DU PONT CENTRAL RESEARCH AND DEVELOPMENT
HASKELL LABORATORY FOR TOXICOLOGY AND INDUSTRIAL MEDICINE

TO: TIMOTHY BINGMAN 571-7761 DATE: MAY 17, 1993
~~DU PONT AGRICULTURAL PRODUCTS~~ Chem
~~E402-3220~~ REO-PIT

PROJECT HR 9660 PART I

TITLE: ABSOLUTE BIOAVAILABILITY OF SOIL LEAD IN RATS AND MICROSWINE

Authority is requested to undertake the study identified above and described as follows:

To conduct a two part study in rats and microswine to determine the absolute bioavailability of soil lead. Details are in the study protocol, provided under separate cover.

This study will be billed in 9 equal installments beginning June, 1993.

. . . . . .

		Forecast month and year	
Amount requested	$ 104,700	Experiment to start:	May, 1993
Previously authorized	$	Experiment to end:	August, 1993
Expended through / /	$	Final report to issue:	February, 1993
General Ledger & Subaccount to be Charged	7035-500183-860100	Request for study made by:	T. Bingman

TESTING X RESEARCH ___ CONSULTING ___
(Check appropriate classification)

. . . . . .

If this request meets with your approval, please enter the GENERAL LEDGER AND SUBACCOUNT TO BE CHARGED, the CLASSIFICATION, and have it PROPERLY AUTHORIZED.

Proposed by: H. Ghantous 5/17/93
Hanan N. Ghantous

Cost Code	Total (%)
19	41.1
23	30.2
24	
25	1.0
30	27.8
31	
32	

Approved by: Matthew S. Bogdanffy 5/18/93
Matthew S. Bogdanffy

Authorized: [signature] 6/29/93
(date)

PLEASE RETURN AUTHORIZED COPY DIRECTLY TO: LAURA WALLS, FINANCIAL SERVICES, HASKELL LABORATORY

___ This is a TSCA 8(d) chemical.
(check if appropriate)

HG/amb

DuPont HLR 671-93

SOIL LEAD
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lustrial Medicine

60-001

DUPONT Date _____

TO:

FROM: HANAN N. GHANTOUS
CR&D
Haskell Laboratory
(302) 451-0834

For Attention	For Information	Forwarded per your Request

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DUP040000520

N 25663.01

Study Title

ABSOLUTE BIOAVAILABILITY OF SOIL LEAD IN RATS AND MICROSWINE

Laboratory Project ID

Haskell Laboratory Report No. 671-93

Author

Hanan N. Ghantous, Ph.D.

Study Completed on

December 9, 1993

Performing Laboratory

E. I. du Pont de Nemours and Company
Haskell Laboratory for Toxicology and Industrial Medicine
Elkton Road, P. O. Box 50
Newark, Delaware 19714

Medical Research Project No. 9660-001

GOOD LABORATORY PRACTICE COMPLIANCE STATEMENT

This study was conducted in compliance with U. S. EPA TSCA Good Laboratory Practice Standards (40 CFR 792) except for the deviations documented and explained in Appendix B. The deviations did not affect the integrity of the study.

Submitter: E. I. du Pont de Nemours and Company

Sponsor: Corporate Remediation Group of
DuPont Chemicals
E. I. du Pont de Nemours and Company

Date

Study Director: H. Ghantous 12/9/93
Hanan N. Ghantous, Ph.D.
Research Toxicologist
Biochemical Toxicology and
Risk Analysis

Company Representative: _____ _____

GENERAL INFORMATION

Substance Tested (Intravenous Administration):	Acetic acid, lead(2+) salt, trihydrate
Synonyms/Codes:	H-20202 Aldrich Cat. No. 31,651-2 Lot #KY08221CY Lead acetate trihydrate Lead(II) acetate trihydrate Lead acetate Acetic acid, lead(2+) salt
Haskell Sample No.:	20202
Purity:	99.999%
C.A.S. Registry No.:	6080-56-4
Substance Tested (Oral Administration):	Soil, from former DuPont Works, Washington State, contaminated with lead
Synonyms/Codes:	H-20204 Soil contaminated with approximately 1,000 ppm lead from former DuPont Works in Washington State Soil contaminated with approximately 1,000 ppm lead from Washington State Soil with lead
Haskell Sample No.:	20204
Purity:	N/A
C.A.S. Registry No.:	None Available
Sponsor:	Corporate Remediation Group of DuPont Chemicals E. I. du Pont de Nemours and Company Bellevue Corporate Center Wilmington, Delaware 19809
Sponsor Contact:	Timothy S. Bingman, D.A.B.T. (412) 257-7761

GENERAL INFORMATION (Continued)

Study Initiated/Completed: 05-17-93 / 12-09-93

Experiments Initiated/Completed:

Rats

5-18-93/5-28-93 (Acute I.V.)
6-08-93/6-18-93 (Acute Oral)
5-18-93/6-28-93 (Subchronic Feed & I.V.)
6-08-93/7-19-93 (Subchronic Feed & Oral)

Microswine

8-10-93/8-20-93 (Acute I.V.)
8-06-93/8-16-93 (Acute Oral)
6-12-93/7-23-93 (Subchronic Feed & I.V.)
6-15-93/7-26-93 (Subchronic Feed & Oral)

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SUMMARY

Objectives of this study were:

- to determine the absolute bioavailability of lead in test soil,
- to determine if subchronic exposure to lead-contaminated soil influences lead bioavailability, and
- to compare the bioavailability in two model species (rat and microswine).

The bioavailabilities of both a *single dose (acute)* and a *single dose after feeding for 30 consecutive days (subchronic)* were determined.

For single-dose (acute) administration, 5 male juvenile microswine and 25 male juvenile rats were each gavaged with test soil (17 g soil/microswine, 1 g soil/rat). Six additional microswine and 25 additional rats were each given a single intravenous injection of lead acetate solution containing a lead dose equivalent to that of the gavage dose.

For the subchronic part of the study, 12 male microswine and 50 male rats were provided with optimized diets of approximately 340 g/microswine/day or 21.5 g/rat/day of a special purified diet mixed with test soil (5% w/w) for 30 days. Microswine and rats were then each divided into two groups of equal size and given either a single intravenous or oral (gavage) dose.

Blood was drawn from both groups of microswine and rats at 0.5, 1, 2, 4, 8, 12, 24, 48, 72, 96, 120, 144, 192, and 240 hours after dosing. The blood samples were analyzed for lead content, and the absolute bioavailability was determined as the ratio of the area under the blood-lead versus time curve (AUC) for the oral /intravenous groups.

Findings from this study were:

- Differences between AUCs of the gavage group and the intravenous group were statistically significant in the acute rat and microswine groups.
- Differences between AUCs of the gavage and intravenous groups were significant in the subchronic rat groups but not the subchronic microswine groups. However, assessment of bioavailability after subchronic exposure appeared to be inaccurate due to the apparent confounding effect of bioaccumulation of lead in blood.
- Single-dose rats absorbed only 4.14% of the lead dose while the single-dose microswine absorbed 20.90%.

- Absolute bioavailability results were:

acute rats	4.14%
subchronic rats	27.83%
acute microswine	20.90%
subchronic microswine	47.13%

In conclusion, based upon (1) the fact that microswine appear to provide a more conservative estimation of bioavailability than rats, and (2) the difficulty in interpreting subchronic data due to bioaccumulative effect, acute administration to microswine appears to be the most appropriate model for lead bioavailability in children.

SIGNATURE PAGE

Authored by: H. Ghantous

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Research Toxicologist
Biochemical Toxicology and
Risk Analysis

Approved by: Matthew S. Bogdanffy by CFR

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Reviewed and
Approved for Issue
by Study Director: H. Ghantous 12/9/93

Hanan N. Ghantous, Ph.D.
Research Toxicologist
Biochemical Toxicology and
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Date Report Issued: December 9, 1993

QUALITY ASSURANCE DOCUMENTATION

Date(s) of Inspection:

Conduct - 6/14,17/93, 7/6,7,13,16,19,23/93, 8/9/93

Records, Report(s) - 5/25/93, 7/7-9/93, 8/9,10/93, 11/19,22,23,27-30/93, 12/1-4,6-8/93

Date(s) Findings Reported to:

Study Director - 7/9/93, 9/1/93, 12/4,8,9/93

Management - 7/19/93, 9/1/93, 12/9/93

Reported by: Robert C. Rhea 12/9/93

Robert C. Rhea, B.S. (date)
Auditor
Quality Assurance

STUDY PERSONNEL

The following individuals participated in the conduct of the study:

Study Director:	Hanan N. Ghantous, Ph.D.
Manager:	Matthew S. Bogdanffy, Ph.D.
Primary Technician:	Christine M. Loeffler
Assisting Technicians:	Michele Aschiero, A.A.S. Walter C. Moll Benjamin Robinson, B.A.
Report Preparation:	John D. Oldham, Jr., B.S.

The following individuals were responsible for the gross pathology examinations and tissue collection from the microswine:

Manager:	Nancy C. Chromey, Ph.D.
Pathologist:	Paul E. Ross, D.V.M.
Coordinator Pathology Operations:	William J. Lynam, A.S.
Pathology Steward:	Merralyn K. Vaillancourt, B.S.

The following individuals were responsible for lead analysis (Jackson Lab):

Coordinator:	Keith A. McCleary, Ph.D.
Technicians:	Billy R. Cooper Ralph Postorivo Michael Sisitka

STUDY PERSONNEL (Continued)

The following individuals were responsible for quality assurance:

Coordinator: Joseph C. Hamill

Auditor: Robert C. Rhea, B.S.

The health status of the animals on study was assessed by the attending Laboratory Veterinarian, Charles E. Cover, V.M.D., with the assistance of James G. Aftosmis, V.M.D. and Carolyn S. VanPelt, D.V.M., Ph.D.

Assistance with statistical analysis was provided by John W. Green, Ph.D., Ph.D.

INTRODUCTION

Recently, DuPont conducted a study aimed at developing a suitable animal model for determining site-specific bioavailability of lead-contaminated soil.

The initial phase of the study, performed previously, was designed to establish the proper dose levels to be used in subsequent phases. Results from this previous phase indicated that subsequent testing should be performed using diet formulations containing 5% of lead-contaminated soils with lead concentrations ranging between 375 and 1500 mg/kg.[1]

A second phase of the study, which is the subject of this report, was designed to establish the absolute bioavailability of lead from the site's soil in both rats and microswine using either single dose or subchronic dosing followed by single-dose exposures.

Rats and microswine were used in order to choose the correct animal model in the conduct of experimental investigation for the purpose of understanding relevance to humans. Weis and LaVelle[2] summarized the considerations to be addressed when choosing an animal model to determine the bioavailability of lead.

OBJECTIVE

The objective of the study was to determine the absolute oral bioavailability of lead from soil in rats and microswine following a single oral (gavage) dose. Absolute oral bioavailability is also to be determined in these species following a subchronic exposure to lead-contaminated soils that are mixed with feed. These results will assist development of a site-specific clean-up goal by providing soil-specific data on gastrointestinal absorption.

MATERIALS AND METHODS

The original protocol and protocol amendments are in Appendix A.

A. Test Substance

Lead acetate trihydrate was supplied by Aldrich, Milwaukee, Wisconsin, and assigned Haskell sample number 20202. The lead-contaminated soil (lead/soil) was supplied by the sponsor and assigned Haskell sample number 20204.

Characterization and mineralogy data of the lead/soil are maintained by the sponsor.

B. Test Species

Ninety juvenile male Crl:CD®BR rats, born approximately 4/19/93, were received from Charles River Laboratories, Inc., Kingston, New York, on 5/11/93. On 5/12/93, these male rats weighed between 33.3 and 65.4 g. Sixty additional juvenile male Crl:CD®BR rats, born approximately 5/10/93, were received from Charles River Laboratories, Inc., Kingston, New York, on 6/01/93. On 6/02/93, these additional male rats weighed between 34.6 and 58.2 g. The Crl:CD®BR rat was selected on the bases of extensive experience with this strain and its suitability with respect to hardiness, longevity, sensitivity, and low incidence of spontaneous disease.

Twelve juvenile male Yucatan microswine, born approximately 4/24/93, were received from Charles River Laboratories, Inc., Windham, Maine, on 6/03/93. On 6/04/93, these male microswine weighed between 3795 and 7008 g. Twelve additional juvenile male Yucatan microswine, born approximately 6/22/93, were received from Charles River Laboratories, Windham, Maine, on 7/29/93. On 7/30/93, these additional male microswine weighed between 3843 and 7688 g. The male Yucatan microswine was selected on the bases of its low body weight, its reported suitability as a model of soil-lead ingestion by children, and its suitability with respect to longevity, hardiness, sensitivity, and low incidence of spontaneous disease.

C. Animal Husbandry

During the pretest period, rats and microswine were housed according to the *Guide for Care and Use of Laboratory Animals*.[3] After assignment to groups, rats and microswine were housed in stainless steel cages. Rat cages were suspended above cage boards; microswine cages were bedded with recyclable, biodegradable shredded paper (Enviro Dri). To improve housing conditions, microswine were provided with recreational equipment (balls) in their cages and occasionally rewarded with Oreo® cookies.

Animal rooms were targeted at a temperature of 23±2°C for rats and 25±2°C for microswine and a relative humidity of 50±10%. On two occasions, the relative humidity of rooms housing rats was beyond 60%; however, these deviations were brief and did not affect the study. Animal rooms that housed rats were artificially illuminated (fluorescent light) on a 12-hour light/dark cycle. Animal rooms that housed microswine were artificially illuminated (fluorescent light) on

a 12-hour light/dark cycle with additional natural light entering through a window at one end of the room.

Haskell Laboratory has an animal health monitoring program which consists of periodic food analysis for contaminants and sampling freshly washed cages and cage racks for bacteria. The program is monitored and administered by the laboratory veterinarian; data are maintained separately from study records. All parameters were found to be within acceptable ranges.

Dosing solutions, prepared diets, components of diets, deionized water, Oreo® cookies, and microswine bedding were analyzed for lead content by DuPont Chemicals at DuPont Chambers Works, Deepwater, New Jersey.

D. Pretest Period

Rats and microswine were quarantined for at least six days. During the pretest period, rats were fed an optimized diet (once per day) of Purina® Certified Rodent Chow® #5002 meal (PCRC) and transferred gradually to Teklad basal mix (95%) for rats (TD 92242) combined with sucrose (5%; sucrose obtained from Acme Markets, Inc.). Microswine were fed an optimized diet (once per day) of microswine diet (Charles River) and transferred gradually to Teklad basal mix (95%) for microswine (TD 93034) combined with sucrose (5%). For rats and microswine, transfer to Teklad basal mix and sucrose was completed by the fifth day of quarantine. Rats and microswine were provided deionized water *ad libitum*.

Rats and microswine were weighed three times during the quarantine period and observed daily with respect to eating habits, weight gain, and any signs of disease or injury. One microswine (Pretest ID #6) was sacrificed *in extremis* because of loss of appetite and, consequently, severe weight loss; no signs of infectious disease were found at necropsy.

E. Assignment to Groups and Study Start

After the quarantine period, rats and microswine were grouped as follows:

- 50 of the 90 rats received on 5/11/93 were selected for study use on the bases of adequate body weight gain and freedom from any clinical signs of disease or injury. The selected rats were divided by computerized, stratified randomization into two groups of 25 rats each. These groups were designated as Groups I and III. The remaining 40 rats were removed from the room and used on other studies.

- 50 of the 60 rats received on 6/01/93 were divided by computerized, stratified randomization into two groups of 25 rats each. These groups were designated as Groups II and IV. In addition, due to a technical error, 5 of the remaining 10 rats were randomly selected to be bled for the 12-hour timepoint for Group II. The remaining 5 rats were removed from the room and used on other studies.

- all of the microswine received on 6/03/93 were divided by computerized, stratified randomization into two groups of six microswine each. These groups were designated as Groups V and VII.

- the remaining 11 (of 12) microswine received on 7/29/93 were divided by computerized, stratified randomization into one group of six microswine (designated as Group VI) and one group of five microswine (designated as Group VIII). In addition, one microswine from each group was fed one hour before dosing; data from these animals are presented separately (at the end of Section C.2).

Groups of rats and microswine were exposed to lead in the following manner:

Group Number	Species	Experiment Start Date	Approximate Age at Start of Experiment (days)	Days Fed Lead in Diet Before Dose	Dosing Method	Composition of Dosing Material
I	Rat	5/18/93	29	0 (Acute)	I.V.	Lead Acetate
II	Rat	6/08/93	29	0 (Acute)	Oral	Lead in Soil
III	Rat	5/18/93	29	30 (Subchronic)	I.V.	Lead Acetate
IV	Rat	6/08/93	29	30 (Subchronic)	Oral	Lead in Soil
V	Microswine	6/12/93	49	30 (Subchronic)	I.V.	Lead Acetate
VI	Microswine	8/10/93	49	0 (Acute)	I.V.	Lead Acetate
VII	Microswine	6/15/93	52	30 (Subchronic)	Oral	Lead in Soil
VIII	Microswine	8/06/93	45	0 (Acute)	Oral	Lead in Soil

F. Diet Preparation, Administration, and Sampling

Subchronic rats and microswine, designated to be fed at least 30 days prior to dose administration, received diets containing test soil contaminated with approximately 1,000 ppm lead. Diets were prepared by combining Teklad basal mix for the particular species with the lead-contaminated soil. The basal mix was designed for use at the 95% level, in conjunction with 5% of the test substance (or sucrose). These ingredients were mixed in a high-speed mixer for three minutes.

On the day of dose administration and until sacrifice, rats and microswine received diets containing sucrose. Diets were prepared by combining Teklad

basal mix (95%) for the particular species with sucrose (5%). These ingredients were mixed in a high-speed mixer for three minutes.

All diets were refrigerated until used or discarded.

During the study, rats were provided approximately 21.5 g/day of test diet and microswine were fed approximately 340 g/day of diet. Rats and microswine were provided deionized water *ad libitum*.

Prior to the start of any of the experiments (5/17/93), samples were collected from the top, middle, and bottom of the mixing bowl containing diet prepared with lead/soil. These samples were analyzed to measure concentration and homogeneity.

Due to a technical error, samples from feed jars were not collected and sent for analysis of lead concentration. Because the samples collected from the mixing bowl were found to be at the proper concentration and homogeneously mixed (see Results and Discussion) and because the lead in the samples used in this study was not expected to degrade, this did not affect the integrity of the study.

G. Dose Preparation, Administration, and Sampling

Oral Dose. Rats designated for oral (gavage) administration (acute or subchronic) received a 2.0 ml suspension of test soil (containing approximately 1,000 ppm lead) and deionized water. Each rat received approximately 1 g test soil within the 2.0 ml dose. Microswine designated for oral (gavage) administration (acute or subchronic) received a 30.0 ml suspension of test soil (containing approximately 1,000 ppm lead) and deionized water. Each microswine received approximately 17 g test soil within the 30.0 ml dose. For rats and microswine, the suspension was constantly stirred until all animals were dosed.

Oral dosing suspensions were analyzed for lead concentration and the measured values were used for calculating lead bioavailability (Table 1). Due to an error, oral dosing suspensions from the rat subchronic groups were not sent for analysis.

Intravenous Dose. Rats and microswine designated for intravenous administration (acute or subchronic) received a 0.1 ml solution of lead acetate and sterile water. The solution was prepared to contain a lead dose equivalent to that contained in the oral (gavage) dose for each species. Rats were dosed intravenously via the tail vein; microswine were dosed intravenously via the ear vein.

Intravenous dosing solutions were analyzed for lead concentration and the measured values were used for calculating lead bioavailability (Table 1). Due to an error, intravenous dosing suspensions from the rat subchronic groups were not sent for analysis.

H. Blood Sampling

Approximately 0.1-0.2 ml of rat venous blood and approximately 0.2-0.5 ml of microswine venous blood were drawn from designated animals at 0.5, 1, 2, 4, 8, 12, 24, 48, 72, 96, 120, 144, 192, and 240 hours after dosing. At each timepoint, all microswine were bled; however, only four or five rats were bled at each timepoint. The groups of 25 rats were randomly divided into five subgroups of five rats each. Each subgroup was bled at two or three non-consecutive timepoints. Because of a death and a tail injury during the first day of bleeding, two subgroups within Group III had only four rats (see *Body Weights, Clinical Observations, and Mortality* section).

Due to an error, no blood was collected from rats at 12 hours after the oral (gavage) dose. In order to analyze blood at this timepoint, five rats were randomly selected from those not originally designated for use on the study. These rats were bled, dosed orally, and bled again 12 hours later.

All blood samples were stored at 4°C and sent to DuPont Chambers Works for analysis.

I. Body Weights, Clinical Observations, and Mortality

All rats and microswine were weighed and carefully examined for clinical signs twice each week.

During the bleeding procedure for Group III (subchronic intravenous group), one rat was accidentally killed and a second rat, which sustained a tail injury, was removed from the study.

J. Food Consumption and Intake of Test Substance

The amount of food consumed by each rat or microswine fed for 30 consecutive days was determined weekly. From these values and body weigh data, intake of the test substance was determined.

K. Sacrifice of Animals

At the end of the experiment, rats bled at 240 hours after dose administration were humanely euthanatized by carbon dioxide asphyxiation, then dissected. The liver and femurs from rats that were dosed at study start were collected, weighed, and frozen. The liver, kidneys, femurs, spleen, and brain from the subchronic rats were also collected, weighed, and frozen. Tissues, other than blood, collected at the time of sacrifice were stored for potential future analyses. Analyses of these tissues is not part of this project.

At the end of the experiment, all surviving microswine were weighed, humanely euthanatized by intravenous injection of Euthanasia-V solution, then given a gross pathological examination. The livers and femurs from microswine that were dosed at study start were collected, weighed, and frozen. The liver, kidneys , femurs, spleen, and brain from the subchronic microswine were collected, weighed, and frozen. Tissues, other than blood, collected at the time of sacrifice were stored for potential future analyses. Analyses of these tissues is not part of this project.

L. Data Analysis

Blood lead concentration was analyzed by graphite furnace atomic absorption spectroscopy (GFAA) at DuPont Chambers Works, Deepwater, New Jersey. These concentrations were used to calculate AUC (area under blood concentration versus time curve) and the absolute oral bioavailability of lead.

Mean body weights, body weight gains, and food consumption were analyzed statistically using the One-Way Analysis of Variance and Dunnett's tests. Blood lead concentrations from rats were analyzed statistically using nonlinear regression analysis. Blood lead concentrations versus time curves (AUCs) from microswine were analyzed statistically using the Student t-test. Statistical significance was judged at alpha = 0.05.

RECORDS AND SAMPLE RETENTION

All original records are retained at Haskell Laboratory, Jackson Laboratory (DuPont Chambers Works), or at the Records Management Center, E. I. du Pont de Nemours and Company, Wilmington, Delaware.

RESULTS AND DISCUSSION

A. Diet Samples

Samples of diet containing lead-contaminated soil were collected at the time the diet was prepared to verify concentration and homogeneity. Homogeneity in the diet was evaluated by calculating the coefficient of variation (C.V. = standard deviation/mean x 100) of the average concentrations measured in samples collected from the top, middle, and bottom of the mixing vessel.

For the rat diet, the mean ± standard deviation of the homogeneity values expressed as the percent of nominal were: 84.66 ± 6.43, 90±5.29, and 74±2, for the top, middle, and bottom samples, respectively. The C.V. was 9.8% (Table 2).

For the microswine diet, the mean ± standard deviation of the homogeneity values expressed as the percent of nominal were: 89.46±3.26, 87.4±3.55, and 94.2±6.88, for top, middle, and bottom samples, respectively. The C.V. was 3.85% (Table 3).

The measured concentration of lead in the bottom samples of the rat diet were lower than expected. However, since the C.V. was less than 10%, the diet mixture was considered homogeneous. (Historical data at Haskell Laboratory indicates that homogeneous diets yield a C.V. of 10% or less.) There were no apparent differences between the top, middle, or bottom samples in the measured concentrations of lead in the microswine diet nor between the top and middle samples of the rat diet, demonstrating that the mixing conditions resulted in a homogeneous distribution of lead in the diets.

Stability testing of the soils were not performed. U.S. EPA Publication SW-846, *Methods for the Analysis of Solid Waste*, specifies a holding time for solid material samples of six months with proper refrigeration for samples being analyzed for lead. Since the lead in the samples used in this study was not expected to degrade during the study period, stability testing was not necessary.

Samples of Teklad basal mix (rat and microswine), sucrose mixed diets, sucrose, Oreo® cookies, swine bedding, deionized water, and lead-contaminated soil were also analyzed. Results are provided in Table 4. Results of these analyses demonstrated that the nominal 1,000 ppm soil was actually 840.9 ppm and no lead was present in the other samples.

B. Dosing Solutions

Dosing solutions were analyzed and the measured lead concentrations were used to calculate the bioavailability of lead. Results are provided in Table 1.

