3 be added to the final report. |

AMENDED

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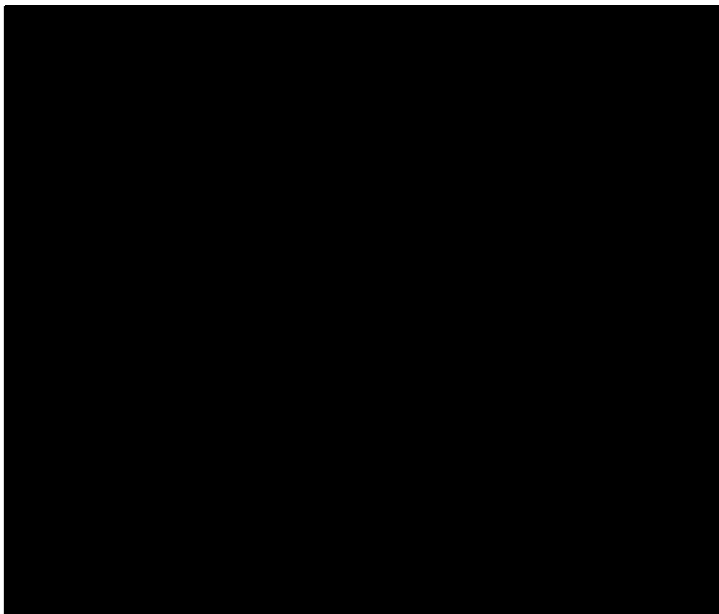
Appendix 11
(continued)

Report Amendment

- | | | |
|----|--|---|
| 8. | Original Report:
Amended Report:
Reason: | Page 39
Appendix 3 was added to the report.
The Sponsor requested that Appendix 3 be added to the final report. |
| 9. | Original Report:
Amended Report:

Reason: | Pages 39-64
Appendix 3 was added to the report, therefore all pages thereafter were renumbered.
The Sponsor requested that Appendix 3 be added to the final report. |

AMENDMENT SIGNATURES:



10 Apr 2007
Date

10 April 07
Date

4-10-07
Date

AMENDED