C. Blood Lead Concentrations, Area Under the Curve, and Absolute Bioavailability

Mean blood lead concentrations are listed in Tables 5-8. Individual animal blood lead concentrations before dosing, after dosing, and during the 30-day feeding phase are listed in Appendices C-E.

Area under the blood lead concentration versus time curve (AUC) for lead was determined by trapezoidal rule using the terminal elimination rate constant (β) to extrapolate from the last blood concentration value (Figures 1-4).

Absolute bioavailability was determined as the ratio of area under the blood-lead concentration versus time curve for the oral/intravenous groups in comparison to the dosage administered (Table 9).

$$\% \text{ Absolute Bioavailability} = \frac{AUC_{oral} \times Dose_{intravenous}}{AUC_{intravenous} \times Dose_{oral}} \times 100$$

1. Single Dose Administration (Acute)

Rats. Area under the mean blood concentration-time curve was determined for rats. Individual AUCs were not determined since only four samples (or less) were drawn from each rat due to the size of the animals used for this study (juveniles). Instead, AUC was evaluated using the group mean data for each timepoint.

The mean AUC for the intravenous dose (Group I) was 534.32 µg(hr)/ml and the mean AUC for the oral (gavage) dose (Group II) was 10.81 µg(hr)/ml. The percent absolute oral bioavailability was 4.14%. The difference between the AUCs of the intravenous and the oral (gavage) doses was significant (Figure 1).

Microswine. Individual animal AUCs were determined for the microswine and are reported in Table 10. The mean AUC for the intravenous dose (Group VI) was 54.09 µg(hr)/ml and the mean AUC for the oral (gavage) dose (Group VIII) was 14.98 µg(hr)/ml . The percent absolute oral bioavailability was 20.90%. The difference between AUCs of the oral (gavage) and intravenous doses was statistically significant (Figure 2).

In both species, the observed C_{max} of the intravenous dose was at the first timepoint (0.5 hr). The C_{max} of the oral (gavage) dose was at 8.0 hrs for rats and at 12.0 hrs for microswine.

2. Single Dose Administration (Subchronic)

After 30 consecutive days of feeding, predosing blood lead levels were higher than predosing levels in the single administration groups of rats and microswine (Appendix C).

Rats. Similar to the single dose administration group, due to the small number of samples drawn from each animal, the individual areas under the blood concentration-time curve were not determined. Instead, AUCs were determined using the group mean for each timepoint. The mean AUC for the intravenous dose (Group III) was 457.94 µg(hr)/ml and the mean AUC for the oral (gavage) dose (Group IV) was 62.22 µg(hr)/ml. The percent absolute oral bioavailability was 27.83%. The difference between the AUC of the oral (gavage) and the intravenous dose was significant (Figure 3).

Microswine. Individual animal AUCs were determined for the microswine and are reported in Table 11. The mean AUC for the intravenous dose (Group V) was 96.95 µg(hr)/ml and the mean AUC for the oral (gavage) dose (Group VII) was 70.02 µg(hr)/ml. The percent absolute oral bioavailability was 47.13%. However, there were no significant differences between AUCs of the oral (gavage) and intravenous doses (Figure 4).

In both species, the observed C_{max} of the intravenous dose was at the first timepoint (0.5 hr). The C_{max} of the oral (gavage) dose was at 8.0 hrs for rats and at 4.0 hrs for microswine.

The apparent absolute oral bioavailability was greater following subchronic exposure compared to acute exposure. However, this finding appears to be an artifact resulting from the remobilization of lead which had bioaccumulated in tissues during subchronic exposure. Therefore, the oral bioavailability determined after subchronic exposure appears to be inaccurate.

Rats and microswine were provided with diets of approximately 21.5 g/rat/day or 340 g/microswine/day at approximately 11:00 a.m. The microswine consumed their diet within one hour while the rats did not consume all their diet until the next day. Therefore, on the day of study start, when the oral (gavage) or intravenous doses were administered (approximately 6:00 a.m.), the rats were considered fed animals while the microswine were fasted for at least 15 hours. Data presented in Sections C.1 and C.2 suggest that decreased bioavailability in rats could be related to the presence of food in the G.I. tract.

To determine if increased bioavailability of soil lead in microswine relative to rats is related to the presence of food in the G.I. tract at the time of gavage

dosing, two microswine were fed their optimized diet one hour before receiving the intravenous or oral (gavage) dose. The AUC for the microswine administered the intravenous dose was 184.86 and for the microswine administered the oral (gavage) dose was 13.05. Calculated absolute bioavailability was 5.33%, which was similar to the acute rat bioavailability (4.14%). The AUC of the intravenous dose of the fed microswine was much higher than the AUC of the intravenous group of the fasted microswine. The AUC of the oral (gavage) dose did not decrease as expected. These results could be due to the deprivation of food which alters the lead distribution rather than affecting absorption. Aungst and Fung, 1981,[4] suggested that alterations in tissue and blood concentration of peptides or amino acids with high affinity for lead could be a causative factor.

D. Body Weights, Body Weight Gains, Food Consumption, Intake of Test Substance, and Clinical Observations

Exposure to lead produced no statistically significant effects on mean body weights (Tables 12-15, Appendix F), body weight gains (Tables 16-19), or food consumption (Tables 20-23) in either species. Mean daily intake values are listed in Tables 24 and 25. No deaths or compound-related clinical observations (Tables 24-27, Appendix G) of toxicity were observed in any group. A few rats had swollen tails or were missing the ends of the tails, due to drawing blood from tail veins. A few other rats had ruffled fur or skin sores. Microswine were salivating before feeding time.

CONCLUSION

In conclusion, on the bases of species sensitivity and interpretability of the single-dose assay results, these results suggest that the determination of the bioavailability of lead in soil following single-dose gavage administration to microswine is a reasonable model, of the methods examined, for determination of lead bioavailability in children. Based on this assay, the test soil exhibited an absolute oral bioavailability of 20.90%.

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TABLES

TABLE 1. CONCENTRATION OF LEAD IN INTRAVENOUS AND ORAL GAVAGE DOSING SOLUTIONS

RATS

		Amount of Lead	Amount of Water
Group I:	Single Intravenous Dose (Acute)	1.27 mg	0.1 ml (sterile)
Group II:	Single Oral Dose (Acute)	0.62 mg	2.0 ml (deionized)
Group III:	Single Intravenous Dose (Subchronic)	---[a]	---[a]
Group IV:	Single Oral Dose (Subchronic)	---[a]	---[a]

MICROSWINE

		Amount of Lead	Amount of Water
Group VI:	Single Intravenous Dose (Acute)	12.17 mg	0.1 ml (sterile)
Group VIII:	Single Oral Dose (Acute)	16.12 mg	30.0 ml (deionized)
Group V:	Single Intravenous Dose (Subchronic)	11.85 mg	0.1 ml (sterile)
Group VII:	Single Oral Dose (Subchronic)	18.16 mg	30.0 ml (deionized)

[a] Due to technical error, dosing solutions for the subchronic rat groups were not sent for analysis. Concentrations from the single dose rat groups were used to calculate the bioavailability for the subchronic rat groups.

TABLE 2. HOMOGENEITY OF LEAD IN DIET PREPARED FOR RATS

Sample Type	Concentration of Lead (ppm) Nominal	Measured	Mean ± S.D.	% Nominal
Top	50	41 46 40	42.33±3.21	84.66±6.43
Middle	50	46 42 47	45.00±2.65	90.00±5.29
Bottom	50	37 36 38	37.00±1.00	74.00±2.00

Coefficient of Variation (%) = 9.8

TABLE 3. HOMOGENEITY OF LEAD IN DIET PREPARED FOR MICROSWINE

Sample Type	Concentration of Lead (ppm) Nominal	Measured	Mean ± S.D.	% Nominal
Top	50	43.6 46.6 44.0	44.73±1.63	89.46±3.26
Middle	50	42.3 45.7 43.1	43.70±1.78	87.40±3.56
Bottom	50	45.8 51.0 44.5	47.10±3.44	94.20±6.88

Coefficient of Variation (%) = 3.86

TABLE 4. MISCELLANEOUS LEAD CONCENTRATIONS

Type of Sample	Concentration of Lead[a]
Deionized Water	0
1000 ppm Lead Soil	840.9 ppm
Sucrose	0
Sucrose Diet Sample	0
Oreo® Cookies	0
Teklad Basal (microswine)	0
Teklad Basal (rat)	0
Swine Bedding	0

[a] Detection limit was <5 ppb.

TABLE 5. MEAN BLOOD LEAD CONCENTRATION (μg/ml) IN MALE RATS (Acute)
Group I: Single Intravenous Dose
Group II: Single Oral Dose

COLLECTION TIME (hrs)	GROUP I MEAN	GROUP I S.D.	GROUP II MEAN	GROUP II S.D.
pre-dose	0.011	0.012	0.022[a]	0.020[a]
0.5	47.360	16.649	0.024	0.006
1.0	32.120	16.849	0.022	0.004
2.0	13.980	5.766	0.049	0.011
4.0	9.680	6.650	0.112	0.060
8.0	3.600	2.256	0.324	0.120
12.0	4.375	3.595	0.145[b]	0.039[b]
24.0	4.840	1.776	0.062	0.037
48.0	2.720	2.753	0.046	0.009
72.0	2.160	1.301	0.030	0.009
96.0	1.320	0.817	0.068	0.014
120.0	1.400	0.707	0.022	0.006
144.0	1.320	0.870	0.023	0.008
192.0	0.500	0.300	0.018	0.008
240.0	0.400	0.071	0.008	0.008

[a] Value does not contain pre-dose data from substitute 12-hour rats.
[b] Substitute animals used for 12-hour collection. Pre-dose mean for these animals was 0.003; standard deviation was 0.005.

TABLE 6. MEAN BLOOD LEAD CONCENTRATION (µg/ml) IN MALE RATS (Subchronic)
Group III: Single Intravenous Dose After Feeding for 30 Consecutive Days
Group IV: Single Oral Dose After Feeding for 30 Consecutive Days

COLLECTION TIME (hrs)	GROUP III MEAN	GROUP III S.D.	GROUP IV MEAN	GROUP IV S.D.
pre-dose	0.072	0.019	0.077	0.029
0.5	18.620	8.221	0.062	0.028
1.0	17.375	3.218	0.074	0.032
2.0	10.560	5.588	0.049	0.022
4.0	6.980	3.152	0.113	0.033
8.0	3.500	2.183	0.146	0.029
12.0	5.360	1.029	0.065	0.025
24.0	1.275	0.850	0.095	0.028
48.0	3.980	3.162	0.091	0.010
72.0	1.440	0.808	0.035	0.025
96.0	0.525	0.350	0.027	0.038
120.0	0.840	0.439	0.023	0.035
144.0	0.750	0.311	0.045	0.017
192.0	0.490	0.162	0.031	0.011
240.0	0.740	0.548	0.059	0.022

TABLE 7. MEAN BLOOD LEAD CONCENTRATION (μg/ml) IN MALE MICROSWINE (Acute)
Group VI: Single Intravenous Dose
Group VIII: Single Oral Dose

COLLECTION TIME (hrs)	GROUP VI MEAN	GROUP VI S.D.	GROUP VIII MEAN	GROUP VIII S.D.
pre-dose	0.041	0.066	0.002	0.003
0.5	1.526	0.698	0.005	0.003
1.0	1.360	0.737	0.016	0.007
2.0	0.554	0.353	0.031	0.020
4.0	0.358	0.107	0.064	0.022
8.0	0.342	0.093	0.072	0.011
12.0	0.352	0.078	0.104	0.026
24.0	0.306	0.146	0.067	0.038
48.0	0.216	0.085	0.043	0.025
72.0	0.305	0.156	0.033	0.009
96.0	0.196	0.125	0.022	0.011
120.0	0.122	0.054	0.039	0.014
144.0	0.109	0.057	0.024	0.011
192.0	0.089	0.034	0.041	0.027
240.0	0.081	0.042	0.018	0.005

TABLE 8. MEAN BLOOD LEAD CONCENTRATION (µg/ml) IN MALE MICROSWINE (Subchronic)
Group V: Single Intravenous Dose After Feeding for 30 Consecutive Days
Group VII: Single Oral Dose After Feeding for 30 Consecutive Days

COLLECTION TIME (hrs)	GROUP V MEAN	GROUP V S.D.	GROUP VII MEAN	GROUP VII S.D.
pre-dose	0.163	0.045	0.182	0.056
0.5	3.828	3.015	0.191	0.041
1.0	1.310	1.142	0.221	0.066
2.0	1.258	1.057	0.224	0.072
4.0	0.827	0.642	0.260	0.091
8.0	0.493	0.407	0.249	0.085
12.0	0.617	0.704	0.234	0.061
24.0	0.312	0.254	0.219	0.060
48.0	0.335	0.220	0.223	0.066
72.0	0.245	0.149	0.189	0.050
96.0	0.285	0.154	0.168	0.033
120.0	0.250	0.129	0.159	0.026
144.0	0.224	0.129	0.139	0.027
192.0	0.167	0.064	0.121	0.022
240.0	0.140	0.054	0.113	0.025

TABLE 9. ABSOLUTE BIOAVAILABILITY IN RATS AND MICROSWINE

	Absolute Bioavailability[a]
Single Dose Rats (Acute)	4.14
Single Dose Rats (Subchronic)	27.83
Single Dose Microswine (Acute)	20.90
Single Dose Microswine (Subchronic)	47.13
Single Dose Fed Microswine[b]	5.33

[a] Absolute bioavailability was determined as the ratio of the area under the blood-lead concentration versus time curve for the oral/intravenous groups in comparison to the dosage size administered.

[b] Because microswine were considered fasted before dosing and rats were not, one microswine administered a single intravenous dose (acute) and one microswine administered a single oral gavage dose (acute) were fed one hour before dosing. The absolute bioavailability of these animals was determined independently of the other microswine.

TABLE 10. AREA UNDER THE BLOOD CONCENTRATION-TIME CURVE (AUC) FOR MICROSWINE (Acute)
Group VI: Single Intravenous Dose
Group VIII: Single Oral Dose

GROUP VI		GROUP VIII	
ANIMAL NUMBER	AUC [μg(hr)/ml]	ANIMAL NUMBER	AUC [μg(hr)/ml]
602	66.43	801	22.22
603	66.35	802	11.48
604	69.25	804	9.70
605	19.83	805	16.51
606	48.63		

Mean AUC for Single Intravenous Dose (Acute) was 54.09 μg(hr)/ml (Figure 3).
Mean AUC for Single Oral Dose (Acute) was 14.98 μg(hr)/ml (Figure 3).

All AUC values were adjusted for dose, then statistically evaluated with the Student t-test (alpha = 0.05). For microswine, the AUCs of the Single Intravenous Dose group (Acute) was significantly different from the AUCs of the Single Oral Dose group(Acute).

TABLE 11. AREA UNDER THE BLOOD CONCENTRATION-TIME CURVE (AUC) FOR MICROSWINE (Subchronic)
Group V: Single Intravenous Dose After Feeding for 30 Consecutive Days
Group VII: Single Oral Dose After Feeding for 30 Consecutive Days

GROUP V		GROUP VII	
ANIMAL NUMBER	AUC [µg(hr)/ml]	ANIMAL NUMBER	AUC [µg(hr)/ml]
501	185.58	701	60.99
502	56.54	702	54.05
503	101.63	703	63.04
504	36.86	704	70.88
505	38.47	705	71.29
506	162.64	706	99.87

Mean AUC for Single Intravenous Dose (Subchronic) was 96.95 µg(hr)/ml (Figure 4).
Mean AUC for Single Oral Dose (Subchronic) was 70.02 µg(hr)/ml (Figure 4).

All AUC values were adjusted for dose, then statistically evaluated with the Student t-test (alpha = 0.05). For microswine, there were no statistically significant differences between AUCs of the Single Intravenous Dose group (Subchronic) and the Single Oral Dose group (Subchronic).

TABLE 12. MEAN BODY WEIGHTS (g) OF MALE RATS (Acute)
Group I: Single Intravenous Dose
Group II: Single Oral Dose

GROUP:	I	II
CONCENTRATION (ppm):	0	0
DAYS ON TEST		
0	86.3(9.3)[a]	82.4(11.7)
7	138.0(15.4)	130.4(15.3)
10	154.0(16.7)	151.5(13.3)

[a] Standard deviation is reported in parentheses.

TABLE 13. MEAN BODY WEIGHTS (g) OF MALE RATS (Subchronic)
Group III: Single Intravenous Dose After Feeding for 30 Consecutive Days
Group IV: Single Oral Dose After Feeding for 30 Consecutive Days

	GROUP: III	IV
APPROXIMATE CONCENTRATION (ppm):	1000	1000
DAYS ON TEST		
0	86.0(8.8)[a]	83.1(10.0)
7	143.2(13.3)	132.8(12.9)
14	194.2(18.3)	181.2(15.2)
21	247.0(22.2)	221.0(15.0)
28	279.7(22.6)	265.2(18.1)
35	307.2(26.2)	300.2(17.9)
41	327.6(26.9)	323.0(20.4)

[a] Standard deviation is reported in parentheses.

TABLE 14. MEAN BODY WEIGHTS (g) OF MALE MICROSWINE (Acute)
Group VI: Single Intravenous Dose
Group VIII: Single Oral Dose

GROUP:	VI	VIII
CONCENTRATION (ppm):	0	0
DAYS ON TEST		
0	6822.3(1354)[a]	6368.6(814)
7	7734.7(1512)	7385.2(825)
10	8318.0(1477)	7870.8(992)

[a] Standard deviation is reported in parentheses.

TABLE 15. MEAN BODY WEIGHTS (g) OF MALE MICROSWINE (Subchronic)
Group V: Single Intravenous Dose After Feeding for 30 Consecutive Days
Group VII: Single Oral Dose After Feeding for 30 Consecutive Days

	GROUP: V	VII
APPROXIMATE CONCENTRATION (ppm):	1000	1000
DAYS ON TEST		
0	6253.8(793)[a]	6776.3(894)
7	8013.3(807)	8652.8(820)
14	8487.3(839)	9029.0(782)
21	9493.2(841)	10006.0(837)
28	10803.0(905)	11142.0(891)
35	11901.0(1051)	12607.0(1143)
41	13094.0(1151)	13498.0(883)

[a] Standard deviation is reported in parentheses.

TABLE 16. MEAN BODY WEIGHT GAINS (g) OF MALE RATS (Acute)
Group I: Single Intravenous Dose
Group II: Single Oral Dose

GROUP:	I	II
CONCENTRATION (ppm):	0	0
DAYS ON TEST		
0- 7	51.7(7.8)[a]	48.0(5.3)
7-10	24.1(2.4)	23.4(3.3)

[a] Standard deviation is reported in parentheses

TABLE 17. MEAN BODY WEIGHT GAINS (g) OF MALE RATS (Subchronic)
Group III: Single Intravenous Dose After Feeding for 30 Consecutive Days
Group IV: Single Oral Dose After Feeding for 30 Consecutive Days

	GROUP: III	IV
APPROXIMATE CONCENTRATION (ppm):	1000	1000
DAYS ON TEST		
0- 7	56.7(7.0)[a]	49.7(5.1)
7-14	51.0(7.6)	48.4(8.4)
14-21	52.8(7.6)	39.8(9.7)
21-28	32.7(7.0)	44.2(10.0)
28-35	27.5(7.1)	35.0(13.6)
35-41	23.4(5.8)	25.5(6.8)

[a] Standard deviation is reported in parentheses.

TABLE 18. MEAN BODY WEIGHT GAINS (g) OF MALE MICROSWINE (Acute)
Group VI: Single Intravenous Dose
Group VIII: Single Oral Dose

GROUP:	VI	VIII
CONCENTRATION (ppm):	0	0
DAYS ON TEST		
0- 7	912.3(259)[a]	1016.6(153)
7-10	583.3(121)	485.6(272)

[a] Standard deviation is reported in parentheses.

TABLE 19. MEAN BODY WEIGHT GAINS (g) OF MALE MICROSWINE (Subchronic)
Group V: Single Intravenous Dose After Feeding for 30 Consecutive Days
Group VII: Single Oral Dose After Feeding for 30 Consecutive Days

	GROUP: V	VII
APPROXIMATE CONCENTRATION (ppm):	1000	1000
DAYS ON TEST		
0- 7	1759.5(231)[a]	1876.5(130)
7-14	474.0(214)	376.2(117)
14-21	1005.8(179)	977.2(211)
21-28	1310.2(190)	1135.8(133)
28-35	1097.2(205)	1464.5(329)
35-41	1193.0(123)	891.2(285)

[a] Standard deviation is reported in parentheses.

TABLE 20. MEAN DAILY FOOD CONSUMPTION (g) BY MALE RATS (Acute)
Group I: Single Intravenous Dose
Group II: Single Oral Dose

GROUP:	I	II
CONCENTRATION (ppm):	0	0
DAYS ON TEST		
0- 7	15.8(2.0)[a]	16.0(2.0)
7-10	19.4(2.4)	19.0(4.4)

[a] Standard deviation is reported in parentheses

Rats were provided with approximately 21.5 g/rat/day of optimized diet.

TABLE 21. MEAN DAILY FOOD CONSUMPTION (g) BY MALE RATS (Subchronic)
Group III: Single Intravenous Dose After Feeding for 30 Consecutive Days
Group IV: Single Oral Dose After Feeding for 30 Consecutive Days

	GROUP: III	IV
APPROXIMATE CONCENTRATION (ppm):	1000	1000
DAYS ON TEST		
0- 7	17.8(1.7)[a]	17.0(1.6)
7-14	21.0(1.7)	19.8(1.6)
14-21	22.7(1.6)[b]	20.6(0.6)
21-28	21.0(1.1)	21.3(0.5)
28-35	20.4(1.5)	20.3(2.1)
35-41	20.8(1.1)	20.9(1.2)

a Standard deviation is reported in parentheses.
b Due to technical error, food consumption value is beyond the 21.5 g/day food allowance.

Rats were provided with approximately 21.5 g/rat/day of optimized diet.

TABLE 22. MEAN DAILY FOOD CONSUMPTION (g) BY MALE MICROSWINE (Acute)
Group VI: Single Intravenous Dose
Group VIII: Single Oral Dose

GROUP:	VI	VIII
CONCENTRATION (ppm):	0	0
DAYS ON TEST		
0- 7	318.7(46.6)[a]	336.5(1.3)
7-10	326.3(29.4)	338.0(0.6)

[a] Standard deviation is reported in parentheses.

Microswine were provided with approximately 340 g/microswine/day of optimized diet.

TABLE 23. MEAN DAILY FOOD CONSUMPTION (g) BY MALE MICROSWINE (Subchronic)
Group V: Single Intravenous Dose After Feeding for 30 Consecutive Days
Group VII: Single Oral Dose After Feeding for 30 Consecutive Days

	GROUP: V	VII
APPROXIMATE CONCENTRATION (ppm):	1000	1000
DAYS ON TEST		
0- 7	328.2(3.9)[a]	330.7(3.2)
7-14	336.8(0.8)	339.0(0.3)
14-21	338.6(0.2)	338.1(0.3)
21-28	338.6(0.4)	338.6(0.4)
28-35	338.5(0.1)	338.7(0.1)
35-41	338.4(0.2)	338.1(0.7)

[a] Standard deviation is reported in parentheses.

Microswine were provided with approximately 340 g/microswine/day of optimized diet.

TABLE 24. MEAN DAILY INTAKE (mg LEAD/kg BODY WEIGHT/day) OF LEAD BY MALE RATS (Subchronic)
Group III: Single Intravenous Dose After Feeding for 30 Consecutive Days
Group IV: Single Oral Dose After Feeding for 30 Consecutive Days

DAYS ON TEST		
0- 7	6.22(0.51)[a]	6.42(0.47)
7-14	5.43(0.46)	5.47(0.37)
14-21	4.62(0.31)	4.67(0.32)
21-28	3.77(0.29)	4.03(0.32)
28-35	3.33(0.22)	3.39(0.34)
35-41	3.18(0.23)	3.25(0.21)

[a] Standard deviation is reported in parentheses.

TABLE 25. MEAN DAILY INTAKE (mg LEAD/kg BODY WEIGHT/day) OF LEAD BY MALE MICROSWINE (Subchronic)
Group V: Single Intravenous Dose After Feeding for 30 Consecutive Days
Group VII: Single Oral Dose After Feeding for 30 Consecutive Days

DAYS ON TEST		
0- 7	2.06(0.20)[a]	1.92(0.17)
7-14	2.00(0.19)	1.89(0.16)
14-21	1.80(0.16)	1.70(0.14)
21-28	1.58(0.14)	1.53(0.12)
28-35	1.43(0.14)	1.35(0.12)
35-41	1.30(0.12)	1.26(0.08)

[a] Standard deviation is reported in parentheses.

TABLE 26. SUMMARY OF CLINICAL OBSERVATIONS IN MALE RATS (Acute)
Group I: Single Intravenous Dose
Group II: Single Oral Dose

	NUMBER OF RATS WITH GIVEN SIGN	
GROUP:	I	II
NUMBER RATS AT STUDY START:	25	25
OBSERVATION		
END OF TAIL MISSING	1 (10)[a]	0
RUFFLED FUR	3 (0)	0
SORE	5 (8)	0
SWOLLEN TAIL	1 (3)	0

[a] Numbers in parentheses are the median days on test when the given signs were first observed.

TABLE 27. SUMMARY OF CLINICAL OBSERVATIONS IN MALE RATS (Subchronic)
Group III: Single Intravenous Dose After Feeding for 30 Consecutive Days
Group IV: Single Oral Dose After Feeding for 30 Consecutive Days

	NUMBER OF RATS WITH GIVEN SI	
GROUP:	III	IV
NUMBER RATS AT STUDY START:	26	25
OBSERVATION		
END OF TAIL MISSING	6 (16)[a]	0
RUFFLED FUR	2 (0)	0
SORE	10 (14)	1 (28)
SPLAYED LEG(S)	1 (17)	0

[a] Numbers in parentheses are the median days on test when the given si were first observed.

TABLE 28. SUMMARY OF CLINICAL OBSERVATIONS IN MALE MICROSWINE (Acute)
Group VI: Single Intravenous Dose
Group VIII: Single Oral Dose

	NUMBER OF MICROSWINE WITH GIVE	
GROUP:	VI	VIII
NUMBER MICROSWINE AT STUDY START:	6	5
OBSERVATION		
DIARRHEA	1 (0)[a]	0

[a] Numbers in parentheses are the median days on test when the given s were first observed.

TABLE 29. SUMMARY OF CLINICAL OBSERVATIONS IN MALE MICROSWINE (Subchroni
Group V: Single Intravenous Dose After Feeding for 30 Consecutive Days
Group VII: Single Oral Dose After Feeding for 30 Consecutive Days

	NUMBER OF MICROSWINE WITH GIV	
GROUP:	V	VII
NUMBER MICROSWINE AT STUDY START:	6	6
OBSERVATION		
SALIVATION	4 (23)[a]	2 (23)

[a] Numbers in parentheses are the median days on test when the given s were first observed.

FIGURES

BLOOD LEAD CONCENTRATION (µg/ml) IN MALE MICROSWINE
AFTER INTRAVENOUS OR ORAL DOSING
Values from microswine fed one hour before dosing

COLLECTION TIME (hrs)	INDIVIDUAL ANIMAL NUMBER AND BLOOD LEAD CONCENTRATION			
	GROUP VI		GROUP VIII	
predose	601	0.006	803	0.000
0.5	601	22.300	803	0.000
1.0	601	12.600	803	0.023
2.0	601	11.200	803	0.033
4.0	601	9.800	803	0.085
8.0	601	1.600	803	0.065
12.0	601	0.910	803	0.118
24.0	601	0.660	803	0.071
48.0	601	0.390	803	0.229
72.0	601	0.440	803	0.044
96.0	601	0.270	803	0.015
120.0	601	0.340	803	0.038
144.0	601	0.232	803	0.010a
192.0	601	0.148	803	0.023
240.0	601	0.195	803	0.009

[a] Value derived from reanalysis of sample.

APPENDIX E. BLOOD LEAD CONCENTRATION DURING THE 30-DAY FEEDING PHASE

ABBREVIATION

RS - Removed from Study

BLOOD LEAD CONCENTRATION (µg/ml) IN MALE RATS
DURING THE 30-DAY FEEDING PHASE
Group III: Single Intravenous Dose After Feeding for 30 Consecutive Days

ANIMAL NUMBER	PRE-FEEDING	FEEDING WEEK 1	FEEDING WEEK 2	FEEDING WEEK 3
301	0.056	-a	0.095	0.054
302	RS (Day 0)			
303	0.008	0.237	0.122	0.096
304	0.019	0.132	0.083	0.099
305	0.009	0.108	0.094	0.059
306	0.005	-a	0.138	0.081
307	0.006	0.077	0.112	0.055
308	0.005	0.090	0.175	0.078
309	0.011	-a	0.195	0.064
310	0.014	0.058	0.122	0.070
311	-a	0.124	0.092	0.084
312	-a	0.065	0.026	0.128
313	0.007	0.048	0.046	0.056
314	0.005	0.070	0.077	0.044
315	0.034	0.062	0.054	0.051
316	0.007	0.100	0.045	0.038
317	0.011	0.039	0.054	0.056
318	RS (Day 0)			
319	0.004	0.103	0.063	0.064
320	0.006	0.051	0.089	0.056
321	0.006	0.121	0.051	0.059
322	0.005	0.059	0.048	0.031
323	0.003	0.132	0.036	0.026
324	0.000	0.046	0.038	0.048
325	0.024	0.064	0.012	0.078

[a] Sample not submitted for anlaysis.

BLOOD LEAD CONCENTRATION (µg/ml) IN MALE RATS
DURING THE 30-DAY FEEDING PHASE
Group IV: Single Oral Dose After Feeding for 30 Consecutive Days

ANIMAL NUMBER	PRE-FEEDING	FEEDING WEEK 1	FEEDING WEEK 2	FEEDING WEEK 3
401	0.009	0.103	0.078	0.120
402	0.000	0.102	0.086	0.055
403	0.000	0.147	0.088	0.089
404	0.000	0.095	0.054	0.057
405	0.000	0.071	0.056	0.120
406	0.008	0.116	0.124	0.126
407	0.006	0.145	0.113	0.109
408	0.000	0.083	0.067	0.064
409	0.000	0.135	0.117	0.157
410	0.000	0.059	0.101	0.080
411	0.000	0.044	0.052	0.096
412	0.000	0.072	0.032	0.050
413	0.000	0.067	0.057	0.036
414	0.007	0.067	0.037	0.053
415	0.006	0.078	0.031	0.034
416	0.005	0.099	0.044	0.129
417	0.005	0.123	0.084	0.108
418	0.000	0.018	0.056	0.045
419	0.000	0.015	0.027	0.045
420	0.000	0.055	0.037	0.105
421	0.007	0.016	0.016	0.096
422	0.000	0.058	0.024	0.067
423	0.000	0.051	0.101	0.082
424	0.000	0.022	0.062	0.135
425	0.000	0.007	0.018	0.067

BLOOD LEAD CONCENTRATION (μg/ml) IN MALE MICROSWINE
DURING THE 30-DAY FEEDING PHASE
Group V: Single Intravenous Dose After Feeding for 30 Consecutive Days

ANIMAL NUMBER	PRE-FEEDING	FEEDING WEEK 1	FEEDING WEEK 2	FEEDING WEEK 3	FEEDING WEEK 4
501	0.030	0.129	0.100	0.258	0.139
502	0.008	0.257	0.190	0.347	0.152
503	0.020	0.323	0.150	0.266	0.225
504	0.034	0.150	0.050	0.155	0.135
505	0.008	0.195	0.140	0.147	0.173
506	0.017	0.160	0.100	0.193	0.195

BLOOD LEAD CONCENTRATION (μg/ml) IN MALE MICROSWINE
DURING THE 30-DAY FEEDING PHASE
Group VII: Single Oral Dose After Feeding for 30 Consecutive Days

ANIMAL NUMBER	PRE-FEEDING	FEEDING WEEK 1	FEEDING WEEK 2	FEEDING WEEK 3
701	0.007	0.190	0.206	0.193
702	0.023	0.130	0.206	0.169
703	0.005	0.080	0.217	0.137
704	0.057	0.170	0.261	0.209
705	0.010	0.180	0.258	0.227
706	0.007	0.290	0.201	0.173

APPENDIX F. INDIVIDUAL BODY WEIGHTS

ABBREVIATIONS

NW - Not Weighed
RS - Removed from Study
SD - Sacrificed by Design

INDIVIDUAL BODY WEIGHTS (g) OF MALE RATS
Group I: Single Intravenous Dose

DAYS ON TEST:	0	3	7	10
ANIMAL #				
536261	83.5	102.8	131.6	SD (DAY 7)
536262	92.5	114.7	152.8	SD (DAY 7)
536263	79.8	95.5	127.3	SD (DAY 8)
536264	102.4	123.8	159.4	SD (DAY 7)
536265	76.3	91.1	113.3	133.4
536266	83.2	99.9	128.1	SD (DAY 7)
536267	79.8	98.9	132.9	SD (DAY 7)
536268	110.7	135.2	170.0	SD (DAY 7)
536269	76.1	91.2	119.3	SD (DAY 7)
536270	81.6	95.4	124.3	SD (DAY 7)
536271	82.0	102.2	128.3	SD (DAY 7)
536272	87.3	102.6	130.5	SD (DAY 7)
536273	76.8	91.8	120.9	144.7
536274	86.8	105.5	135.9	161.3
536275	72.3	91.5	125.9	SD (DAY 7)
536276	97.0	123.5	157.5	SD (DAY 7)
536277	77.0	92.7	126.2	SD (DAY 8)
536278	91.3	122.2	165.0	SD (DAY 8)
536279	83.0	97.9	134.5	SD (DAY 8)
536280	92.8	116.9	151.8	177.6
536281	78.9	96.6	127.7	153.2
536282	94.6	114.4	150.6	SD (DAY 7)
536283	95.3	111.0	146.6	SD (DAY 7)
536284	93.7	116.0	151.2	SD (DAY 7)
536285	82.7	103.4	137.2	SD (DAY 8)

INDIVIDUAL BODY WEIGHTS (g) OF MALE RATS
Group II: Single Oral Dose

DAYS ON TEST:	0	3	7	10
ANIMAL #				
537350	79.1	99.1	125.5	141.4
537351	87.6	107.9	140.0	SD (DAY 7)
537352	71.4	90.3	118.5	SD (DAY 7)
537353	96.3	118.3	141.2	163.2
537354	62.9	78.5	100.3	SD (DAY 7)
537355	86.3	102.3	135.4	SD (DAY 7)
537356	77.8	96.1	122.2	146.3
537357	88.8	108.8	141.7	166.5
537358	71.8	89.7	109.4	136.8
537359	89.9	109.3	139.4	SD (DAY 7)
537360	81.6	102.3	137.4	162.6
537361	87.2	104.1	138.0	163.9
537362	71.9	90.9	123.0	SD (DAY 7)
537363	106.6	127.1	158.9	SD (DAY 7)
537364	68.6	84.5	110.4	132.5
537365	109.0	130.9	163.2	SD (DAY 7)
537366	72.8	93.5	121.9	SD (DAY 7)
537367	88.6	110.8	145.7	SD (DAY 7)
537368	66.7	85.7	114.6	SD (DAY 7)
537369	92.5	113.5	146.4	SD (DAY 7)
537370	78.9	97.8	128.9	SD (DAY 7)
537371	81.7	95.2	124.6	SD (DAY 7)
537372	75.5	90.2	118.7	139.7
537373	90.8	97.3	136.1	161.8
537374	74.7	90.9	117.6	SD (DAY 7)

INDIVIDUAL BODY WEIGHTS (g) OF MALE RATS
Group III: Single Intravenous Dose After Feeding for 30 Consecutive Days

DAYS ON TEST:	0	3	7	10	14	17	21	24
ANIMAL #								
536286	100.6	126.4	165.9	186.4	213.5	240.7	283.7	284.2
536287	86.6	RS (DAY	0)					
536288	101.2	126.8	155.5	180.5	210.6	244.2	275.1	280.3
536289	87.1	112.1	150.2	171.9	200.9	229.0	254.8	261.8
536290	91.3	114.3	151.2	177.4	207.9	235.0	264.5	270.6
536291	87.0	109.8	140.6	175.3	206.8	228.9	264.0	272.5
536292	86.8	109.8	133.8	150.4	172.8	191.5	219.8	237.8
536293	75.3	92.7	127.5	151.2	175.5	211.9	240.2	253.0
536294	80.7	109.0	145.5	165.7	200.7	221.9	256.4	269.0
536295	86.1	107.2	143.3	165.3	201.0	224.2	245.9	264.5
536296	86.9	108.8	134.6	157.2	184.1	198.6	235.6	243.7
536297	71.4	90.3	112.6	131.6	159.0	172.8	200.4	210.5
536298	94.5	115.4	147.6	163.6	192.3	207.7	241.7	252.4
536299	86.5	110.6	155.0	183.7	216.5	246.1	272.1	281.9
536300	90.1	110.8	143.4	170.1	199.6	226.2	255.7	267.1
536301	79.0	99.0	131.1	157.3	177.4	205.8	236.3	247.7
536302	87.7	109.4	143.1	161.0	187.8	209.8	237.7	245.6
536303	71.7	RS (DAY	0)					
536304	93.0	118.5	156.8	185.3	209.7	238.1	264.5	279.4
536305	77.0	96.3	134.8	155.2	185.7	210.8	239.5	254.9
536306	95.5	121.6	159.1	185.2	214.4	233.7	253.9	262.4
536307	78.9	103.8	140.7	165.6	194.1	216.3	245.0	257.2
536308	81.2	101.9	136.7	157.0	183.8	204.4	229.6	242.9
536309	73.1	94.8	121.8	138.7	152.6	168.6	195.0	206.4
536310	100.3	127.9	163.9	181.5	220.9	240.8	269.7	281.0
536311	NW	RS (DAY	0)					

INDIVIDUAL BODY WEIGHTS (g) OF MALE RATS
Group III: Single Intravenous Dose After Feeding for 30 Consecutive Days

DAYS ON TEST:	28	31	35	38	41
ANIMAL #					
536286	305.2	318.5	337.8	346.6	353.1
536287					
536288	307.6	320.9	337.8	SD (DAY 35)	
536289	284.8	297.2	312.5	329.3	340.0
536290	290.7	303.5	322.2	332.5	337.0
536291	297.6	312.5	327.1	340.6	350.1
536292	263.7	279.9	301.6	318.7	324.8
536293	281.2	301.0	305.3	311.1	344.1
536294	289.9	304.1	322.4	336.4	351.4
536295	287.9	303.9	312.8	316.5	337.5
536296	263.5	275.1	286.1	302.2	312.2
536297	236.9	254.8	258.4	273.7	280.6
536298	257.1	267.9	285.6	302.8	311.2
536299	306.6	317.1	330.0	337.1	359.3
536300	293.1	307.6	312.2	330.0	334.4
536301	270.1	281.1	302.2	305.8	316.2
536302	262.2	269.5	286.0	SD (DAY 35)	
536303					
536304	306.4	323.2	327.5	SD (DAY 35)	
536305	277.1	289.9	310.2	304.5	330.2
536306	283.3	297.4	320.0	337.2	339.6
536307	281.4	294.7	309.4	326.4	334.1
536308	263.5	284.4	298.6	305.1	322.1
536309	220.5	232.0	225.7	240.8	246.8
536310	302.2	317.1	333.3	SD (DAY 35)	
536311					

INDIVIDUAL BODY WEIGHTS (g) OF MALE RATS
Group IV: Single Oral Dose After Feeding for 30 Consecutive Days

DAYS ON TEST:	0	3	7	10	14	17	21	24
ANIMAL #								
537375	87.4	107.8	130.2	148.3	180.0	198.7	222.9	238.4
537376	72.8	89.5	115.4	131.6	154.4	169.5	202.0	220.0
537377	96.5	118.3	153.3	180.7	209.9	227.2	248.8	270.7
537378	70.9	91.7	120.2	142.9	175.0	195.0	220.4	240.8
537379	87.1	109.2	145.0	173.8	176.0	206.7	224.1	245.7
537380	74.7	92.1	118.8	140.9	170.2	193.7	210.4	227.3
537381	85.8	107.9	141.2	166.0	195.8	192.8	220.3	253.4
537382	77.0	98.8	117.6	158.4	185.9	202.2	223.0	234.4
537383	89.2	107.8	135.9	160.0	189.7	205.9	222.1	250.9
537384	76.8	96.2	125.1	141.8	167.8	188.2	212.1	229.1
537385	82.1	99.5	130.5	151.0	178.3	201.6	223.6	244.2
537386	75.8	97.7	132.9	157.2	184.7	203.6	226.9	251.0
537387	86.5	109.5	138.8	163.2	194.9	221.4	245.1	265.6
537388	66.3	82.2	112.3	131.2	163.0	187.4	212.2	230.9
537389	95.2	116.6	146.3	162.3	187.7	206.2	225.7	244.2
537390	73.6	93.6	124.2	147.9	176.0	195.0	220.0	239.2
537391	92.2	114.8	147.1	172.9	199.3	219.8	234.0	259.4
537392	81.9	103.1	138.4	146.3	193.3	210.2	226.4	257.4
537393	104.9	123.6	153.1	173.8	205.0	218.7	219.8	253.1
537394	76.3	95.2	126.3	144.0	173.6	194.9	199.0	229.2
537395	77.5	94.8	126.4	147.1	155.6	179.3	204.5	232.4
537396	69.2	90.3	110.8	133.5	154.0	169.4	183.4	203.8
537397	99.1	123.1	151.5	178.2	193.5	229.4	251.6	273.1
537398	91.0	106.9	143.2	162.3	186.9	185.6	221.1	242.2
537399	88.4	107.9	135.6	160.6	178.8	205.3	225.9	246.2

INDIVIDUAL BODY WEIGHTS (g) OF MALE RATS
Group IV: Single Oral Dose After Feeding for 30 Consecutive Days

DAYS ON TEST:	28	31	35	38	41
ANIMAL #					
537375	256.3	277.5	295.5	309.6	SD (DAY 38)
537376	232.0	244.2	266.3	279.6	301.7
537377	296.0	321.1	336.0	SD (DAY 35)	
537378	273.0	290.3	313.0	320.7	SD (DAY 38)
537379	268.6	293.9	318.3	321.8	SD (DAY 38)
537380	251.0	267.6	284.9	300.1	300.7
537381	276.4	295.9	314.5	317.0	SD (DAY 38)
537382	264.2	289.7	294.9	305.7	320.2
537383	269.9	287.8	299.3	306.5	319.4
537384	261.5	284.4	299.9	314.6	334.5
537385	269.6	288.0	276.2	289.5	SD (DAY 38)
537386	280.0	303.7	319.5	326.8	342.9
537387	287.3	317.7	303.9	307.6	SD (DAY 38)
537388	253.2	272.3	289.0	295.1	SD (DAY 38)
537389	263.0	277.0	288.4	298.1	SD (DAY 38)
537390	269.7	285.3	306.1	313.5	329.2
537391	285.5	302.7	280.5	SD (DAY 35)	
537392	278.2	296.9	319.3	SD (DAY 35)	
537393	273.0	288.4	302.5	SD (DAY 35)	
537394	254.0	276.8	297.7	304.3	SD (DAY 38)
537395	262.8	288.0	302.9	312.4	SD (DAY 38)
537396	205.3	243.7	263.9	278.3	289.1
537397	272.9	314.1	327.6	331.4	346.7
537398	265.5	287.1	312.0	325.9	345.3
537399	260.7	280.7	293.1	SD (DAY 35)	

INDIVIDUAL BODY WEIGHTS (g) OF MALE MICROSWINE
Group VI: Single Intravenous Dose

DAYS ON TEST:	0	4	7	10
ANIMAL #				
17	6534	6722	7030	7690
18	7013	7623	8110	8600
19	6660	7297	7566	8320
20	6910	7578	8063	8480
21	4788	5052	5513	6130
22	9029	9718	10126	10688

INDIVIDUAL BODY WEIGHTS (g) OF MALE MICROSWINE
Group VIII: Single Oral Dose

DAYS ON TEST:	0	4	7	10
ANIMAL #				
23	5698	6175	6732	6736
24	7233	7916	8312	8915
25	5916	6325	6704	7245
26	7277	7852	8255	8903
27	5719	6408	6923	7555

INDIVIDUAL BODY WEIGHTS (g) OF MALE MICROSWINE
Group V: Single Intravenous Dose After Feeding for 30 Consecutive Days

DAYS ON TEST:	0	4	7	11	14	18	21	25
ANIMAL #								
5	5365	6291	7416	7421	8118	8821	9437	10518
6	6137	6867	8096	7987	8619	9204	9713	10641
7	6331	6742	7796	7876	8495	9091	9461	10423
8	6364	6825	8055	7692	8355	8788	9310	10022
9	5666	6029	7217	6841	7387	7815	8205	8892
10	7660	8409	9500	9660	9950	10476	10833	11706

DAYS ON TEST:	28	32	35	39	41
ANIMAL #					
5	10940	11856	12201	13476	13362
6	11094	12048	12403	13309	13525
7	10982	11869	12186	13340	13439
8	10535	11230	11407	12526	12589
9	9250	9982	10073	11192	11113
10	12019	12984	13133	14404	14533

INDIVIDUAL BODY WEIGHTS (g) OF MALE MICROSWINE
Group VII: Single Oral Dose After Feeding for 30 Consecutive Days

DAYS ON TEST:	0	4	7	11	14	18	21	25
ANIMAL #								
11	6267	7557	8232	8177	8674	9075	9307	10084
12	7802	8943	9499	9488	9972	10393	10882	11650
13	6000	7188	7810	7735	8151	8663	9063	9792
14	6495	7850	8368	8393	8840	9393	9883	10845
15	6084	7385	8157	8081	8521	9287	9762	10560
16	8010	9190	9851	9663	10016	10676	11140	11864

DAYS ON TEST:	28	32	35	39	41
ANIMAL #					
11	10262	10540	11411	12243	12511
12	11924	12430	13200	14530	13916
13	10237	11007	11543	12242	12740
14	11150	11900	12551	13328	13624
15	10843	11550	12434	12901	13250
16	12436	13360	14500	14521	14945

APPENDIX G. INDIVIDUAL CLINICAL OBSERVATIONS AND MORTALITY

INDIVIDUAL CLINICAL OBSERVATIONS AND MORTALITY IN MALE RATS
Group I: Single Intravenous Dose

ANIMAL #	OBSERVATION	FIRST DAY OBSERVED	LAST DAY OBSERVED
536261	NO ABNORMALITIES DETECTED SACRIFICED BY DESIGN TEST DAY 7		
536262	NO ABNORMALITIES DETECTED SACRIFICED BY DESIGN TEST DAY 7		
536263	SORE BASE TAIL SACRIFICED BY DESIGN TEST DAY 8	8	8
536264	NO ABNORMALITIES DETECTED SACRIFICED BY DESIGN TEST DAY 7		
536265	NO ABNORMALITIES DETECTED SACRIFICED BY DESIGN TEST DAY 10		
536266	NO ABNORMALITIES DETECTED SACRIFICED BY DESIGN TEST DAY 7		
536267	NO ABNORMALITIES DETECTED SACRIFICED BY DESIGN TEST DAY 7		
536268	NO ABNORMALITIES DETECTED SACRIFICED BY DESIGN TEST DAY 7		
536269	RUFFLED FUR SACRIFICED BY DESIGN TEST DAY 7	0	7

INDIVIDUAL CLINICAL OBSERVATIONS AND MORTALITY IN MALE RATS
Group I: Single Intravenous Dose

ANIMAL #	OBSERVATION	FIRST DAY OBSERVED	LAST DAY OBSERVED
536270	NO ABNORMALITIES DETECTED SACRIFICED BY DESIGN TEST DAY 7		
536271	NO ABNORMALITIES DETECTED SACRIFICED BY DESIGN TEST DAY 7		
536272	NO ABNORMALITIES DETECTED SACRIFICED BY DESIGN TEST DAY 7		
536273	END OF TAIL MISSING SACRIFICED BY DESIGN TEST DAY 10	10	10
536274	NO ABNORMALITIES DETECTED SACRIFICED BY DESIGN TEST DAY 10		
536275	RUFFLED FUR SACRIFICED BY DESIGN TEST DAY 7	0	7
536276	NO ABNORMALITIES DETECTED SACRIFICED BY DESIGN TEST DAY 7		
536277	NO ABNORMALITIES DETECTED SACRIFICED BY DESIGN TEST DAY 8		
536278	NO ABNORMALITIES DETECTED SACRIFICED BY DESIGN TEST DAY 8		

INDIVIDUAL CLINICAL OBSERVATIONS AND MORTALITY IN MALE RATS
Group I: Single Intravenous Dose

ANIMAL #	OBSERVATION	FIRST DAY OBSERVED	LAST DAY OBSERVED
536279	SORE BASE TAIL	8	8
	SACRIFICED BY DESIGN TEST DAY 8		
536280	SORE BASE TAIL	10	10
	SACRIFICED BY DESIGN TEST DAY 10		
536281	RUFFLED FUR	0	10
	SORE TAIL	3	10
	SWOLLEN TAIL	3	10
	SACRIFICED BY DESIGN TEST DAY 10		
536282	NO ABNORMALITIES DETECTED		
	SACRIFICED BY DESIGN TEST DAY 7		
536283	NO ABNORMALITIES DETECTED		
	SACRIFICED BY DESIGN TEST DAY 7		
536284	NO ABNORMALITIES DETECTED		
	SACRIFICED BY DESIGN TEST DAY 7		
536285	SORE BASE TAIL	8	8
	SACRIFICED BY DESIGN TEST DAY 8		

INDIVIDUAL CLINICAL OBSERVATIONS AND MORTALITY IN MALE RATS
Group II: Single Oral Dose

ANIMAL #	OBSERVATION	FIRST DAY OBSERVED	LAST DAY OBSERVED
537350	NO ABNORMALITIES DETECTED SACRIFICED BY DESIGN TEST DAY 10		
537351	NO ABNORMALITIES DETECTED SACRIFICED BY DESIGN TEST DAY 7		
537352	NO ABNORMALITIES DETECTED SACRIFICED BY DESIGN TEST DAY 7		
537353	NO ABNORMALITIES DETECTED SACRIFICED BY DESIGN TEST DAY 10		
537354	NO ABNORMALITIES DETECTED SACRIFICED BY DESIGN TEST DAY 7		
537355	NO ABNORMALITIES DETECTED SACRIFICED BY DESIGN TEST DAY 7		
537356	NO ABNORMALITIES DETECTED SACRIFICED BY DESIGN TEST DAY 10		
537357	NO ABNORMALITIES DETECTED SACRIFICED BY DESIGN TEST DAY 10		
537358	NO ABNORMALITIES DETECTED SACRIFICED BY DESIGN TEST DAY 10		

INDIVIDUAL CLINICAL OBSERVATIONS AND MORTALITY IN MALE RATS
Group II: Single Oral Dose

ANIMAL #	OBSERVATION	FIRST DAY OBSERVED	LAST DAY OBSERVED
537359	NO ABNORMALITIES DETECTED SACRIFICED BY DESIGN TEST DAY 7		
537360	NO ABNORMALITIES DETECTED SACRIFICED BY DESIGN TEST DAY 10		
537361	NO ABNORMALITIES DETECTED SACRIFICED BY DESIGN TEST DAY 10		
537362	NO ABNORMALITIES DETECTED SACRIFICED BY DESIGN TEST DAY 7		
537363	NO ABNORMALITIES DETECTED SACRIFICED BY DESIGN TEST DAY 7		
537364	NO ABNORMALITIES DETECTED SACRIFICED BY DESIGN TEST DAY 10		
537365	NO ABNORMALITIES DETECTED SACRIFICED BY DESIGN TEST DAY 7		
537366	NO ABNORMALITIES DETECTED SACRIFICED BY DESIGN TEST DAY 7		
537367	NO ABNORMALITIES DETECTED SACRIFICED BY DESIGN TEST DAY 7		

INDIVIDUAL CLINICAL OBSERVATIONS AND MORTALITY IN MALE RATS
Group II: Single Oral Dose

ANIMAL #	OBSERVATION	FIRST DAY OBSERVED	LAST DAY OBSERVED
537368	NO ABNORMALITIES DETECTED SACRIFICED BY DESIGN TEST DAY 7		
537369	NO ABNORMALITIES DETECTED SACRIFICED BY DESIGN TEST DAY 7		
537370	NO ABNORMALITIES DETECTED SACRIFICED BY DESIGN TEST DAY 7		
537371	NO ABNORMALITIES DETECTED SACRIFICED BY DESIGN TEST DAY 7		
537372	NO ABNORMALITIES DETECTED SACRIFICED BY DESIGN TEST DAY 10		
537373	NO ABNORMALITIES DETECTED SACRIFICED BY DESIGN TEST DAY 10		
537374	NO ABNORMALITIES DETECTED SACRIFICED BY DESIGN TEST DAY 7		

INDIVIDUAL CLINICAL OBSERVATIONS AND MORTALITY IN MALE RATS
Group III: Single Intravenous Dose After Feeding for 30 Consecutive Days

ANIMAL #	OBSERVATION	FIRST DAY OBSERVED	LAST DAY OBSERVED
536286	SORE NECK	14	41
	SORE BACK	24	41
	SACRIFICED BY DESIGN TEST DAY 41		
536287	NO ABNORMALITIES DETECTED		
	REMOVED FROM STUDY TEST DAY 0		
536288	NO ABNORMALITIES DETECTED		
	SACRIFICED BY DESIGN TEST DAY 35		
536289	NO ABNORMALITIES DETECTED		
	SACRIFICED BY DESIGN TEST DAY 41		
536290	NO ABNORMALITIES DETECTED		
	SACRIFICED BY DESIGN TEST DAY 41		
536291	SORE NECK	28	41
	SORE BACK	28	35
	SORE BOTH SHOULDER(S)	28	28
	SACRIFICED BY DESIGN TEST DAY 41		
536292	NO ABNORMALITIES DETECTED		
	SACRIFICED BY DESIGN TEST DAY 41		

INDIVIDUAL CLINICAL OBSERVATIONS AND MORTALITY IN MALE RATS
Group III: Single Intravenous Dose After Feeding for 30 Consecutive Days

ANIMAL #	OBSERVATION	FIRST DAY OBSERVED	LAST DAY OBSERVED
536293	RUFFLED FUR	0	24
	SORE TAIL	14	41
	END OF TAIL MISSING	21	41
	SACRIFICED BY DESIGN TEST DAY 41		
536294	END OF TAIL MISSING	10	41
	SORE TAIL	10	41
	SORE LEFT SHOULDER(S)	28	41
	SACRIFICED BY DESIGN TEST DAY 41		
536295	END OF TAIL MISSING	10	41
	SORE TAIL	10	41
	SPLAYED LEGS	17	17
	SACRIFICED BY DESIGN TEST DAY 41		
536296	END OF TAIL MISSING	10	41
	SORE TAIL	14	41
	SACRIFICED BY DESIGN TEST DAY 41		
536297	SORE NECK	14	41
	SORE BOTH EAR(S)	21	41
	END OF TAIL MISSING	21	41
	SORE TAIL	21	41
	SORE HEAD	21	41
	SACRIFICED BY DESIGN TEST DAY 41		

INDIVIDUAL CLINICAL OBSERVATIONS AND MORTALITY IN MALE RATS
Group III: Single Intravenous Dose After Feeding for 30 Consecutive Days

ANIMAL #	OBSERVATION	FIRST DAY OBSERVED	LAST DAY OBSERVED
536298	RUFFLED FUR	0	41
	SACRIFICED BY DESIGN TEST DAY 41		
536299	SPLAYED LEGS	17	24
	SORE ABDOMEN	28	31
	SACRIFICED BY DESIGN TEST DAY 41		
536300	NO ABNORMALITIES DETECTED		
	SACRIFICED BY DESIGN TEST DAY 41		
536301	NO ABNORMALITIES DETECTED		
	SACRIFICED BY DESIGN TEST DAY 41		
536302	NO ABNORMALITIES DETECTED		
	SACRIFICED BY DESIGN TEST DAY 35		
536303	NO ABNORMALITIES DETECTED		
	REMOVED FROM STUDY TEST DAY 0		
536304	SORE FACE	14	35
	SORE NECK	14	35
	SORE BACK	21	35
	SACRIFICED BY DESIGN TEST DAY 35		
536305	NO ABNORMALITIES DETECTED		
	SACRIFICED BY DESIGN TEST DAY 41		

INDIVIDUAL CLINICAL OBSERVATIONS AND MORTALITY IN MALE RATS
Group III: Single Intravenous Dose After Feeding for 30 Consecutive Days

ANIMAL #	OBSERVATION	FIRST DAY OBSERVED	LAST DAY OBSERVED
536306	NO ABNORMALITIES DETECTED		
	SACRIFICED BY DESIGN TEST DAY 41		
536307	NO ABNORMALITIES DETECTED		
	SACRIFICED BY DESIGN TEST DAY 41		
536308	NO ABNORMALITIES DETECTED		
	SACRIFICED BY DESIGN TEST DAY 41		
536309	END OF TAIL MISSING	28	41
	SORE TAIL	28	41
	SACRIFICED BY DESIGN TEST DAY 41		
536310	NO ABNORMALITIES DETECTED		
	SACRIFICED BY DESIGN TEST DAY 35		
536311	REMOVED FROM STUDY TEST DAY 0		

INDIVIDUAL CLINICAL OBSERVATIONS AND MORTALITY IN MALE RATS
Group IV: Single Oral Dose After Feeding for 30 Consecutive Days

ANIMAL #	OBSERVATION	FIRST DAY OBSERVED	LAST DAY OBSERVED
537375	NO ABNORMALITIES DETECTED SACRIFICED BY DESIGN TEST DAY 38		
537376	NO ABNORMALITIES DETECTED SACRIFICED BY DESIGN TEST DAY 41		
537377	NO ABNORMALITIES DETECTED SACRIFICED BY DESIGN TEST DAY 35		
537378	NO ABNORMALITIES DETECTED SACRIFICED BY DESIGN TEST DAY 38		
537379	NO ABNORMALITIES DETECTED SACRIFICED BY DESIGN TEST DAY 38		
537380	NO ABNORMALITIES DETECTED SACRIFICED BY DESIGN TEST DAY 41		
537381	NO ABNORMALITIES DETECTED SACRIFICED BY DESIGN TEST DAY 38		
537382	NO ABNORMALITIES DETECTED SACRIFICED BY DESIGN TEST DAY 41		
537383	NO ABNORMALITIES DETECTED SACRIFICED BY DESIGN TEST DAY 41		

INDIVIDUAL CLINICAL OBSERVATIONS AND MORTALITY IN MALE RATS
Group IV: Single Oral Dose After Feeding for 30 Consecutive Days

ANIMAL #	OBSERVATION	FIRST DAY OBSERVED	LAST DAY OBSERVED
537384	NO ABNORMALITIES DETECTED SACRIFICED BY DESIGN TEST DAY 41		
537385	NO ABNORMALITIES DETECTED SACRIFICED BY DESIGN TEST DAY 38		
537386	SORE RIGHT SHOULDER(S) SACRIFICED BY DESIGN TEST DAY 41	28	28
537387	NO ABNORMALITIES DETECTED SACRIFICED BY DESIGN TEST DAY 38		
537388	NO ABNORMALITIES DETECTED SACRIFICED BY DESIGN TEST DAY 38		
537389	NO ABNORMALITIES DETECTED SACRIFICED BY DESIGN TEST DAY 38		
537390	NO ABNORMALITIES DETECTED SACRIFICED BY DESIGN TEST DAY 41		
537391	NO ABNORMALITIES DETECTED SACRIFICED BY DESIGN TEST DAY 35		
537392	NO ABNORMALITIES DETECTED SACRIFICED BY DESIGN TEST DAY 35		

INDIVIDUAL CLINICAL OBSERVATIONS AND MORTALITY IN MALE RATS
Group IV: Single Oral Dose After Feeding for 30 Consecutive Days

ANIMAL #	OBSERVATION	FIRST DAY OBSERVED	LAST DAY OBSERVED
537393	NO ABNORMALITIES DETECTED SACRIFICED BY DESIGN TEST DAY 35		
537394	NO ABNORMALITIES DETECTED SACRIFICED BY DESIGN TEST DAY 38		
537395	NO ABNORMALITIES DETECTED SACRIFICED BY DESIGN TEST DAY 38		
537396	NO ABNORMALITIES DETECTED SACRIFICED BY DESIGN TEST DAY 41		
537397	NO ABNORMALITIES DETECTED SACRIFICED BY DESIGN TEST DAY 41		
537398	NO ABNORMALITIES DETECTED SACRIFICED BY DESIGN TEST DAY 41		
537399	NO ABNORMALITIES DETECTED SACRIFICED BY DESIGN TEST DAY 35		

INDIVIDUAL CLINICAL OBSERVATIONS AND MORTALITY IN MALE MICROSWINE
Group VI: Single Intravenous Dose

ANIMAL #	OBSERVATION	FIRST DAY OBSERVED	LAST DAY OBSERVED
17	NO ABNORMALITIES DETECTED SACRIFICED BY DESIGN TEST DAY 10		
18	NO ABNORMALITIES DETECTED SACRIFICED BY DESIGN TEST DAY 10		
19	NO ABNORMALITIES DETECTED SACRIFICED BY DESIGN TEST DAY 10		
20	NO ABNORMALITIES DETECTED SACRIFICED BY DESIGN TEST DAY 10		
21	DIARRHEA SACRIFICED BY DESIGN TEST DAY 10	0	10
22	NO ABNORMALITIES DETECTED SACRIFICED BY DESIGN TEST DAY 10		

INDIVIDUAL CLINICAL OBSERVATIONS AND MORTALITY IN MALE MICROSWINE
Group VIII: Single Oral Dose

ANIMAL #	OBSERVATION	FIRST DAY OBSERVED	LAST DAY OBSERVED
23	NO ABNORMALITIES DETECTED SACRIFICED BY DESIGN TEST DAY 10		
24	NO ABNORMALITIES DETECTED SACRIFICED BY DESIGN TEST DAY 10		
25	NO ABNORMALITIES DETECTED SACRIFICED BY DESIGN TEST DAY 10		
26	NO ABNORMALITIES DETECTED SACRIFICED BY DESIGN TEST DAY 10		
27	NO ABNORMALITIES DETECTED SACRIFICED BY DESIGN TEST DAY 10		

INDIVIDUAL CLINICAL OBSERVATIONS AND MORTALITY IN MALE MICROSWINE
Group V: Single Intravenous Dose After Feeding for 30 Consecutive Days

ANIMAL #	OBSERVATION	FIRST DAY OBSERVED	LAST DAY OBSERVED
5	SALIVATION SACRIFICED BY DESIGN TEST DAY 41	25	41
6	SALIVATION SACRIFICED BY DESIGN TEST DAY 41	18	41
7	SALIVATION SACRIFICED BY DESIGN TEST DAY 41	21	41
8	NO ABNORMALITIES DETECTED SACRIFICED BY DESIGN TEST DAY 41		
9	NO ABNORMALITIES DETECTED SACRIFICED BY DESIGN TEST DAY 41		
10	SALIVATION SACRIFICED BY DESIGN TEST DAY 41	25	41

INDIVIDUAL CLINICAL OBSERVATIONS AND MORTALITY IN MALE MICROSWINE
Group VII: Single Oral Dose After Feeding for 30 Consecutive Days

ANIMAL #	OBSERVATION	FIRST DAY OBSERVED	LAST DAY OBSERVED
11	SALIVATION	21	41
	SACRIFICED BY DESIGN TEST DAY 41		
12	NO ABNORMALITIES DETECTED		
	SACRIFICED BY DESIGN TEST DAY 41		
13	NO ABNORMALITIES DETECTED		
	SACRIFICED BY DESIGN TEST DAY 41		
14	SALIVATION	25	41
	SACRIFICED BY DESIGN TEST DAY 41		
15	NO ABNORMALITIES DETECTED		
	SACRIFICED BY DESIGN TEST DAY 41		
16	NO ABNORMALITIES DETECTED		
	SACRIFICED BY DESIGN TEST DAY 41		

APPENDIX H. ANALYSIS OF LEAD IN BLOOD, SOIL, AND ASSOCIATED MATERIALS

KEY TO DATA APPENDIX

Blood samples were labeled with a code letter to identify the dose and species, followed by the animal identification number, followed by a hyphen, followed by the timepoint (in hours) for which the blood was collected. An example would be

B211-12.0

where the code letter is *B*, the animal number is *211*, and the timepoint is *12.0* hrs.

Timepoints labeled as PRE are identifying the predosing samples. Timepoints listed as dates are identifying the blood samples during the 30-day feeding period (subchronic).

The explanations of the Code Letters are listed below:

- A - Single Dose Intravenous Rats (Acute)
- B - Single Dose Oral (Gavage) Rats (Acute)
- C - Single Dose Intravenous Rats (Subchronic)
- D - Single Dose Oral (Gavage) Rats (Subchronic)
- E - Single Dose Intravenous Microswine (Acute)
- F - Single Dose Oral (Gavage) Microswine (Acute)
- G - Single Dose Intravenous Microswine (Subchronic)
- H - Single Dose Oral (Gavage) Microswine (Subchronic)

Note: The supporting data for this report contains an analysis of Purina Rat Chow. This is incorrect. Teklad basal mix (95%) for rats (TD 92242) was submitted for analysis.

FIGURE 1. MEAN BLOOD LEAD CONCENTRATION IN MALE RATS (Acute)

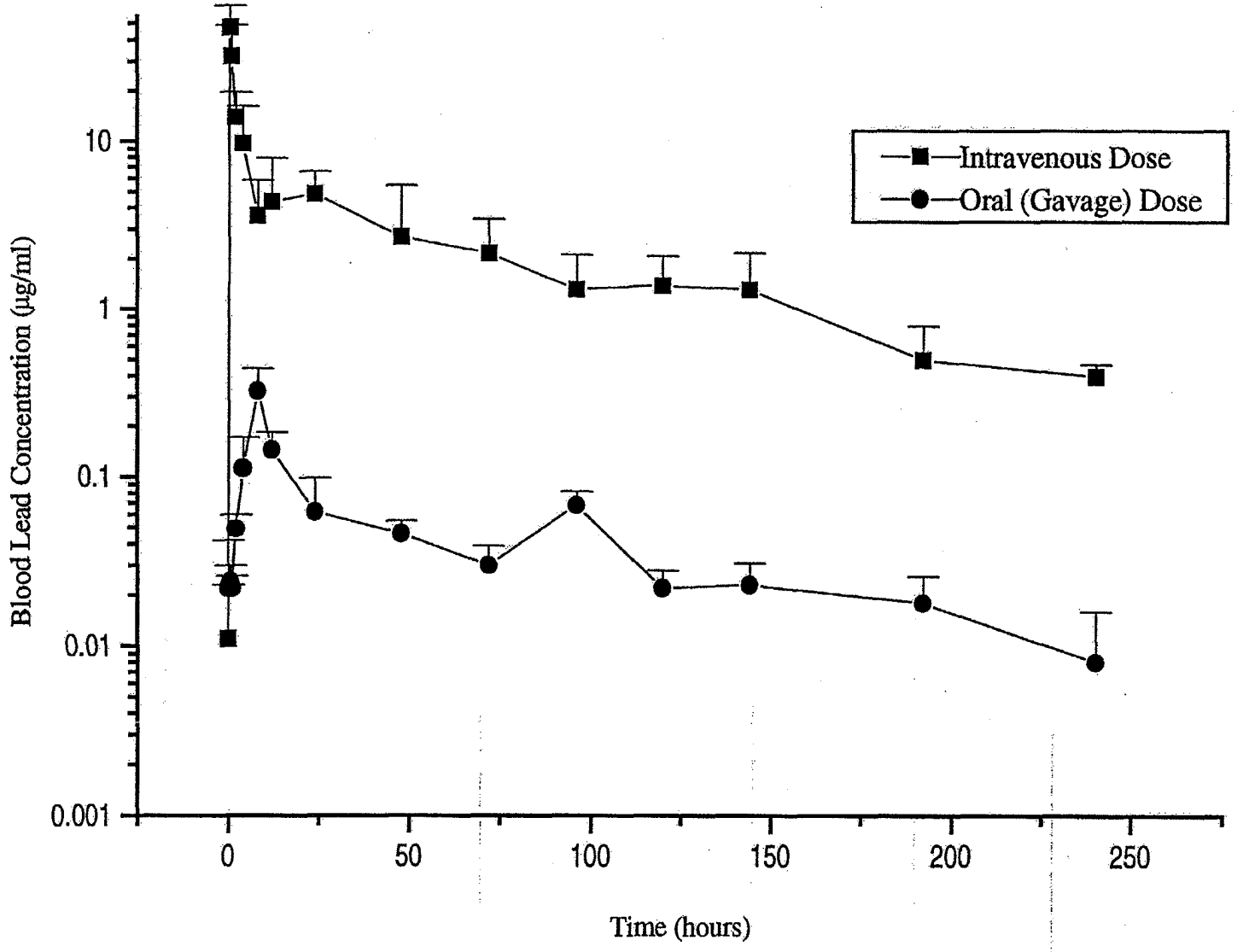


FIGURE 2. MEAN BLOOD LEAD CONCENTRATION IN MALE MICROSWINE (Acute)

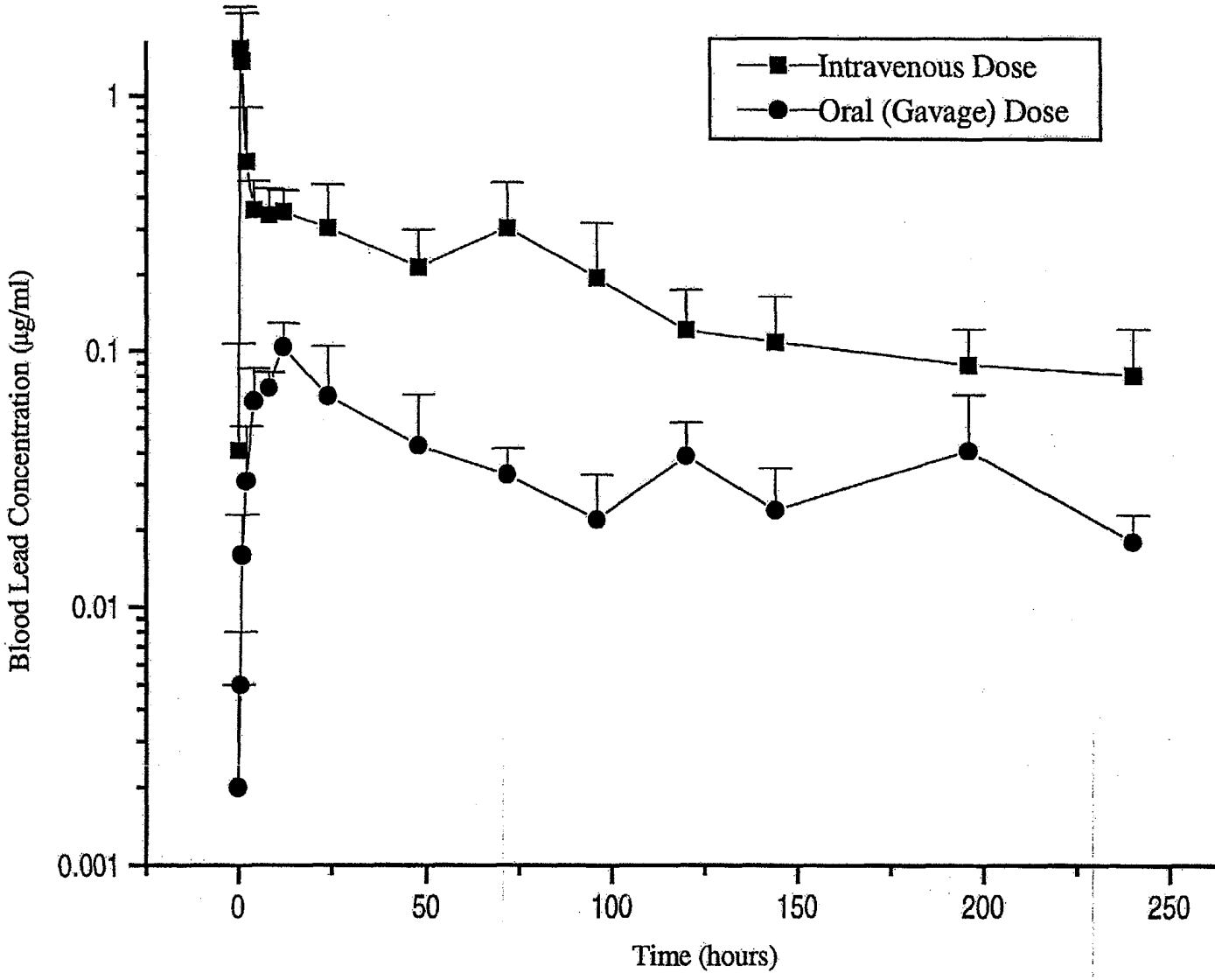


FIGURE 3. MEAN BLOOD LEAD CONCENTRATION IN MALE RATS (Subchronic)

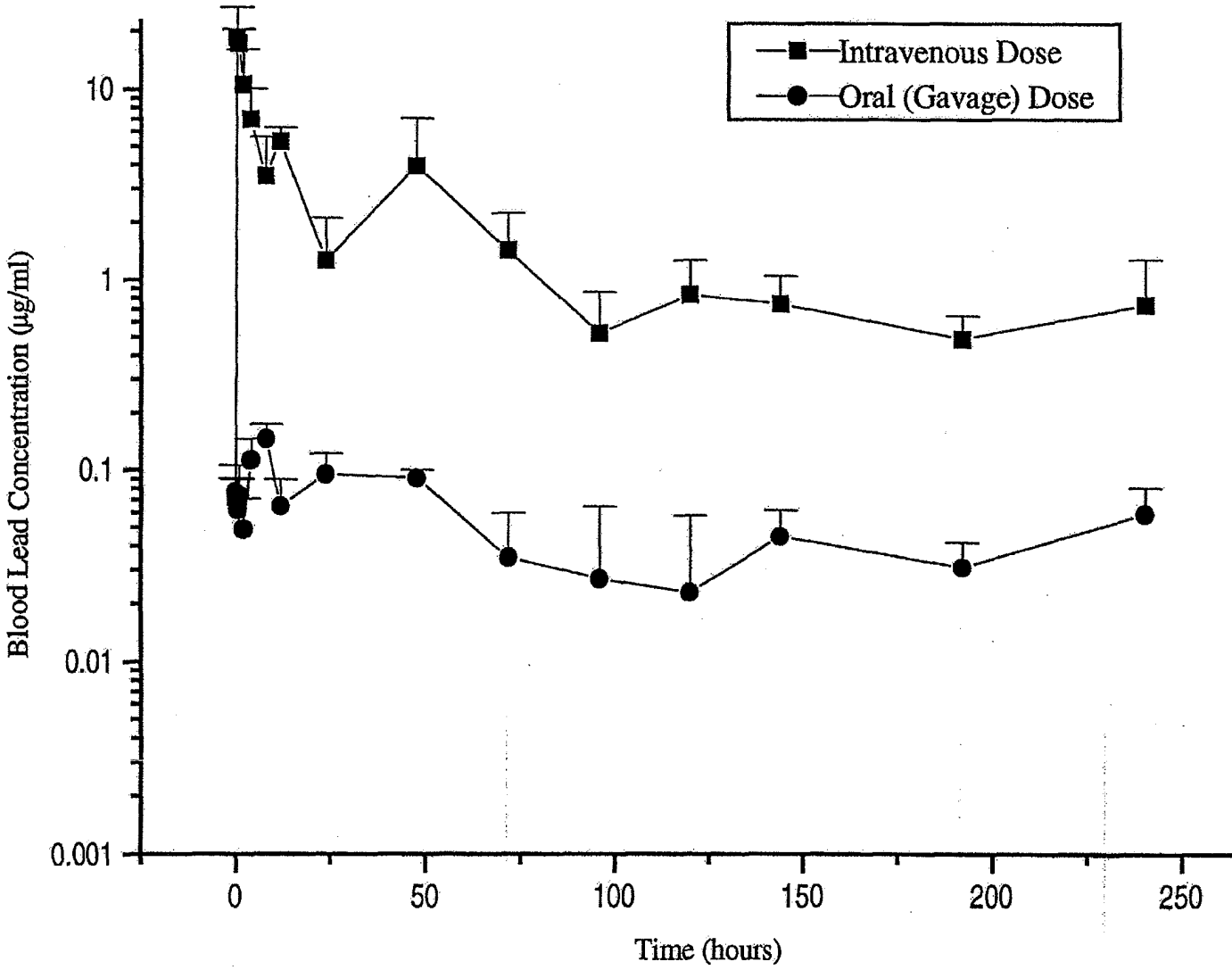
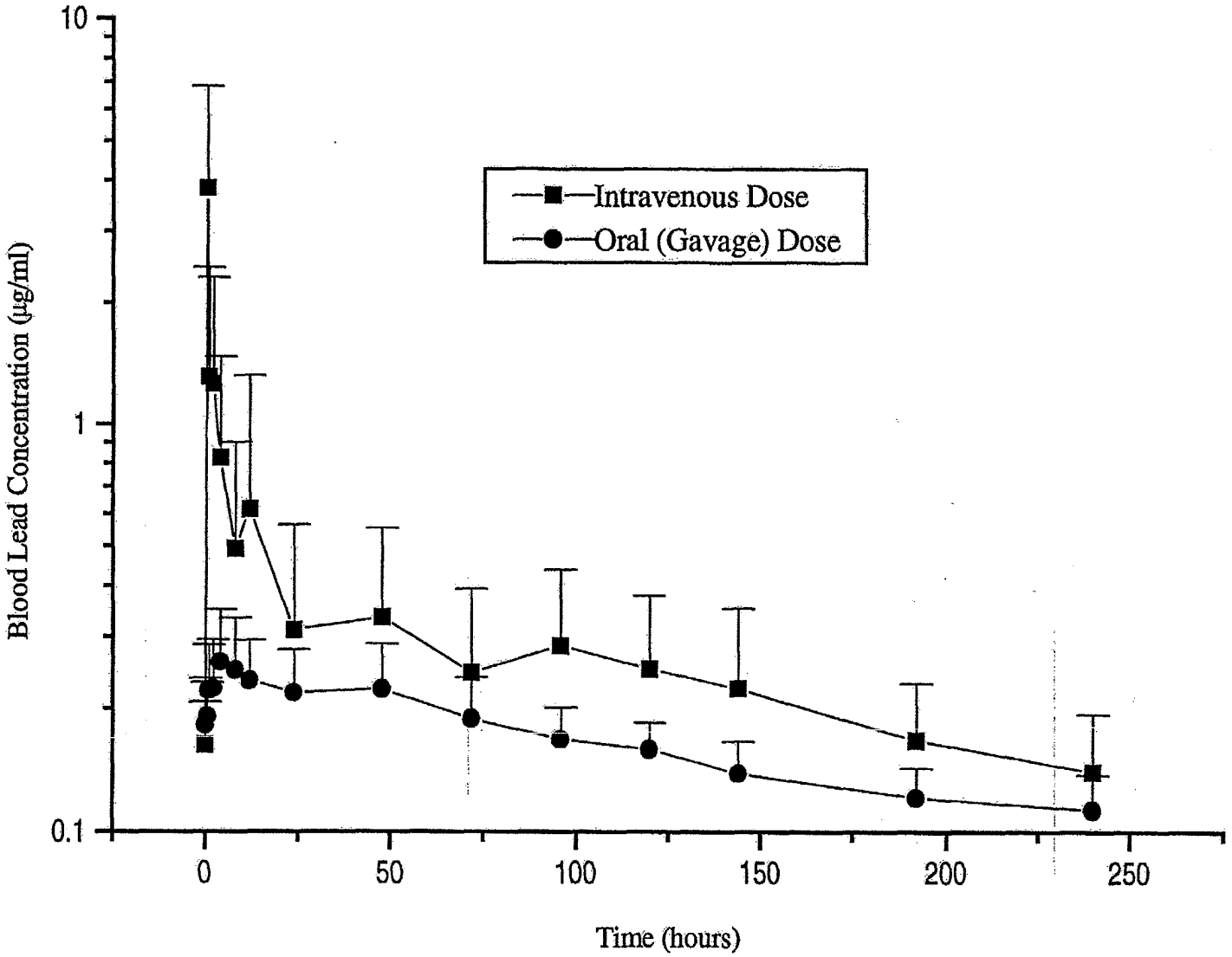


FIGURE 4. MEAN BLOOD LEAD CONCENTRATION IN MALE MICROSWINE (Subchronic)



APPENDIX A. PROTOCOL AND PROTOCOL AMENDMENTS

ABSOLUTE BIOAVAILABILITY OF SOIL LEAD IN RATS AND MICROSWINE

MEDICAL RESEARCH PROJECT NO. 9660

PROTOCOL

Stine-Haskell Animal Welfare Committee No. CBT51-P

ABSOLUTE BIOAVAILABILITY OF SOIL LEAD IN RATS AND MICROSWINE

MEDICAL RESEARCH PROJECT NO. 9660

OBJECTIVE

The objective of this study is to determine the absolute bioavailability of lead from soil in rats and microswine to aid in developing a site specific clean-up goal.

SPONSOR AND TEST FACILITY

This study is sponsored by the Corporate Remediation Group of DuPont Chemicals, Bellevue Corporate Center, Wilmington, DE 19809. The study will be conducted at Haskell Laboratory for Toxicology and Industrial Medicine, E. I. du Pont de Nemours and Company, Newark, Delaware, in accordance with all applicable Good Laboratory Practices. The sponsor's approval was effective the date the sponsor signed the Medical Research (MR) project.

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STUDY DESIGN

A. Single Dose Administration

- o Fifty male rats and ten male microswine will be used.
- o A predosing blood sample will be drawn from each animal for levels of background lead.
- o Twenty-five rats and five microswine will be dosed orally (gavage) with test soil containing 1,000 ppm lead (approximately 1 g soil/rat and 17 g soil/microswine).
- o Twenty-five rats and five microswine will be given a single intravenous injection of lead acetate solution containing lead-dose equivalent to that contained in the gavage dose.

Single Dose Administration (continued)

- o Approximately 0.1-0.3 ml of blood will be drawn from the rats (each rat will be used to collect three blood samples only) and approximately 0.5 ml of blood will be drawn from each microswine at 0.5, 1, 2, 4, 8, 12, 24, 48, 72, 96, 120, 144, 192, and 240 hours after dosing.
- o Blood samples will be collected in lead-free tubes containing EDTA and stored at 4°C until analysis.
- o At sacrifice, liver and bone (both cortical and trabecular as well as whole femur) will be collected, weighed, and frozen at -20°C.

B. Single Dose Administration After Feeding For 30 Consecutive Days

- o Fifty male rats and ten male microswine will be used.
- o A predosing blood sample will be drawn from each animal for levels of background lead, followed by one sample/week for the duration of 30 days.
- o All animals will be provided with optimized diet of approximately 21.5 g/rat/day or 340 g/microswine/day of a special purified diet mixed with test soil containing 1,000 ppm lead, 5% w/w, for 30 days.
- o After 30 days, blood samples will be drawn from each animal for levels of background lead and the animals will be divided into two groups.
- o Twenty-five rats and five microswine will be dosed orally (gavage) with test soil containing 1,000 ppm lead (approximately 1 g soil/rat and 17 g soil/microswine).
- o Twenty-five rats and five microswine will be given a single intravenous injection of lead acetate solution containing lead-dose equivalent to that contained in the gavage dose.
- o Approximately 0.1-0.3 ml of blood will be drawn from the rats (each rat will be used to collect three blood samples only) and approximately 0.5 ml of blood will be drawn from microswine at 0.5, 1, 2, 4, 8, 12, 24, 48, 72, 96, 120, 144, 192, and 240 hours after dosing.
- o Blood samples will be collected in lead-free tubes containing EDTA and stored at 4°C until analysis.
- o At sacrifice, liver, kidney, brain, spleen, and bone (both cortical and trabecular as well as whole femur) will be collected, weighed, and frozen at -20°C.

MATERIALS AND METHODS

A. Test Material

The test materials will be supplied by Aldrich, Milwaukee, WI 53733, U.S.A. The lead acetate trihydrate will be assigned Haskell Laboratory No. 20202. The lead/soil will be assigned Haskell Laboratory No. 20204. Characterization and mineralogy data of the test soils will be maintained by the sponsor.

B. Test Species

Male Crl:CD®BR rats, approximately 21 days of age, with body weights in the range of approximately 40 to 65 grams will be acquired from Charles River Laboratories, Inc., Kingston, New York. The Crl:CD®BR rat has been selected on the bases of extensive experience with this strain and its suitability with respect to hardiness, longevity, sensitivity, and low incidence of spontaneous disease.

Male Yucatan microswine, approximately 30 days of age, with body weights in the range of approximately 3 to 6 kilograms will be acquired from Charles River Laboratories, Inc., Wilmington, Massachusetts. The weanling Charles River microswine has been selected on the bases of its low body weight, its suitability as a model of soil-lead ingestion by children, and its suitability with respect to longevity, hardiness, sensitivity, and low incidence of spontaneous disease.

C. Pretest Procedures

Rats and microswine will be housed according to the Guide for Care and Use of Laboratory Animals. (NIH Publications No. 86-23, Revised 1985)

The quarantine period will be approximately one week, during which rats will be fed optimized diet (once per day) of irradiated Purina® Certified

Rodent Chow (PCRC) #5002 meal and transferred gradually to Teklad basal mix (95%) for rats. Microswine will be fed optimized diet (twice per day) of microswine diet (Charles River) and transferred gradually to Teklad basal mix (95%) for microswine. Rats and microswine will be provided deionized water ad libitum, weighed three times, and observed with respect to eating habits, weight gain, and any signs of disease or injury.

During the test period, all rats and microswine will be fed the diet of their respective treatment groups.

Animals that die or are sacrificed in extremis during the quarantine period will be necropsied to check for the presence of disease. Animals will be released by the laboratory veterinarian at the end of the quarantine period.

Haskell Laboratory has an animal health monitoring program which consists of periodic food and water analyses for contaminants, sampling freshly washed cages and cage racks for bacteria, and sampling non-study animals housed in the study room for pathogens. The program is monitored and administered by the laboratory veterinarian; data are maintained separately from study records.

D. Assignment to Groups

Healthy animals will be assigned to groups by computerized, stratified randomization. Rats will be housed individually while microswine will be housed two per cage. Each animal will be assigned its own Haskell animal number, and permanently identified by a number marked on the rat's tail or the microswine's ear.

E. Animal Husbandry

After assignment to groups, animals will be identified with a cage card and housed in stainless steel cages. All rats and microswine will be provided optimized test diets of approximately 21.5 g/rat/day and 340 g/microswine/day. Deionized water will be provided ad libitum.

Animal rooms will be targeted at a temperature of 23 ± 2°C and a relative humidity of 50 ± 10%.

F. Diet Preparation and Analysis

During the test period, rats and microswine in each group will be fed the following purified diets:

- o Rat Basal mix (95%) (TD 92242)
- o Swine Basal mix (95%) (TD 93034)

These diets will be custom formulated by their manufacturers so that each diet contains complete normal nutrition for each species when the diet is diluted (weight/weight) with test soil containing 1,000 ppm lead or with sucrose. These diets are sucrose-free so that the soil added will replace the sucrose rather than diluting the complete mixed diet. The diets will contain < 0.1 ug Pb/g.

Lead/soil or sucrose will be added to the diets and thoroughly mixed for a period of time that is adequate to assure homogeneous distribution in the diet. Once prepared, diets will be refrigerated until used.

Sucrose mixed diets will be given to all animals during quarantine and the single dose administration group (Group A in Study Design). The lead/soil mixed diet will be given to the other group for 30 days (Group B in Study Design). After 30 days, Group B animals will be given sucrose-mixed diets for the rest of the study period.

Before the beginning of the study, samples will be collected from the diet prepared with lead/soil. These samples will be analyzed to verify concentration and homogeneity in the test diets.

Homogeneity samples will be collected from the top, middle, and bottom of the diet mixer. Samples will be frozen until analyzed.

Once during the study, samples will be collected from randomly selected feed jars to verify concentration of lead in the feeders. Samples will be collected from each species. Backup samples will be taken on the same day from other feeders in the same species and analyzed if needed. Feeder samples and backup samples will be frozen on the day of collection.

G. Body Weights

All rats and microswine will be weighed twice per week unless experimental findings warrant a more infrequent weighing schedule.

H. Food Consumption and Intake of Test Material

The amount of food consumed by each microswine will be determined twice per week throughout the study. The amount of food consumed by each rat will be determined weekly throughout the study. From these determinations and body weight data, average test animal daily food consumption, and intake of test material will be calculated.

I. Dosing

Rats will be dosed intravenously via the tail vein, while microswine will be dosed via the ear vein. Sterile water will be used as a vehicle for intravenous administration. Oral doses to both species will be administered by gavage. Deionized water will be used as a vehicle.

The route of administration of the lead-contaminated soil will be by oral gavage and/or feeding since ingestion of soil lead activity presents one of the principle direct pathways for exposure to children. Intravenous administration of lead acetate will be performed in order to calculate area under the curve (AUC) and absolute bioavailability of lead.

J. Blood Sampling

Approximately 0.1-0.3 ml of rat venous blood and 0.5-1 ml of microswine venous blood will be drawn from each designated animal at 0, 0.5, 1, 2, 4, 8, 12, 24, 48, 72, 96, 120, 144, 192, and 240 hours after dosing. Blood will be collected into a lead-free tube containing EDTA and refrigerated until the time of preparation for analysis.

K. Sacrifice of Animals

At the end of the experiment, the animals will be humanely euthanized. Animals will be necropsied, liver and bone will be collected from the single dose administered animals (Group A in Study Design) and liver, bone, kidney, spleen, and brain will be collected from the animals fed lead/soil for 30 days (Group B in Study Design). All tissues will be weighed, frozen at approximately -20°C, and stored for potential future analyses.

L. Blood Analysis

The blood lead concentration will be analyzed by graphite furnace atomic absorption spectroscopy (GFAA), at DuPont Chambers Works. Dosing solutions, diets, and drinking water will also be analyzed.

M. Data Analysis

The blood lead concentrations determined by atomic absorption will be used to calculate AUC (area under blood concentration versus time curve) and absolute bioavailability of lead. Individual animal data as well as the mean for five animals in each group will be reported.

N. Statistical Methods

Body weights, body weight gain, and food consumption will be analyzed by one-way analysis of variance. Appropriate statistical analysis will be performed on mean AUC values between oral and intravenous administrations.

SAFETY AND HOUSEKEEPING

Good housekeeping practices will be used to avoid contamination of work areas and potential health hazards. Gloves will be worn when handling formulation, blood, animals, animal tissues, or analytical standards. Animal carcasses, feces, and diet will be incinerated.

RECORDS AND SAMPLE RETENTION

All original records will be retained at Haskell Laboratory or at the Records Management Center, E. I. du Pont de Nemours and Company, Wilmington, Delaware.

PROTOCOL APPENDIX I

MEDICAL RESEARCH PROJECT NO. 9660

Study Personnel and Study Dates

STUDY PERSONNEL

Study Sponsor: Corporate Remediation Group of DuPont Chemicals
E. I. du Pont de Nemours and Company
Bellevue Corporate Center
Wilmington, Delaware 19809

Contact: Timothy S. Bingman, D.A.B.T.
Consultant
(302)571-7761

Study Director: Hanan N. Ghantous, Ph.D.
Research Toxicologist

STUDY DATES

Proposed Experiment Start:	May, 1993
Proposed Sponsor Approval:	Date the Sponsor signed the MR Project
Proposed Experiment Completion:	August, 1993
Proposed Report Issue Date:	February, 1994

ABSOLUTE BIOAVAILABILITY OF SOIL LEAD IN RATS AND MICROSWINE

MEDICAL RESEARCH PROJECT NO. 9660

PROTOCOL

Date

Approved: H. Ghantous 5/17/93
Hanan N. Ghantous
Study Director

Matthew S. Bogdanffy 5/18/93
Matthew S. Bogdanffy
Manager
Biochemical Toxicology

cc: P. E. Ross
G. S. Elliott
T. S. Bingman (REO-PIT)
K. Mc Cleary
M. S. Bogdanffy
R. C. Rhea

DUPONT CENTRAL RESEARCH AND DEVELOPMENT
HASKELL LABORATORY FOR TOXICOLOGY
AND INDUSTRIAL MEDICINE

June 28, 1993

TO: MEDICAL RESEARCH PROJECT NO. MR 9660-001

ABSOLUTE BIOAVAILABILITY OF SOIL LEAD IN RATS AND MICROSWINE

PROTOCOL AMENDMENT #1

The protocol is amended as follows:

1. Page 2, Study Design, Section A. Single Dose Administration, replace "Fifty male rats and ten male microswine will be used." with "Fifty male rats and twelve male microswine will be used."

Reason: To obtain better statistical analysis of results.

2. Page 2, Study Design, Section A. Single Dose Administration, replace "Twenty-five rats and five microswine will be dosed orally..." with "Twenty-five rats and six microswine will be dosed orally...".

Reason: To obtain better statistical analysis of results.

3. Page 2, Study Design, Section A. Single Dose Administration, replace "Twenty-five rats and five microswine will be given..." with "Twenty-five rats and six microswine will be given...".

Reason: To obtain better statistical analysis of results.

4. Page 3, Section B. Single Dose Administration After Feeding For 30 Consecutive Days, replace "Fifty male rats and ten male microswine..." with "Fifty male rats and twelve male microswine..."

Reason: To obtain better statistical analysis of results.

5. Page 3, Section B. Single Dose Administration After Feeding For 30 Consecutive Days, replace "Twenty-five rats and five microswine will be dosed orally..." with "Twenty-five rats and six microswine will be dosed orally...".

Reason: To obtain better statistical analysis of results.

6. Page 3, Section B. Single Dose Administration After Feeding For 30 Consecutive Days, replace "Twenty-five rats and five microswine will be given..." with "Twenty-five rats and six microswine will be given...".

Reason: To obtain better statistical analysis of results.

7. Page 4, Pretest Procedures, second paragraph, delete "irradiated" from first sentence.

Reason: The animals are not being feed irradiated chow. The study is being conducted in Building 1 and irradiated chow is not received there.

8. Page 5, second sentence, replace "(twice per day)" with "(once a day)".

Reason: More convenient for individual food consumption calculations.

9. Page 5, Section D. Assignment to Groups, second sentence, replace "housed two per cage" with "housed individually".

Reason: Animals will be housed individually to calculate individual food consumption.

10. Page 6, Section E. Animal Husbandry, second paragraph should read "Animal rooms will be targeted at a temperature of 23 ± 2°C for rats and 25 ± 2°C for microswine and a relative humidity of 50 ± 10%."

Reason: Microswine should be kept at a higher temperature than rats.

11. Page 6, Section E. Animal Husbandry, after second paragraph, insert the following paragraph: Animal rooms that will house rats will be artificially illuminated (fluorescent light) on a 12-hour light/dark cycle. Animal rooms that will house microswine will be artificially illuminated (fluorescent light) on a 12-hour light/dark cycle with additional natural light being available through a window at one end of the room.

Reason: Microswine do not need to be on a 12 hour light cycle. The animals should be housed in conditions similar to a barn.

12. Page 2, Section A. Single Dose Administration, "Blood from the single dose administered group of rats was collected in lead-free tubes containing EDTA. Heparin containing tubes were used for collecting blood from the other groups.

Reason: EDTA caused complications in analyzing blood lead by atomic absorption.

13. Page 6, Section E. Animal Husbandry, add the following paragraph "Microswine were provided with recreational equipment (balls) in their cages.

Reason: To improve housing conditions. Balls were added after consulting with Linda Panepinta, Swine Research Consultant.

14. Page 6, Section F. Diet Preparation and Analysis, the following should be added "Microswine were given Oreo cookies after blood sampling.

Reason: Linda Panepinta, Swine Research Consultant, suggested the administration of Oreo cookies after blood sampling as a reward.

15. Page 7, first paragraph, the following sentence should be added "Diet samples will be refrigerated, not frozen until analysis.

Reason: Analysis was done within a short period of time after sampling.

16. "Due to technical error, the 12 hr. interval blood collection was not taken for the single dose gavage group of rats. Five male rats from the animals not used on the study were randomly selected, prebled, dosed by gavage, and bled after 12 hrs.

Reason: Technical error.

17. Page 8, Section K. Sacrifice of Animals, Replace the final sentence with "Tissues from rats bled at the 240 hr. time point will be saved at sacrifice.

Reason: Tissues from 5 rats only are needed for statistical analysis.

Date

Approved: H. N. Ghantous July 20/1993

Hanan N. Ghantous
Study Director

Matthew S. Bogdanffy 7/26/93

Matthew S. Bogdanffy
Manager
Biochemical Toxicology

cc: P. E. Ross
G. S. Elliott
T. S. Bingman (REO-PIT)
K. Mc Cleary
M. S. Bogdanffy
R. C. Rhea

TO: MEDICAL RESEARCH PROJECT NO. 9660-001

The following changes are made to the original protocol:

page 2 *STUDY DESIGN*
A. Single Dose Administration
first and third bullets

Change '...and ten male microswine...' to '...and nine male microswine...' in the first bulleted item. Change '...and five microswine will be dosed orally (gavage)...' to '...and four microswine will be dosed orally (gavage)...' in the third bulleted item. One microswine became ill and was sacrificed *in extremis*.

page 4 *MATERIALS AND METHODS*
A. Test Material
paragraph 1, sentence 1

Replace the first sentence with: 'Lead acetate will be supplied by Aldrich, Milwaukee, WI 53733, U.S.A. and the test soils will be supplied by the sponsor.' This sentence was written incorrectly. It was always the intent to receive the lead acetate from Aldrich and the test soils from the sponsor.

page 7 *MATERIALS AND METHODS*
H. Food Consumption and Intake of Test Material
paragraph 1, sentence 1

Change '...food consumed by each microswine will be determined twice per week...' to '...food consumed by each microswine will be determined weekly...' For easier data comparison, microswine and rats were maintained on the same schedule for food consumption determination.

page 7 *MATERIALS AND METHODS*
I. Dosing
paragraph 1

Add the following sentences: 'One microswine designated for intravenous administration and one microswine designated for oral (gavage) administration will be fed one hour before dosing. Data from these animals will be reported separately.' The microswine ate all of their food immediately, while the rats tended to have food remaining in their food jars up to the time of dosing. Therefore, microswine were considered 'fasted' while the rats were considered 'fed.' To evaluate if being 'fed' made a difference, one microswine from each type of single dose administration was fed one hour before dosing.

page 8 *MATERIALS AND METHODS*
J. Blood Sampling
paragraph 1

Insert the following sentence after the original first sentence: 'For microswine in the single dose administration group, syringes will be washed with heparin before blood collection.' The blood collected from microswine that received a single dose after feeding for 30 consecutive days was clotting. The heparin wash was added to prevent this from occurring in the single dose administration group.

page 9 *RECORDS AND SAMPLE RETENTION*
paragraph 1, sentence 1

Change '...will be retained at Haskell Laboratory or at the Records Management Center,...' to '...will be retained at Haskell Laboratory, Jackson Laboratory (DuPont Chambers Works), or at the Records Management Center,...' Records and samples from the analyses for lead will be maintained at Jackson Laboratory.

Approved by: _____ 11/23/93
Hanan N. Ghantous, Ph.D. (date)
Study Director

_____ 11/23/93
Matthew S. Bogdanffy, Ph.D. (date)
Manager
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cc: T.S. Bingman
K.A. McCleary
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APPENDIX B. GLP DEVIATIONS

The following GLP deviations occurred in this study (MR 9660-001):

1. The analytical notebooks include a few write-overs and other changes where the original entry is not legible.

2. Approximately 15 (of over 4,000) original analytical instrument printouts cannot be located.

3. Due to publicly available data on lead, the stability of the test substance was not determined.

4. Some Standard Operation Procedures (SOPs) concerning microswine procedures were not issued when the procedures were performed. However, SOPs, based on the methods used for this study, have now been issued.

APPENDIX C. BLOOD LEAD CONCENTRATION BEFORE INTRAVENOUS OR ORAL DOS

ABBREVIATION

RS - Removed from Study

BLOOD LEAD CONCENTRATION (µg/ml) IN MALE RATS BEFORE INTRAVENOUS OR ORAL DOSING

GROUP I		GROUP II		GROUP II-1[a]		GROUP III		GROUP IV	
101	0.010	201	0.034	7400	0.010	301	0.100	401	0.101
102	0.000	202	0.105	7401	0.000	302	RS (Day 0)	402	0.061
103	0.000	203	0.023	7402	0.000	303	0.106	403	0.086
104	0.000	204	0.026	7403	0.000	304	0.095	404	0.063
105	0.006	205	0.007	7404	0.013	305	0.068	405	0.133
106	0.000	206	0.032			306	0.080	406	0.088
107	0.006	207	0.042			307	0.079	407	0.103
108	0.009	208	0.016			308	0.066	408	0.076
109	0.044	209	0.023			309	0.098	409	0.155
110	0.010	210	0.016			310	0.065	410	0.062
111	0.050	211	0.023			311	0.057	411	0.075
112	0.013	212	0.016			312	0.077	412	0.089
113	0.013	213	0.012			313	0.082	413	0.055
114	0.010	214	0.013			314	0.042	414	0.066
115	0.015	215	0.024			315	0.046	415	0.061
116	0.010	216	0.020			316	0.069	416	0.063
117	0.006	217	0.026			317	0.066	417	0.079
118	0.012	218	0.015			318	RS (Day 0)	418	0.052
119	0.012	219	0.021			319	0.087	419	0.041
120	0.005	220	0.016			320	0.092	420	0.060
121	0.016	221	0.024			321	0.053	421	0.059
122	0.012	222	0.008			322	0.037	422	0.041
123	0.015	223	0.000			323	0.051	423	0.124
124	0.000	224	0.000			324	0.069	424	0.089
125	0.000	225	0.000			325	0.081	425	0.052

[a] 12-hour bleed inadvertently missed. Five extra animals (reported here) were selected to be dosed and bled after 12 hours.

BLOOD LEAD CONCENTRATION (μg/ml) IN MALE MICROSWINE
BEFORE INTRAVENOUS OR ORAL DOSING

GROUP V		GROUP VI		GROUP VII		GROUP VIII	
501	0.203	601a	0.006	701	0.169	801	0.007
502	0.201	602	0.027	702	0.119	802	0.005
503	0.194	603	0.000	703	0.145	803a	0.000
504	0.145	604	0.140	704	0.168	804	0.000
505	0.090	605	0.016	705	0.224	805	0.000
506	0.143	606	0.007	706	0.255		

[a] Fed one hour before dose administration.

APPENDIX D. BLOOD LEAD CONCENTRATION AFTER INTRAVENOUS OR ORAL DOSI

ABBREVIATION

RS - Removed from Study

BLOOD LEAD CONCENTRATION (μg/ml) IN MALE RATS
AFTER INTRAVENOUS OR ORAL DOSING
Group I: Single Intravenous Dose

COLLECTION TIME (hrs)	INDIVIDUAL ANIMAL NUMBER AND BLOOD LEAD CONCENTRATION									
	SAMPLE 1		SAMPLE 2		SAMPLE 3		SAMPLE 4		SAMPLE 5	
0.5	102	39.8	106	68.9	110	24.3	116	54.6	122	49.2
1.0	108	18.6	109	24.6	112	60.5	123	22.8	124	34.1
2.0	101	15.0	104	15.1	107	19.1	111	16.6	115	4.1
4.0	105	11.0	113	7.7	114	6.6	120	2.6	121	20.3
8.0	103	3.1	117	4.5	118	6.5	119	3.6	125	0.3
12.0	104	6.2	107	8.5	111	0.8	115	2.0	101	--a
24.0	108	6.4	109	3.3	112	6.6	123	5.2	124	2.7
48.0	103	0.3	117	2.0b	118	6.8b	119	4.1	125	0.4
72.0	105	3.8	113	1.8	114	3.1	120	1.6	121	0.5
96.0	102	2.4	106	1.4	110	0.1	116	1.3	122	1.4
120.0	108	2.0	109	1.1	112	2.3	123	0.9	124	0.7
144.0	101	0.8	104	1.0	107	1.6	111	2.7	113	0.5
192.0	103	0.2	117	0.6	118	0.9	119	0.6	125	0.2
240.0	105	0.5	113	0.4	114	0.4	120	0.3	121	0.4c

[a] Sample not sent for analysis.
[b] Value derived from reanalysis of sample.
[c] Value derived from second reanalysis of sample.

BLOOD LEAD CONCENTRATION (µg/ml) IN MALE RATS
AFTER INTRAVENOUS OR ORAL DOSING
Group II: Single Oral Dose

COLLECTION TIME (hrs)	INDIVIDUAL ANIMAL NUMBER AND BLOOD LEAD CONCENTRATION									
	SAMPLE 1		SAMPLE 2		SAMPLE 3		SAMPLE 4		SAMPLE 5	
0.5	221	0.015	220	0.021	214	0.024	213	0.031	205	0.029
1.0	216	0.022	202	0.023	222	0.020	206	0.029	210	0.018
2.0	208	0.059	224	0.053	209	0.057	212	0.047	223	0.031
4.0	204	0.071	215	0.068	211	0.197	207	0.071	201	0.155
8.0	203	0.290	217	0.320	218	0.530	219	0.250	225	0.230
12.0	7400 a	0.136	7401 a	0.113	7402 a	0.154	7403 a	0.208	7404 a	0.113
24.0	202	0.125	206	0.055	210	0.059	216	0.030	222	0.043
48.0	209	0.054	208	0.059	223	0.041	224	0.039	212	0.039
72.0	201	0.036	204	0.034	207	0.030	211	0.035	215	0.015
96.0	203	0.050	217	0.082	218	0.336b	219	0.064	225	0.074
120.0	205	0.022	213	0.032	214	0.016	220	0.021	221	0.017
144.0	202	0.032	206	0.016	210	0.030	216	0.017	222	0.018
192.0	201	0.011	204	0.030	207	0.024	211	0.016	215	0.012
240.0	208	0.009	209	0.012	212	0.000	223	0.000	224	0.018

[a] 12-hour bleed inadvertently missed. Five extra animals (reported here) were dosed and bled after 12 hours.
[b] Value considered to be an error; not included in mean bioavailability calculation.

BLOOD LEAD CONCENTRATION (µg/ml) IN MALE RATS
AFTER INTRAVENOUS OR ORAL DOSING
Group III: Single Intravenous Dose After Feeding for 30 Consecutive Days

COLLECTION TIME (hrs)	INDIVIDUAL ANIMAL NUMBER AND BLOOD LEAD CONCENTRATION									
	SAMPLE 1		SAMPLE 2		SAMPLE 3		SAMPLE 4		SAMPLE 5	
0.5	321	14.2	320	11.9	314	19.4	313	32.5	305	15.1
1.0	316	20.7	322	13.0	306	18.3	310	17.5	--a	
2.0	308	7.5	324	19.1	309	4.3	312	12.2	323	9.7
4.0	304	6.6	301	12.2	307	4.4	311	4.6	315	7.1
8.0	303	6.1	317	3.2	319	0.8	325	3.9	--a	
12.0	305	4.5	313	5.3	314	5.8	320	4.2	321	6.8
24.0	306	0.4	310	2.1	316	1.9	322	0.7	--a	
48.0	308	2.2	309	1.2	312	7.9	323	1.7	324	6.9
72.0	301	2.8	304	1.4	307	0.7	311	1.3	315	1.0
96.0	303	0.7	317	0.4	319	0.1	325	0.9	--a	
120.0	305	0.6	313	1.6	314	0.5	320	0.7	321	0.8
144.0	306	0.7	310	0.6	316	1.2	322	0.5	--a	
192.0	301	0.7	304	0.5	307	0.4	311	0.5	315	0.3
240.0	308	0.4	309	0.6	312	0.4	323	0.6	324	1.7

[a] Because two rats were removed from the study, these timepoints included only four samples.

BLOOD LEAD CONCENTRATION (µg/ml) IN MALE RATS
AFTER INTRAVENOUS OR ORAL DOSING
Group IV: Single Oral Dose After Feeding for 30 Consecutive Days

COLLECTION TIME (hrs)	INDIVIDUAL ANIMAL NUMBER AND BLOOD LEAD CONCENTRATION									
	SAMPLE 1		SAMPLE 2		SAMPLE 3		SAMPLE 4		SAMPLE 5	
0.5	405	0.108	413	0.045	414	0.040	420	0.069	421	0.047
1.0	402	0.089	406	0.048	410	0.115	416	0.079	422	0.037
2.0	408	0.084	409	0.053	412	0.036	423	0.026	424	0.045
4.0	401	0.120	404	0.158	407	0.114	411	0.109	415	0.065
8.0	403	0.124	417	0.190	418	0.160	419	0.121	425	0.133
12.0	405	0.101	413	0.032	414	0.058	420	0.062	421	0.072
24.0	402	0.050	406	0.102	410	0.123	416	0.110	422	0.092
48.0	408	0.096	409	0.083	412	0.083	423	0.103	424	0.469a
72.0	401	0.016	404	0.014	407	0.074	411	0.025	415	0.044
96.0	403	0.082	417	0.051	418	0.000	419	0.000	425	0.000
120.0	405	0.000	413	0.000	414	0.078	420	0.000	421	0.038
144.0	401	0.060	404	0.024	407	0.064	411	0.042	415	0.033
192.0	402	0.019	406	0.045	410	0.029	416	0.039	422	0.025
240.0	408	0.038	409	0.167a	412	0.065	423	0.086	424	0.045

[a] Value considered to be an error; not included in mean bioavailability calculation.

BLOOD LEAD CONCENTRATION (µg/ml) IN MALE MICROSWINE
AFTER INTRAVENOUS OR ORAL DOSING
Group VI: Single Intravenous Dose

COLLECTION TIME (hrs)	INDIVIDUAL ANIMAL NUMBER AND BLOOD LEAD CONCENTRATION									
	SAMPLE 1		SAMPLE 2		SAMPLE 3		SAMPLE 4		SAMPLE 5	
0.5	602	1.850	603	1.280	604	2.070	605	0.410	606	2.020
1.0	602	1.140	603	1.850	604	2.230	605	0.300	606	1.280
2.0	602	0.530	603	0.290	604	1.160	605	0.320	606	0.470
4.0	602	0.300a	603	0.400a	604	0.460a	605	0.200a	606	0.430a
8.0	602	0.270	603	0.470	604	0.390	605	0.240	606	0.340
12.0	602	0.310	603	0.370	604	0.470	605	0.260	606	0.350
24.0	602	0.450	603	0.350	604	0.420	605	0.110	606	0.200
48.0	602	0.230	603	0.320	604	0.250	605	0.190	606	0.090
72.0	602	1.980b	603	0.480	604	0.320	605	0.100	606	0.320
96.0	602	0.370	603	0.180	604	0.230	605	0.020	606	0.180
120.0	602	0.190	603	0.140	604	0.140	605	0.050	606	0.090
144.0	602	0.267b	603	0.136	604	0.165	605	0.032	606	0.101
192.0	602	0.118	603	0.088	604	0.102	605	0.032	606	0.106
240.0	602	0.086	603	0.109	604	0.125	605	0.016	606	0.069

[a] Value derived from reanalysis of sample.
[b] Value considered to be an error; not included in mean bioavailability calculation.

BLOOD LEAD CONCENTRATION (µg/ml) IN MALE MICROSWINE
AFTER INTRAVENOUS OR ORAL DOSING
Group VIII: Single Oral Dose

COLLECTION TIME (hrs)	INDIVIDUAL ANIMAL NUMBER AND BLOOD LEAD CONCENTRATION							
	SAMPLE 1		SAMPLE 2		SAMPLE 3		SAMPLE 4	
0.5	801	0.007	802	0.000	804	0.007	805	0.006
1.0	801	0.019	802	0.007	804	0.023	805	0.016
2.0	801	0.027	802	0.010	804	0.059	805	0.028
4.0	801	0.096	802	0.049	804	0.057	805	0.052
8.0	801	0.079	802	0.078	804	0.056	805	0.074
12.0	801	0.133	802	0.073	804	0.096	805	0.115
24.0	801	0.074	802	0.015	804	0.074	805	0.105
48.0	801	0.031	802	0.036	804	0.025	805	0.079
72.0	801	0.026	802	0.040	804	0.023	805	0.041
96.0	801	0.020	802	0.036	804	0.010	805	0.022
120.0	801	0.031	802	0.055	804	0.030	805	0.098
144.0	801	0.014a	802	0.021a	804	0.022a	805	0.039a
192.0	801	0.081	802	0.026	804	0.033	805	0.025
240.0	801	0.022	802	0.013	804	0.013	805	0.022

[a] Value derived from reanalysis of sample.

BLOOD LEAD CONCENTRATION (µg/ml) IN MALE MICROSWINE
AFTER INTRAVENOUS OR ORAL DOSING
Group V: Single Intravenous Dose After Feeding for 30 Consecutive Days

COLLECTION TIME (hrs)	INDIVIDUAL ANIMAL NUMBER AND BLOOD LEAD CONCENTRATION					
	SAMPLE 1	SAMPLE 2	SAMPLE 3	SAMPLE 4	SAMPLE 5	SAMPLE 6
0.5	501 7.350	502 1.400	503 2.730	504 0.990	505 2.620	506 7.880
1.0	501 3.040	502 0.170	503 0.980	504 0.310	505 2.330	506 1.030
2.0	501 1.170	502 0.320	503 1.250	504 0.280	505 1.340	506 3.190
4.0	501 0.760	502 0.390	503 0.370	504 0.290	505 1.220	506 1.930
8.0	501 0.310	502 0.180	503 0.880	504 0.130	505 0.340	506 1.120
12.0	501 0.270	502 0.120	503 0.860	504 0.190	505 0.310	506 1.950
24.0	501 0.240	502 0.220	503 0.240	504 0.110	505 0.240	506 0.820
48.0	501 0.360	502 0.250	503 0.650	504 0.070	505 0.160	506 0.520
72.0	501 0.122	502 0.240	503 0.201	504 0.069	505 0.376	506 0.460
96.0	501 0.313	502 0.227	503 0.305	504 0.110	505 0.225	506 0.554
120.0	501 0.239	502 0.147	503 0.372	504 0.112	505 0.193	506 0.438
144.0	501 0.225	502 0.057	503 0.206	504 0.115	505 0.369	506 0.372
192.0	501 0.193	502 0.085	503 0.224	504 0.087	505 0.195	506 0.219
240.0	501 0.195	502 0.110	503 0.135	504 0.069	505 0.120	506 0.212

BLOOD LEAD CONCENTRATION (µg/ml) IN MALE MICROSWINE
AFTER INTRAVENOUS OR ORAL DOSING
Group VII: Single Oral Dose After Feeding for 30 Consecutive Days

COLLECTION TIME (hrs)	INDIVIDUAL ANIMAL NUMBER AND BLOOD LEAD CONCENTRATION				
	SAMPLE 1	SAMPLE 2	SAMPLE 3	SAMPLE 4	SAMPLE 5
0.5	701 0.255	702 0.177	703 0.180	704 0.200	705 0.130	706 0.203
1.0	701 0.288	702 0.112	703 0.282	704 0.210	705 0.190	706 0.242
2.0	701 0.222	702 0.207	703 0.142	704 0.170	705 0.258	706 0.347
4.0	701 0.158	702 0.142	703 0.317	704 0.366	705 0.275	706 0.304
8.0	701 0.340	702 0.268	703 0.322	704 0.268	705 0.160	706 0.133
12.0	701 0.267	702 0.316	703 0.234	704 0.187	705 0.255	706 0.143
24.0	701 0.318	702 0.220	703 0.216	704 0.224	705 0.203	706 0.131
48.0	701 0.263	702 0.302	703 0.200	704 0.137	705 0.164	706 0.271
72.0	701 0.159	702 0.224	703 0.114	704 0.204	705 0.181	706 0.254
96.0	701 0.172	702 0.189	703 0.104	704 0.176	705 0.199	706 0.168
120.0	701 0.122	702 0.146	703 0.161	704 --[a]	705 0.183	706 0.181
144.0	701 0.144	702 0.097	703 0.128	704 0.172	705 0.163	706 0.132
192.0	701 0.116	702 0.101	703 0.097	704 0.127	705 0.130	706 0.157
240.0	701 0.100	702 0.091	703 0.095	704 0.111	705 0.118	706 0.160

[a] Inadequate amount of blood collected; sample not submitted for analysis.

Analysis of Pb in Blood, Soil, and Associated Materials
in Support of Haskell Lab Medical Research Project No. MR 9660

Author
Keith A. McCleary

Study Completed:
8/30/93

Performed By:
DuPont Specialty Chemicals
Trace Metals Analysis Group
Quality Control Laboratory
Chambers Works
Deepwater, NJ 08023

Signature: Keith A McCleary 12/8/93
Keith A. McCleary, Ph.D.

Summary

The purpose of this study was to determine the amount of lead that was contained in samples associated with MR9660. There were essentially three type of samples: blood (rat and microswine), aqueous (drinking water, dosing solutions), and solid (soil, bedding, etc.). Blood samples were prepared by dilution with a modifier solution (to digest sample and break viscosity) and analyzed using Graphite Furnace Atomic Absorption Spectrometry (GFAAS). Aqueous and solid samples were prepared using microwave assisted digestion and analyzed using Inductively Coupled Plasma Atomic Emission Spectometry (ICP-AES). Generally, all samples were run in duplicate and average answers reported. In the case of the blood, each sample and duplicate was spiked and checked for recovery. If the spike recovery was greater than 10% in error, a one point standard additions calculation was performed. Greater than 1000 samples were analyzed throughout the duration of this study.

Methodology

The descriptions listed below are summaries of the actual methods used to analyze samples. The actual methods are documented in the Procedures Notebook, archived at QCL and copies are available upon request.

Sample Preparation: Blood

Blood samples were received in small polyethylene vails. Approximate sample volume was 100-300 uL. 100 uL of blood is diluted to 1.0 mL with modifier solution. The modifier solution is a solution of nitric acid (digests sample), Triton X-100 (surfactant, breaks viscosity), methanol (antifoaming agent), and ammonium phosphate (GFAAS modifier) in water.

Sample Analysis: Blood

Blood samples are analyzed using Graphite Furnace Atomic Absorption Spectrometry (GFAAS). In GFAAS, a 20 microliter volume of sample + modifier is injected into a small graphite tube. This tube is electromagnetically heated to dry, char, and atomize the sample. A hollow cathode lamp (HCL) shines light corresponding to the atomic emission of Pb (283.3 nm) through the tube. As free Pb atoms are generated by the sample atomization, they absorb the radiation, attenuating the amount of signal from the HCL. The degree of attenuation of the HCL radiation is proportional to the concentration of Pb in the sample. Calibration standards of 10, 20 ,and 30 ng/mL are prepared by serial dilution of a 1000 ug/mL stock. Each blood sample is analyzed twice. Each sample and duplicate is then spiked with 10ng Pb and reanalyzed to check for recovery. If recovery is less than 90% or greater than 110%, a one point standard addition calculation is

performed to determine the amount of Pb in the sample. Each result is the average of the two replicates.

Sample Preparation: Aqueous Samples

Samples that are primarily aqueous are prepared by acidifying 10.00 mL of sample with 2.00 mL concentrated HNO3. The sample is then diluted to a final volume of 20.00 mL and analyzed by ICP-AES.

Sample Preparation: Solid Samples

Solid Samples are prepared by microwave assisted digestion. 0.1 - 0.2 g of sample are digested with 5.00 mL concentrated HNO3 in a Teflon lined pressure vessel. The vessels are placed in a microwave digestion apparatus and cooked for 10 minutes at 300@W power. The samples are allowed to cool and are then filtered into a 50 mL volumetric flask and diluted to volume with deionized water. Samples are analyzed for Pb content by ICP-AES.

Sample Analysis : Aqueous and Solid Samples

These samples are analyzed by Inductively Coupled Plasma - Atomic Emission Spectrometry (ICP-AES). The prepared samples are pumped into an sample introduction system where a fine spray of aerosol is generated. This sample aerosol flows along a stream of argon gas into the plasma. The plasma is a highly energetic discharge sustained in argon by applied radio-frequency power in excess of 1000 W. This discharge is energetic enough to desolvate, atomize, and excite the sample species to radiative emission. The intensity of the characteristic emission wavelength of Pb (220.35 nm) is proportional to the amount of Pb in the sample.

Results

The results for all samples submitted under MR9660 are listed immediately following this page.

APPENDIX H. ANALYSIS OF LEAD IN BLOOD, SOIL, AND ASSOCIATED MATERIALS

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Sample	Lead ng/mL		Sample	Lead ug/mL		Sample	Lead ug/mL		Sample	Lead ug/mL	
a-101	10		102-0.5	39.8		104-12	6.2		A101-144	0.8	
a-102	<5		106-0.5	68.9		107-12	8.5		A104-144	1	
a-103	<5		110-0.5	24.3		111-12	0.8		A107-144	1.6	
a-104	<5		116-0.5	54.6		115-12	1.97		A111-144	2.7	
a-105	6		122-0.5	49.2					A113-144	0.5	
a-106	<5					A108-24	6.4				
a-107	6		108-1	18.6		A109-24	3.3		A103-192	0.2	
a-108	9		109-1	24.6		A112-24	6.6		A117-192	0.6	
a-109	44		112-1	60.5		A123-24	5.2		A118-192	0.9	
a-110	10		123-1	22.8		A124-24	2.7		A119-192	0.6	
a-111	50		124-1	34.1					A125-192	0.2	
a-112	13					A103-48	0.3				
a-113	13		101-2	15		A117-48	10.3		A105-240	0.46	
a-114	10		104-2	15.1		A118-48	13.2		A113-240	0.36	
a-115	15		107-2	19.1		A119-48	4.1		A114-240	0.4	
a-116	10		111-2	16.6		A125-48	0.4		A120-240	0.32	
a-117	6		115-2	4.1					A121-240	5.9	
a-118	12					A105-72	3.8				
a-119	12		105-4	11		A113-72	1.8		STABILITY		
a-120	5		113-4	7.7		A114-72	3.1		A105-240s	0.45	
a-121	16		114-4	6.6		A120-72	1.8		A113-240s	0.46	
a-122	12		120-4	2.6		A121-72	0.5		A114-240s	0.5	
a-123	15		121-4	20.3					A120-240s	0.22	
a-124	<5					A102-96	2.4		A121-240s	0.54	
a-125	<5		103-8	3.1		A106-96	1.4				
			117-8	4.5		A110-96	0.1		REPEATS		
			118-8	6.5		A116-96	1.3		A107-12	6.34	
			119-8	3.6		A122-96	1.4		A117-48	1.99	
			125-8	0.3					A118-48	6.79	
						A108-120	2				
						A109-120	1.1		A121-240(6/2)		0.2
						A112-120	2.3		A121-240(6/7)		0.3
						A123-120	0.9				
						A124-120	0.7				

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Sample	Pb, ng/mL	Sample	Pb, ng/mL	Sample	Pb, ng/mL	Sample	Pb, ng/mL
B201-PRE	34	B221-0.5	15	B202-24	125	B202-144	32
B202-PRE	105	B220-0.5	21	B206-24	55	B206-144	16
B203-PRE	23	B214-0.5	24	B210-24	59	B210-144	30
B204-PRE	26	B213-0.5	31	B216-24	30	B216-144	17
B205-PRE	7	B205-0.5	29	B222-24	43	B222-144	18
B206-PRE	32						
B207-PRE	42	B216-1	22	B209-48	54	B201-192	11
B208-PRE	16	B202-1	23	B208-48	59	B204-192	30
B209-PRE	23	B222-1	20	B223-48	41	B207-192	24
B210-PRE	16	B206-1	29	B224-48	39	B211-192	16
B211-PRE	23	B210-1	18	B212-48	39	B215-192	12
B212-PRE	16						
B213-PRE	12	B208-2	59	B201-72	36	B208-240	9
B214-PRE	13	B224-2	53	B204-72	34	B209-240	12
B215-PRE	24	B209-2	57	B207-72	30	B212-240	<5
B216-PRE	20	B212-2	47	B211-72	35	B223-240	<5
B217-PRE	26	B223-2	31	B215-72	15	B224-240	18
B218-PRE	15						
B219-PRE	21	B204-4	71	B203-96	50	II-1-7400pre	10
B220-PRE	16	B215-4	68	B217-96	82	II-1-7401pre	<5
B221-PRE	24	B211-4	197	B218-96	336	II-1-7402pre	<5
B222-PRE	8	B207-4	71	B219-96	64	II-1-7403pre	<5
B223-PRE	<5	B201-4	155	B225-96	74	II-1-7404pre	13.1
B224-PRE	<5						
B225-PRE	<5	B203-8	290	B205-120	22	II-1-7400-12	136
		B217-8	320	B213-120	32	II-1-7401-12	113
		B218-8	530	B214-120	16	II-1-7402-12	154
		B219-8	250	B220-120	21	II-1-7403-12	208
		B225-8	230	B221-120	17	II-1-7404-12	113

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Sample	Pb, ng/mL	Sample	Pb, ng/mL	Sample	Pb, ng/mL	Sample	Pb, ng/mL	Sample	Pb, ng/mL
C301	56			C301 8/3	95	C301 6/10	54	C301 6/17	100
C303	8	C303 5/27	237	C303 6/3	122	C303 6/10	96	C303 6/17	106
C304	19	C304 5/27	132	C304 6/3	83	C304 6/10	99	C304 6/17	95
C305	9	C305 5/27	108	C305 6/3	94	C305 6/10	59	C305 6/17	68
C306	5			C306 6/3	138	C306 6/10	81	C306 6/17	80
C307	6	C307 5/27	77	C307 6/3	112	C307 6/10	55	C307 6/17	79
C308	5	C308 5/27	90	C308 6/3	175	C308 6/10	78	C308 6/17	66
C309	11			C309 6/3	195	C309 6/10	64	C309 6/17	98
C310	14	C310 5/27	58	C310 6/3	122	C310 6/10	70	C310 6/17	66
		C311 5/27	124	C311 6/3	92	C311 6/10	84	C311 6/17	57
		C312 5/27	65	C312 6/3	26	C312 6/10	128	C312 6/17	77
C313	7	C313 5/27	48	C313 6/3	46	C313 6/10	56	C313 6/17	82
C314	5	C314 5/27	70	C314 6/3	77	C314 6/10	44	C314 6/17	42
C315	34	C315 5/27	62	C315 6/3	54	C315 6/10	51	C315 6/17	46
C316	7	C316 5/27	100	C316 6/3	45	C316 6/10	38	C316 6/17	69
C317	11	C317 5/27	39	C317 6/3	54	C317 6/10	56	C317 6/17	66
C319	4	C319 5/27	103	C319 6/3	63	C319 6/10	64	C319 6/17	87
C320	6	C320 5/27	51	C320 6/3	89	C320 6/10	66	C320 6/17	92
C321	6	C321 5/27	121	C321 6/3	51	C321 6/10	69	C321 6/17	53
C322	5	C322 5/27	59	C322 6/3	48	C322 6/10	31	C322 6/17	37
C323	3	C323 5/27	132	C323 6/3	36	C323 6/10	26	C323 6/17	51
C324	<2	C324 5/27	46	C324 6/3	38	C324 6/10	48	C324 6/17	69
C325	24	C325 5/27	64	C325 6/3	12	C325 6/10	78	C325 6/17	81

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Sample	Pb,ug/mL	Sample	Pb,ug/mL	Sample	Pb,ug/mL
C321 0.5	14.2	C306-24	0.4	C301-192	0.74
C320 0.5	11.9	C310-24	2.1	C304-192	0.53
C314 0.5	19.4	C316-24	1.9	C307-192	0.43
C313 0.5	32.5	C322-24	0.7	C311-192	0.45
C305 0.5	15.1			C315-192	0.28
		C308-48	2.2		
C316 1.0	20.7	C309-48	1.2	C308-240	0.41
C322 1.0	13	C312-48	7.9	C309-240	0.64
C306 1.0	18.3	C323-48	1.7	C312-240	0.37
C310 1.0	17.5	C324-48	6.9	C323-240	0.58
				C324-240	1.72
C308 2.0	7.5	C301-72	2.8		
C324 2.0	19.1	C304-72	1.4		
C309 2.0	4.3	C307-72	0.7		
C312 2.0	12.2	C311-72	1.3		
C323 2.0	9.7	C315-72	1		
C304 4.0	6.6	C303-96	0.7		
C301 4.0	12.2	C317-96	0.4		
C307 4.0	4.4	C319-96	0.1		
C311 4.0	4.6	C325-96	0.9		
C315 4.0	7.1				
		C305-120	0.6		
C303-8	6.1	C313-120	1.6		
C317-8	3.2	C314-120	0.5		
C319-8	0.8	C320-120	0.7		
C325-8	3.9	C321-120	0.8		
C305-12	4.5	C308-144	0.7		
C313-12	5.3	C310-144	0.6		
C314-12	5.8	C316-144	1.2		
C320-12	4.2	C322-144	0.5		
C321-12	6.8				

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Sample	Pb, ug/mL
Rat Gavage DOSING SOLUTION	
GROUP B	311.9
GROUP B	310.9
DEIONIZED WATER	
	<5
1000 PPM Pb SOIL	
	840.9
SUCROSE	
	<5
Swine IV Single Dose Sampl	
1a	116.6mg/g
2a	126.8mg/g
swine gavage	
7/16/93 30 Day	
10a	0.425 mg/g
11a	0.348 mg/g
YAM 12/8/93	

Sample	Pb, ug/mL
SUCROSE DIET SAMPLE	
	<5
OREO COOKIES	
	<5
TEKLAD BASAL	
SWINE	<5
PURINA RAT CHOW	
	<5
SWINE BEDDING	
	<5
DI H2O	<1
Pb ACETATE (RAT IV SOLN)	
1.27%	
(400mg/20mL)	
Swine IV 30 Day Solution	
118.6 mg/g	
Swine single gavage 8/93	
3a	0.339mg/g
4a	0.347mg/g

Sample	Pb, mg/kg
#1 top of mixer 5/17/93	
1a	41
2b	46
3c	40
#1 middle of mixer 5/17/93	
4a	46
5b	42
6c	47
#1 bottom mixer 5/17/93	
7a	37
8b	38
9c	38
top mixer 6/17/93	
1a	43.6
2a	46.6
3a	44
middle mixer 6/17/93	
4a	42.3
5a	45.7
6a	43.1
bottom mixer 6/17/93	
7a	45.6
8a	51
9a	44.5

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Sample	Pb, ng/mL	Sample	Pb, ng/mL	Sample	Pb, ng/mL	Sample	Pb, ng/mL	Sample	Pb, ng/mL
C401-PRE	9	D401 6/17	103	D401 6/24	78	D401 7/1	120	D401 7/8	101
C402-PRE	<5	D402 6/17	102	D402 6/24	88	D402 7/1	55	D402 7/8	61
C403-PRE	<5	D403 6/17	147	D403 6/24	88	D403 7/1	89	D403 7/8	86
C404-PRE	<5	D404 6/17	95	D404 6/24	54	D404 7/1	57	D404 7/8	63
C405-PRE	<5	D405 6/17	71	D405 6/24	56	D405 7/1	120	D405 7/8	133
C406-PRE	8	D406 6/17	116	D406 6/24	124	D406 7/1	126	D406 7/8	88
C407-PRE	6	D407 6/17	145	D407 6/24	113	D407 7/1	109	D407 7/8	103
C408-PRE	<5	D408 6/17	83	D408 6/24	67	D408 7/1	64	D408 7/8	76
C409-PRE	<5	D409 6/17	135	D409 6/24	117	D409 7/1	157	D409 7/8	155
C410-PRE	<5	D410 6/17	59	D410 6/24	101	D410 7/1	80	D410 7/8	62
C411-PRE	<5	D411 6/17	44	D411 6/24	52	D411 7/1	96	D411 7/8	75
C412-PRE	<5	D412 6/17	72	D412 6/24	32	D412 7/1	50	D412 7/8	89
C413-PRE	<5	D413 6/17	67	D413 6/24	57	D413 7/1	38	D413 7/8	55
C414-PRE	7	D414 6/17	67	D414 6/24	37	D414 7/1	53	D414 7/8	66
C415-PRE	6	D415 6/17	78	D415 6/24	31	D415 7/1	34	D415 7/8	61
C416-PRE	5	D416 6/17	99	D416 6/24	44	D416 7/1	129	D416 7/8	63
C417-PRE	5	D417 6/17	123	D417 6/24	84	D417 7/1	108	D417 7/8	79
C418-PRE	<5	D418 6/17	18	D418 6/24	56	D418 7/1	45	D418 7/8	52
C419-PRE	<5	D419 6/17	15	D419 6/24	27	D419 7/1	45	D419 7/8	41
C420-PRE	<5	D420 6/17	55	D420 6/24	37	D420 7/1	105	D420 7/8	60
C421-PRE	7	D421 6/17	16	D421 6/24	16	D421 7/1	98	D421 7/8	59
C422-PRE	<5	D422 6/17	68	D422 6/24	24	D422 7/1	67	D422 7/8	41
C423-PRE	<5	D423 6/17	51	D423 6/24	101	D423 7/1	82	D423 7/8	124
C424-PRE	<5	D424 6/17	22	D424 6/24	62	D424 7/1	135	D424 7/8	89
C425-PRE	<5	D425 6/17	7	D425 6/24	18	D425 7/1	67	D425 7/8	52

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Sample	Pb,ng/mL	Sample	Pb,ng/mL	Sample	Pb,ng/mL
D405-0.5	108	D405-12	101	D405-120	<5
D413-0.5	45	D413-12	32	D413-120	<5
D414-0.5	40	D414-12	58	D414-120	78
D420-0.5	69	D420-12	62	D420-120	<5
D421-0.5	47	D421-12	72	D421-120	38
D402-1.0	89	D402-24	50	D401-144	60
D406-1.0	48	D406-24	102	D404-144	24
D410-1.0	115	D410-24	123	D407-144	64
D416-1.0	79	D416-24	110	D411-144	42
D422-1.0	37	D422-24	92	D415-144	33
D408-2	84	D408-48	98	D402-192	19
D409-2	53	D409-48	83	D406-192	45
D412-2	36	D412-48	83	D410-192	29
D423-2	26	D423-48	103	D416-192	39
D424-2	45	D424-48	469	D422-192	25
D401-4	120	D401-72	16	D408-240	38
D404-4	158	D404-72	14	D409-240	167
D407-4	114	D407-72	74	D412-240	65
D411-4	109	D411-72	25	D423-240	86
D415-4	65	D415-72	44	D424-240	45
D403-8	124	D403-96	82		
D417-8	190	D417-96	51		
D418-8	180	D418-96	<5		
D419-8	121	D419-96	<5		
D425-8	133	D425-96	<5		

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Sample	Pb, ng/mL	Sample	Pb, ng/mL	Sample	Pb, ug/mL	Sample	Pb, ug/mL	Sample	Pb, ng/mL	Sample	Pb, ng/mL
G501-PRE(a)	30	G501 7/7	139	G501-0.5	7.35	G501-8	0.31	G501-72	122	G501-192	193
G502-PRE(a)	8	G502 7/7	152	G502-0.5	1.4	G502-8	0.18	G502-72	240	G502-192	85
G503-PRE(a)	20	G503 7/7	225	G503-0.5	2.73	G503-8	0.88	G503-72	201	G503-192	224
G504-PRE(a)	34	G504 7/7	135	G504-0.5	0.99	G504-8	0.13	G504-72	69	G504-192	87
G505-PRE(a)	8	G505 7/7	173	G505-0.5	2.62	G505-8	0.34	G505-72	376	G505-192	195
G506-PRE(a)	17	G506 7/7	195	G506-0.5	7.88	G506-8	1.12	G506-72	460	G506-192	219
G501 6/16	129	G501 PRE(b)	203	G501-1	3.04	G501-12	0.27	G501-96	313	G501-240	195
G502 6/16	257	G502 PRE(b)	201	G502-1	0.17	G502-12	0.12	G502-96	227	G502-240	110
G503 6/16	323	duplicate	185	G503-1	0.98	G503-12	0.86	G503-96	305	G503-240	135
G504 6/16	150	G503 PRE(b)	194	G504-1	0.31	G504-12	0.19	G504-96	110	G504-240	69
G505 6/16	195	G504 PRE(b)	145	G505-1	2.33	G505-12	0.31	G505-96	225	G505-240	120
G506 6/16	160	G505PRE(b)	90	G506-1	1.03	G506-12	1.95	G506-96	554	G506-240	212
		G506 PRE(b)	143								
G501 6/23	100			G501-2	1.17	G501-24	0.24	G501-120	239	REPEATS (ug/mL)	
G502 6/23	190			G502-2	0.32	G502-24	0.22	G502-120	147	G503-12	0.58
G503 6/23	150			G503-2	1.25	G503-24	0.24	G503-120	372	G506-2	5.43*
G504 6/23	60			G504-2	0.28	G504-24	0.11	G504-120	112	G501-1	3
G505 6/23	140			G505-2	1.34	G505-24	0.24	G505-120	193	G505-1	5.33*
G506 6/23	100			G506-2	3.19	G506-24	0.82	G506-120	438	G501-0.5	4.40*
										G502-0.5	0.26*
G501 6/30	258			G501-4	0.76	G501-48	0.36	G501-144	225	G503-0.5	4.37*
G502 6/30	347			G502-4	0.39	G502-48	0.25	G502-144	57	G504-0.5	0.59*
G503 6/30	266			G503-4	0.37	G503-48	0.65	G503-144	206	G506-0.5	17.8*
G504 6/30	155			G504-4	0.29	G504-48	0.07	G504-144	115	*DENOTES	
G505 6/30	147			G505-4	1.22	G505-48	0.16	G505-144	389	CLOTTING	
G506 6/30	193			G506-4	1.93	G506-48	0.52	G506-144	372		
										CANNOT REPEAT	
										G502-1	G505-0.5
(a) denotes predose prior to weekly testing										G503-1	
(b) denotes predose prior to hourly testing										G504-1	
										G506-1	
										G503-8	

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Sample	Pb, ng/mL	Sample	Pb, ng/mL	Sample	Pb, ng/mL	Sample	Pb, ng/mL	Sample	Pb, ng/mL
H701-PRE	7	H701-PRE	169	H701-4	158	H701-48	263	H701-144	144
H702-PRE	23	H702-PRE	119	H702-4	142	H702-48	302	H702-144	97
H703-PRE	5	H703-PRE	145	H703-4	317	H703-48	200	H703-144	128
H704-PRE	57	H704-PRE	168	H704-4	366	H704-48	137	H704-144	172
H705-PRE	10	H705-PRE	224	H705-4	275	H705-48	164	H705-144	163
H706-PRE	7	H706-PRE	255	H706-4	304	H706-48	271	H706-144	132
H701 6/23	190	H701-0.5	255	H701-8	340	H701-72	159	H701-192	116
H702 6/23	130	H702-0.5	177	H702-8	268	H702-72	224	H702-192	101
H703 6/23	80	H703-0.5	180	H703-8	322	H703-72	114	H703-192	97
H704 6/23	170	H704-0.5	200	H704-8	268	H704-72	204	H704-192	127
H705 6/23	180	H705-0.5	130	H705-8	160	H705-72	181	H705-192	130
H706 6/23	290	H706-0.5	203	H706-8	133	H706-72	254	H706-192	157
H701 6/30	206	H701-1	288	H701-12	267	H701-96	172	H701-240	100
H702 6/30	208	H702-1	112	H702-12	316	H702-96	189	H702-240	91
H703 6/30	217	H703-1	282	H703-12	234	H703-96	104	H703-240	95
H704 6/30	281	H704-1	210	H704-12	197	H704-96	176	H704-240	111
H705 6/30	258	H705-1	190	H705-12	255	H705-96	199	H705-240	118
H706 6/30	201	H706-1	242	H706-12	143	H706-96	188	H706-240	160
H701 7/7	193	H701-2	222	H701-24	316	H701-120	122		
H702 7/7	169	H702-2	207	H702-24	220	H702-120	146		
H703 7/7	137	H703-2	142	H703-24	216	H703-120	161		
H704 7/7	209	H704-2	170	H704-24	224	H704-120	See Note		
H705 7/7	227	H705-2	258	H705-24	203	H705-120	183		
H706 7/7	173	H706-2	347	H706-24	131	H706-120	181		

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APPENDIX H. ANALYSIS OF LEAD IN BLOOD, SOIL, AND ASSOCIATED MATERIALS

Comprehensive Blood Report
PB_BL_CP.XLS

Sample	Pb, ng/mL	Sample	Pb, ng/mL	Sample	Pb, ng/mL	Repeat	Pb, ng/mL
F801 PRE	7	F801-12	133	F801-144	206	F803-48	65.1
F802 PRE	5	F802-12	73	F802-144	162	F801-144	14.4
F803 PRE	<5	F803-12	118	F803-144	147	F802-144	21.2
F804 PRE	<5	F804-12	96	F804-144	727	F803-144	9.9
F805 PRE	<5	F805-12	115	F805-144	33	F804-144	21.9
						F805-144	38.5
F801 0.5	7	F801-24	74	F801-192	81		
F802 0.5	<5	F802-24	15	F802-192	26		
F803 0.5	<5	F803-24	71	F803-192	23		
F804 0.5	7	F804-24	74	F804-192	33		
F805 0.5	6	F805-24	105	F805-192	25		
F801-1	19	F801-48	31	F801-240	22		
F802-1	7	F802-48	36	F802-240	13		
F803-1	23	F803-48	229	F803-240	9		
F804-1	23	F804-48	25	F804-240	13		
F805-1	16	F805-48	79	F805-240	22		
F801-2	27	F801-72	26				
F802-2	10	F802-72	40				
F803-2	33	F803-72	44				
F804-2	59	F804-72	23				
F805-2	28	F805-72	41				
F801-4	96	F801-96	20				
F802-4	49	F802-96	36				
F803-4	85	F803-96	15				
F804-4	57	F804-96	10				
F805-4	52	F805-96	22				
F801-8	79	F801-120	31				
F802-8	78	F802-120	55				
F803-8	65	F803-120	38				
F804-8	56	F804-120	30				
F805-8	74	F805-120	98				

12/8/93
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APPENDIX H. ANALYSIS OF LEAD IN BLOOD, SOIL, AND ASSOCIATED MATERIALS

Comprehensive Blood Report
PB_BL_CP.XLS

Sample	Pb, ng/mL	Sample	Pb, ug/mL	Sample	Pb, ug/mL	Sample	Pb, ng/mL	REPEATS	Pb, ug/mL
E601-PRE	6	E601-4	9.8	E601-48	0.39	E601-144	232	E601-0.5	22.35
E602-PRE	27	E602-4	1.9	E602-48	0.23	E602-144	267	E601-1	21.51
E603-PRE	<5	E603-4	6.3	E603-48	0.32	E603-144	136	E601-2	16.7
E604-PRE	14	E604-4	4.1	E604-48	0.25	E604-144	185		
E605-PRE	16	E605-4	clotted	E605-48	0.19	E605-144	32	REPEATS	Pb, ug/mL
E606-PRE	7	E606-4	5.3	E606-48	0.09	E606-144	101	E601-4	2.6
								E602-4	0.3
Sample	Pb, ug/mL	E601-8	1.6	E601-72	0.44	E601-192	148	E603-4	0.4
E601-0.5	22.3	E602-8	0.27	E602-72	1.98	E602-192	118	E604-4	0.46
E602-0.5	1.85	E603-8	0.47	E603-72	0.48	E603-192	88	E605-4	0.2
E603-0.5	1.28	E604-8	0.39	E604-72	0.32	E604-192	102	E606-4	0.43
E604-0.5	2.07	E605-8	0.24	E605-72	0.1	E605-192	32		
E605-0.5	0.41	E606-8	0.34	E606-72	0.32	E606-192	106		
E606-0.5	2.02								
		E601-12	0.91	E601-96	0.27	E601-240	195		
E601-1	12.6	E602-12	0.31	E602-96	0.37	E602-240	88		
E602-1	1.14	E603-12	0.37	E603-96	0.18	E603-240	109		
E603-1	1.85	E604-12	0.47	E604-96	0.23	E604-240	125		
E604-1	2.23	E605-12	0.26	E605-96	0.02	E605-240	18		
E605-1	0.3	E606-12	0.35	E606-96	0.18	E606-240	69		
E606-1	1.28								
		E601-24	0.66	E601-120	0.34				
E601-2	11.2	E602-24	0.45	E602-120	0.19				
E602-2	0.53	E603-24	0.35	E603-120	0.14				
E603-2	0.29	E604-24	0.42	E604-120	0.14				
E604-2	1.18	E605-24	0.11	E605-120	0.05				
E605-2	0.32	E606-24	0.2	E606-120	0.09				
E606-2	0.47								

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Biochemical Toxicology and Risk Analysis
Haskell Laboratory for Toxicology and Industrial Medicine

To: MR FILE 9660

From: Hanan N. Ghantous HG
Study Director

Notebooks used in this study:

E-78163, E-78163-AA, -BA, -CA, -DA
E-78164, E-78164-AA, -BA, -CA, -DA

Haskell Laboratory Report No.: 671-93

Name of Report: Absolute Bioavailability of Soil Lead in Rats and Microswine

Distribution of Report: Timothy Bingman
(6 bound, 1 unbound, all single-sided)
Hanan Ghantous (1 bound, ~~double-sidedD~~)
2 bound HG 12/10/93

Medical Research Project No.: 9660-001
Haskell Sample No.: 20202 and 20204
Haskell Test Code: 467
Haskell Report No.: 671-93

==========

COMPLETE THIS PORTION AFTER REPORT IS SIGNED,
BUT BEFORE REPORT IS SENT TO INFORMATION SERVICES

Does the summary from this report qualify for the ICARE database? yes (no)
If yes, who made the electronic submittal? _____
If yes when was the electronic submittal sent? __/__/__

TO: INFORMATION SECTION PLEASE DESTROY ALL OLD FORMS

HASKELL LABORATORY TEST SUBSTANCE EVALUATION FORM

i. Fill out a test substance evaluation form as completely as possible for each test substance submitted
ii. If a test substance is being submitted for mutagenicity testing, pay special attention to Sections 6, 8 and 9.
iii. Unless otherwise requested, test substances will be returned to submitter within six months after the final report is written.

Haskell No.: 20202
(to be assigned by Haskell Personnel)

1. Chemical Abstracts nomenclature: Acetic Acid, Lead (2+) salt
Correction by PWB 6/2/93

Chemical Abstracts Registry No.: 6080-56-4

2. Other name(s): Lead acetate TRIHYDRATE

3. Cost Center identification code and/or lot number: KY 08221 CY

4. Structural formula:

$Pb(C_2H_3O_2)_3H_2O$

(a) Molecular weight 379.33

5. Composition of mixtures by % (include all additives and solvents): N/A

6. Purity 99.999 % (a) Impurities present: N/A

7. Physical characteristics:
(a) Color: White (b) Form: Crystalline Solid (c) m.p.: 75°C
(d) b.p. at 760 mm Hg.: 93° to 100° C (e) Density: N/A (f) pH: 6.0 at 25
(g) Vapor pressure at 25 degrees C: N/A 37 degrees C: N/A (h) Vapor density N/A
UNKNOWN (attach curve if available)
(i) Maximum process temp. N/A in presence/absence of air. (delete one)

TO: INFORMATION SECTION PLEASE DESTROY ALL OLD FORMS

HASKELL LABORATORY TEST SUBSTANCE EVALUATION FORM

i. Fill out a test substance evaluation form as completely as possible for each test substance submitted
ii. If a test substance is being submitted for mutagenicity testing, pay special attention to Sections 6, 8 and 9.
iii. Unless otherwise requested, test substances will be returned to submitter within six months after the final report is written.

Haskell No.: 20204
(to be assigned by Haskell Personnel)

1. Chemical Abstracts nomenclature: NA

Chemical Abstracts Registry No.: NA

2. Other name(s): SOIL CONTAMINATED WITH 1000 PPM LEAD FROM A SITE IN WASHINGTON STATE.

3. Cost Center identification code and/or lot number: NA

4. Structural formula: NA

(a) Molecular weight NA

5. Composition of mixtures by % (include all additives and solvents): NA

6. Purity NA % (a) Impurities present: NA

7. Physical characteristics: NA
(a) Color: Brown (b) Form: Solid (c) m.p.: N/A
(d) b.p. at 760 mm Hg.: N/A (e) Density: N/A (f) pH: N/A
(g) Vapor pressure at 25 degrees C: N/A 37 degrees C: N/A (h) Vapor density N/A
UNKNOWN (attach curve if available) NA
(i) Maximum process temp. _____ in presence/absence of air. (delete one)

DUP040000665

LOUISIANA STATE UNIVERSITY MEDICAL CENTER
1542 Tulane Avenue
New Orleans, LA 70112-2822
Telephone: (504) 568-4750
FAX: (504) 568-4633

LSUMC

Department of Surgery

May 3, 1993

Dr. Hanan Ghantous
Stein Laboratory (DuPont)
Building 1
Elkton Road
Newark, DE 19714

Dear Dr. Ghantous:

Please find enclosed 2 small keys for the lock on the container of 19.5 Kg DuPont test soil "P" that I am shipping to you via 2-day Federal Express per Tim Bingman's (DuPont) request. This test soil is from our original stock of test soil obtained from Hazen Research, Inc., Project # 7910-08.

Sincerely,

Duane M. Smith, Jr., Ph.D.
Department of Surgery
1542 Tulane Avenue
New Orleans, LA 70112
(504) 568-4763

TEST

REFERENCE NO. ..RF- 011-139-000-2 (### ### ### #)

*NAME ..NM- Acetic Acid, lead(2+) salt, trihydrate

*REGISTRY NO. ..RN- 6080-56-4 (######-##-#, zero-filled)

*SYNONYMS ..SY- Lead acetate trihydrate / Lead(II) acetate trihydrate / Lead acetate / Acetic acid, lead(2+) salt

SPONSORING DEPT. (see DE word list) ..DE- CHEM

Distribution:
K. D. Dastur (1)
W. J. Brock (1)
E. L. Holloway (1)
R. V. Daum (1)

MEDICAL RESEARCH ..MR-MR-9660-001

SAMPLE NO. ..SN- 20202

Other Codes: Aldrich Cat. No. 31,651-2 Lot# KY08221CY

SAMPLE R'CD DATE ..SA- 930511 (YYMMDD)

TEST TYPE (see TT wordlist) ..TT-999 MISCL

LMR
MSB/HNG
Sample Room

COMMENTS ..CO-

		Report Nos. (HL-#-YY D+.)	Date (YYMMDD.)	Notebook & Pages (E-# P. #-#.)
HASKELL REPORT	..HR-HL-	_____.	_____.	_____.
(Outside rpts)	..HR-HLO-	_____.	_____.	_____.
(Shell rpts)	..HR-SR-	_____.	_____.	_____.
CLINICAL REPORT	..CRA-	_____.	_____.	_____.
PATHOLOGY REPORT	..PRA-	_____.	_____.	_____.
DOCUMENT NUMBER	..DN-	_____.	_____.	_____.

+ Input a "D" one space after the Haskell Report No. if and only if the report includes a chemical which is a TSCA 8(D) chemical.

* Computer entered Completed (__) 1/16/87

TEST

REFERENCE NO. ..RF- (### ### ### #)

*NAME ..NM- Soils, Washington State, contaminated with lead

*REGISTRY NO. ..RN- NA (######-##-#, zero-filled)

*SYNONYMS ..SY- Soil contaminated with 1000 ppm lead from a site in Washington State / Soil contaminated with 1000 ppm lead from Washington Statew / Soil with lead

SPONSORING DEPT. (see DE word list) ..DE- CHEM

Distribution: K. D. Dastur (1)
W. J. Brock (1)
E. L. Holloway (1)
R. V. Daum (1)

MEDICAL RESEARCH ..MR-MR-9660-001

SAMPLE NO. ..SN- 20204

Other Codes: NA (Note: NA was not defined by the sponsor)

SAMPLE R'CD DATE ..SA- 930512 (YYMMDD)

TEST TYPE (see TT wordlist) ..TT-999 MISCL

LMR
RWR/HNG
Sample Room

COMMENTS ..CO-

		Report Nos. (HL-#-YY D+.)	Date (YYMMDD.)	Notebook & Pages (E-# P. #-#.)
HASKELL REPORT	..HR-HL-	_____.	_____.	_____.
(Outside rpts)	..HR-HLO-	_____.	_____.	_____.
(Shell rpts)	..HR-SR-	_____.	_____.	_____.
CLINICAL REPORT	..CRA-	_____.	_____.	_____.
PATHOLOGY REPORT	..PRA-	_____.	_____.	_____.
DOCUMENT NUMBER	..DN-	_____.	_____.	_____.

+ Input a "D" one space after the Haskell Report No. if and only if the report includes a chemical which is a TSCA 8(D) chemical.

* Computer entered Completed (__) 1/16/87

SHIPPER'S DECLARATION FOR DANGEROUS GOODS

Shipper L.S.U.M.C./DUPONT
Surgery
1542 Tulane Ave.
New Orleans, LA 70112

Air Waybill No. 8739535313
Page 1 of 1 Pages
Shipper's Reference Number *(optional)*

Consignee Dr. Hanan Ghantous
Stein Laboratory (DuPont)
Bldg. 1, Elkton Rd.
Newark, DE 19714

PHB

Two completed and signed copies of this Declaration must be handed to the operator

WARNING

Failure to comply in all respects with the applicable Dangerous Goods Regulations may be in breach of the applicable law, subject to legal penalties. This Declaration must not, in any circumstances, be completed and/or signed by a consolidator, a forwarder or an IATA cargo agent.

TRANSPORT DETAILS

This shipment is within the limitations prescribed for *(delete non-applicable)*

PASSENGER AND CARGO AIRCRAFT	~~CARGO AIRCRAFT ONLY~~

Airport of Departure NEW ORLEANS

Airport of Destination PHILADELPHIA

Shipment type *(delete non-applicable)*

NON-RADIOACTIVE	~~RADIOACTIVE~~

NATURE AND QUANTITY OF DANGEROUS GOODS

Dangerous Goods Identification				Quantity and type of packing	Packing Inst.	Authorization
Proper Shipping Name	Class or Division	UN or ID No.	Subsidiary Risk			
Lead Compounds, Soluble, N.O.S. (Lead Contaminated Soil) POISON	6.1	UN 2291		2 plastic drums x 10Kg each	Y619 III	Limited Quantity

Additional Handling Information

24Hr Emergency phone # (504)361-5284

I hereby declare that the contents of this consignment are fully and accurately described above by proper shipping name and are classified, packed, marked and labelled, and are in all respects in the proper condition for transport by air according to the applicable International and National Government Regulations.

Name/Title of Signatory Asst.
Duane M Smith Jr.Ph.D./Prof.
Place and Date
LSUMC, 1542 Tulane Ave.,N.O.
Signature *(see warning above)* 5/11/98

49-FS-C4 Revised 1/8 DUP040000669

ML-100
REV. 12/92

E. I. du Pont de Nemours and Company

PURCHASE ORDER

PURCHASE ORDER NO. | ALT. NO.

RELEASE NUMBER

PAGE OF

SITE NO. | TAX CODE | DIRECT ALL INQUIRIES ▶

ISSUED BY

DATE OF ORDER

REQUIRED DELIVERY DATE | PROMISED DELIVERY DATE

TERMS OF PAYMENT

F.O.B. ☐ DEST. (DEL'D) ☐ SHIP PT.

☐ PREPAID ☐ OTHER (SEE BELOW)

☐ PREPAID ADDED ☐ COLLECT*

TITLE OF GOODS | (If Ship Pt. Show City & State) | FREIGHT TERMS

VENDOR CODE

PAYEE CODE

Aldrich

SHIP TO

E. I. du Pont de Nemours and Company
STINE-HASKELL RESEARCH CENTER
P.O. BOX 30, ELKTON ROAD
NEWARK, DE 19711

ORDER NO.

SHIP VIA:

RECEIPTS	SHIPMENT NUMBER 1	2	3
DATE RECEIVED			
CHECKED BY			

FREIGHT			
DATE SHIPPED			
VIA			
PRO. NO. or CAR NO.			
F.B. WGT.			
FREIGHT CHARGES			
NO. AND/OR KIND PKGS.			

INVOICE			
DATE RECEIVED			
AMOUNT			
CHECK NO.			
DATE			

TO RECORD ADDITIONAL SHIPMENTS SEE REVERSE SIDE ▶

ITEM	QUANTITY	UNIT	DESCRIPTION (SHOW OR ATTACH COMPLETE SPECIFICATIONS)	PRICE	QUANTITY RECEIVED 1	2	3
A	1	100g	31,651-2 lead (II) Acetate trihydrate	58.80			

CONFIRMING TO: ▶ BY: DATE:

REQUISITIONED BY	EXTENSION	REQUISITION APPROVED BY	DATE	AUTH. LEVEL	DATE DELIVERY REQ'D	ESTIMATED WEIGHT	ESTIMATED COST
M. DeSato	65278	Michele K. DeSato	3/25/93		ASAP		58.80

ITEM NO.	GENERAL LEDGER ACCOUNT/SUB ACCOUNTS	DETAIL ACCOUNTING	AMOUNT
	7035-500 106 860 100		

H-1 Rm 1207

DELIVERY SPOT #

LAST ORDER NO. OR SUGGESTED SELLER & ADDRESS

Aldrich FAX 800 962-9591

REASON SELLER SELECTED ☐ LOWEST PRICE ☐ BETTER QUALITY ☐ REQUIRED DESIGN ☐ ONLY KNOWN SOURCE ☐ CONTRACT/P.A.

ORDER APPROVED BY | ORDER EXECUTED BY

22522

REQDUP040000670

NumCaps

THIS IS THE ENTRY SCREEN FOR NEW COMPOUNDS
THAT HAVE BEEN RECEIVED. ALL ENTRIES MUST FIT
WITHIN THEIR AREA.
HASKELL NUMBER:20202
AMOUNT RECEIVED 125.2GG FOR UNITS USE-
SAMPLE WAS RECEIVED 05/11/93 SEF DATE 05/07/93

SUBMITTER CHRISTINE LOEFFLER
LOCATION HASKELL DEPT. CHEM

TEST ARTICLE LEAD ACETATE CODE# CAT#31,651-2(ALDRICH
LOT# KY08221CY N.B.
CAS# 6080-56-4

HAZARD CODE
HAZARDS

COMMENTS MATERIAL-WHITE, CRYSTALLINE SOLID

ZDDDDDDDDDDDDDDDDDDDDDDDDDDDDDDDDDDDDDDDDDDDDDDDDDDDDDDDDDDDDDDDDDDDDDD?
3 USE "PAGE-DOWN" TO FLIP TO NEXT PAGE 3
3 USE "ESC" TO ABORT 3
@DDDDDDDDDDDDDDDDDDDDDDDDDDDDDDDDDDDDDDDDDDDDDDDDDDDDDDDDDDDDDDDDDDDDDDY

NumCaps

THERE IS SPACE FOR TWO SYNONYMS-EACH MUST FIT ON A LINE

LEAD(II) ACETATE TRIHYDRATE, 99.999%

9th CI NAME

ZDDDDDDDDDDDDDDDDDDDDDDDDDDDDDDDDDDDDDDDDDDDDDDDDDDDDDDDDDDDDDDDDDDDDDD?
3 USE "CTRL-END" TO ACCEPT ALL FIELDS AND EXIT 3
3 USE "ESC" TO ABORT 3
@DDDDDDDDDDDDDDDDDDDDDDDDDDDDDDDDDDDDDDDDDDDDDDDDDDDDDDDDDDDDDDDDDDDDDDY

NumCaps

THIS IS THE ENTRY SCREEN FOR NEW COMPOUNDS
THAT HAVE BEEN RECEIVED. ALL ENTRIES MUST FIT
WITHIN THEIR AREA.
HASKELL NUMBER:20204
AMOUNT RECEIVED COMMENT FOR UNITS USE-
SAMPLE WAS RECEIVED 05/12/93 SEF DATE 05/12/93

SUBMITTER TIM BINGMAN
LOCATION REO-PIT DEPT. CHEM

TEST ARTICLE SOIL W/LEAD CODE#
LOT# N.B.
CAS#

HAZARD CODE 2291
HAZARDS POISON

COMMENTS 1 OF 2 =11,199 GRM GROSS, 2 OF 2 =10,774 GRM GROSS

ZDDDDDDDDDDDDDDDDDDDDDDDDDDDDDDDDDDDDDDDDDDDDDDDDDDDDDDDDDDDDDDD?
3 USE "PAGE-DOWN" TO FLIP TO NEXT PAGE 3
3 USE "ESC" TO ABORT 3
@DDDDDDDDDDDDDDDDDDDDDDDDDDDDDDDDDDDDDDDDDDDDDDDDDDDDDDDDDDDDDDDY

NumCaps

THERE IS SPACE FOR TWO SYNONYMS-EACH MUST FIT ON A LINE

SOIL CONTAMINATED W/ 1000 PPM LEAD FROM WASHINGTON STATE

9th CI NAME

ZDDDDDDDDDDDDDDDDDDDDDDDDDDDDDDDDDDDDDDDDDDDDDDDDDDDDDDDDDDDDDDD?
3 USE "CTRL-END" TO ACCEPT ALL FIELDS AND EXIT 3
3 USE "ESC" TO ABORT 3
@DDDDDDDDDDDDDDDDDDDDDDDDDDDDDDDDDDDDDDDDDDDDDDDDDDDDDDDDDDDDDDDY

DUPONT CENTRAL RESEARCH AND DEVELOPMENT
HASKELL LABORATORY FOR TOXICOLOGY
AND INDUSTRIAL MEDICINE

Date: 8/5/93

TO: STUDY DIRECTOR, H. GHANTOUS

QUARANTINE RELEASE FORM

The shipment of animals described below has been examined, meets the health status criteria set forth by the Manager, Animal Resources, and is suitable for study.

DATE RECEIVED: 7/29/93

SPECIES: 120 YUCATAN MICROPIGS (2) BORN 6/93

HASKELL ANIMAL NUMBERS: # 17 - 28

SUPPLIER: CHARLES RIVER LABORATORIES, INC

TO BE USED ON STUDY: MR - 9660

RELEASED FROM QUARANTINE: 8/5/93

Charles E. Cover, VMD, ACLAM
Manager, Animal Resources

CENTRAL RESEARCH AND DEVELOPMENT DEPARTMENT
HASKELL LABORATORY FOR TOXICOLOGY
AND INDUSTRIAL MEDICINE

cc: Quality Assurance

Date: 6-10-93

TO: STUDY DIRECTOR, Havan Chanteus

QUARANTINE RELEASE FORM

The shipment of animals described below has been examined, meets the health status criteria set forth by the Consultant, Animal Resources, and is suitable for study.

DATE RECEIVED: 6-3-93

SPECIES: 12 ♂ Yucatan Microswine D.O.B. 4-24-93

HASKELL ANIMAL NUMBERS: 5-16

SUPPLIER: Charles River Laboratories

TO BE USED ON STUDY: MR 91660 HC 89

RELEASED FROM QUARANTINE: 6-9-93

Due to Unavailability of Form on Release Date, animals were Released on Legal sheets for 6-7-93 SEE Notebook E7811 BA for initials and Date of 6/10/93

DUP040000674

CENTRAL RESEARCH AND DEVELOPMENT DEPARTMENT
HASKELL LABORATORY FOR TOXICOLOGY
AND INDUSTRIAL MEDICINE

cc: Quality Assurance

Date: 6-7-93

TO: STUDY DIRECTOR, Hanan Ghantous

QUARANTINE RELEASE FORM

The shipment of animals described below has been examined, meets the health status criteria set forth by the Consultant, Animal Resources, and is suitable for study.

DATE RECEIVED: 6-1-93

SPECIES: CRL;CD 60 ♂ D.O.B. 5-10Δ-93

HASKELL ANIMAL NUMBERS: 537350-537409

SUPPLIER: Charles River Laboratories

TO BE USED ON STUDY: MR 9660

RELEASED FROM QUARANTINE: 6/7/93

Δ According to shipping form, rats were born 5/10/93. JED 12/06/93

C. Van Deest, DVM

DUP040000675

CENTRAL RESEARCH AND DEVELOPMENT DEPARTMENT
HASKELL LABORATORY FOR TOXICOLOGY
AND INDUSTRIAL MEDICINE

Revision of QRF 5/24/93

Date: 5/25/93

TO: STUDY DIRECTOR, HANAN GHANTOUS

QUARANTINE RELEASE FORM

The shipment of animals described below has been examined, meets the health status criteria set forth by the Consultant, Animal Resources, and is suitable for study.

DATE RECEIVED: 5/11/93

SPECIES: 90♂ Crl:CD®BR rats, 4/19/93

HASKELL ANIMAL NUMBERS: #536261-536350

SUPPLIER: CHARLES RIVER LABORATORIES, INC

TO BE USED ON STUDY: MR-9660

RELEASED FROM QUARANTINE: 5/17/93 *

* Only 50 of the 90 rats were used for the study. These 50 rats were weighed on 5/17/93 and released from quarantine. The additional 40 rats were not weighed at this time, however weights and clinical signs taken 5/25/93 indicate that these rats also met the criteria for release from quarantine.

Charles E. Cover, V.M.D.
Consultant, Animal Resources

CCover 5/25/93

DUP040000676

CENTRAL RESEARCH AND DEVELOPMENT DEPARTMENT
HASKELL LABORATORY FOR TOXICOLOGY
AND INDUSTRIAL MEDICINE

cc: Quality Assurance

Date: 5-24-93

TO: STUDY DIRECTOR, Hanan Ghantous

QUARANTINE RELEASE FORM

The shipment of animals described below has been examined, meets the health status criteria set forth by the Consultant, Animal Resources, and is suitable for study.

DATE RECEIVED: 5-11-93

SPECIES: Rats CRL:CD®BR(90 ♂)

HASKELL ANIMAL NUMBERS: 5362161 - 5363350

SUPPLIER: Charles River Laboratories

TO BE USED ON STUDY: MR 96160

RELEASED FROM QUARANTINE: 5-17-93

SEE Legalsheets FOR original signature FOR Release ON 5/17/93
FORM unavailable at time of Release ON 5/17/93 & 5/24/93

C. Tex Fleet DVM, PhD

DUP040000677

HASKELL LAB REPORTS: WRITING AIDS

(Please return this sheet with the final report.)

Please use the names, codes, synonyms and composition supplied below when writing your reports. When pathology or clinical pathology work is scheduled, pass a copy of this form on to the appropriate persons in those groups.

MR-9660-001 SAMPLE # 20204

CHEMICAL NAME USED FOR HASKELL LAB'S CENTRAL FILES:
(Use in the "MATERIAL TESTED" section)

Soils, Washington State, contaminated with lead

NAME TO BE USED IN THE REPORT:

H-20204

OTHER CODES: NA (Note: NA was not defined by the sponsor)

SUBMITTER'S NOTEBOOK NO.: None submitted

SYNONYMS:
- Soil contaminated with 1000 ppm lead from a site in Washington State
- Soil contaminated with 1000 ppm lead from Washington State
- Soil with lead

CAS REGISTRY NUMBER: None available

PURITY: NA (Note: NA was not defined by the sponsor)

COMPOSITION: NA (Note: NA was not defined by the sponsor)

CONTAMINANTS: NA (Note: NA was not defined by the sponsor)

==========

PREPARED BY: Paul W. Grube DATE: 2 June 1993

PWG

TO: MEDICAL RESEARCH PROJECT NO. 9660-001

The following changes are made to the original protocol:

page 2 *STUDY DESIGN*
A. Single Dose Administration
first and third bullets

Change '...and ten male microswine...' to '...and nine male microswine...' in the first bulleted item. Change '...and five microswine will be dosed orally (gavage)...' to '...and four microswine will be dosed orally (gavage)...' in the third bulleted item. One microswine became ill and was sacrificed *in extremis*.

page 4 *MATERIALS AND METHODS*
A. Test Material
paragraph 1, sentence 1

Replace the first sentence with: 'Lead acetate will be supplied by Aldrich, Milwaukee, WI 53733, U.S.A. and the test soils will be supplied by the sponsor.' This sentence was written incorrectly. It was always the intent to receive the lead acetate from Aldrich and the test soils from the sponsor.

page 7 *MATERIALS AND METHODS*
H. Food Consumption and Intake of Test Material
paragraph 1, sentence 1

Change '...food consumed by each microswine will be determined twice per week...' to '...food consumed by each microswine will be determined weekly...' For easier data comparison, microswine and rats were maintained on the same schedule for food consumption determination.

page 7 *MATERIALS AND METHODS*
I. Dosing
paragraph 1

Add the following sentences: 'One microswine designated for intravenous administration and one microswine designated for oral (gavage) administration will be fed one hour before dosing. Data from these animals will be reported separately.' The microswine ate all of their food immediately, while the rats tended to have food remaining in their food jars up to the time of dosing. Therefore, microswine were considered 'fasted' while the rats were considered 'fed.' To evaluate if being 'fed' made a difference, one microswine from each type of single dose administration was fed one hour before dosing.

page 8 *MATERIALS AND METHODS*
J. Blood Sampling
paragraph 1

Insert the following sentence after the original first sentence: 'For microswine in the single dose administration group, syringes will be washed with heparin before blood collection.' The blood collected from microswine that received a single dose after feeding for 30 consecutive days was clotting. The heparin wash was added to prevent this from occurring in the single dose administration group.

page 9 *RECORDS AND SAMPLE RETENTION*
paragraph 1, sentence 1

Change '...will be retained at Haskell Laboratory or at the Records Management Center,...' to '...will be retained at Haskell Laboratory, Jackson Laboratory (DuPont Chambers Works), or at the Records Management Center,...' Records and samples from the analyses for lead will be maintained at Jackson Laboratory.

Approved by: H. G. Ghantous 11/23/93

Hanan N. Ghantous, Ph.D. (date)
Study Director

Matthew S. Bogdanffy 11/23/93

Matthew S. Bogdanffy, Ph.D. (date)
Manager
Biochemical Toxicology and Risk Analysis

cc: T.S. Bingman
K.A. McCleary
R.C. Rhea

Memorandum

To: Hanan Ghantous, H-1

From: Keith A. McCleary, Jackson Lab

Date: December 8, 1993

Subject: Final Report, Lead in Blood Analysis

Cc: C. J. Hensler, Jackson Lab
M. S. Bogdanffy, Haskell Toxicology
R. C. Rhea, Haskell Quality Assurance
R. L. Sobocinski, QCL

Enclosed please find three copies of the final report for the analysis of Pb in blood as conducted by the Du Pont Specialty Chemicals' Trace Metals Analysis Group as part of Medical Research Project No. MR 9660: "Absolute Bioavailability of Soil Lead in Rats and Microswine".

A copy of this report is to be kept with those materials relating to the analyses performed (SOP's, methods, and copies of sample logs) under GLP guidelines here at Jackson Lab. If you have any questions, please feel free to call me (Ducom 540-2467).

Note: Due to the pagination procedure used, a copy of this report was used in the final report.

John Aldham
12/10/93

Analysis of Pb in Blood, Soil, and Associated Materials in Support of Haskell Lab Medical Research Project No. MR 9660

Author

Keith A. McCleary

Study Completed:

8/30/93

Performed By:

DuPont Specialty Chemicals

Trace Metals Analysis Group

Quality Control Laboratory

Chambers Works

Deepwater, NJ 08023

Signature: Keith A. McCleary 12/8/93

Keith A. McCleary, Ph.D.

Summary

The purpose of this study was to determine the amount of lead that was contained in samples associated with MR9660. There were essentially three type of samples: blood (rat and microswine), aqueous (drinking water, dosing solutions), and solid (soil, bedding, etc.). Blood samples were prepared by dilution with a modifier solution (to digest sample and break viscosity) and analyzed using Graphite Furnace Atomic Absorption Spectrometry (GFAAS). Aqueous and solid samples were prepared using microwave assisted digestion and analyzed using Inductively Coupled Plasma Atomic Emission Spectometry (ICP-AES). Generally, all samples were run in duplicate and average answers reported. In the case of the blood, each sample and duplicate was spiked and checked for recovery. If the spike recovery was greater than 10% in error, a one point standard additions calculation was performed. Greater than 1000 samples were analyzed throughout the duration of this study.

Methodology

The descriptions listed below are summaries of the actual methods used to analyze samples. The actual methods are documented in the Procedures Notebook, archived at QCL and copies are available upon request.

Sample Preparation: Blood

Blood samples were received in small polyethylene vails. Approximate sample volume was 100-300 uL. 100 uL of blood is diluted to 1.0 mL with modifier solution. The modifier solution is a solution of nitric acid (digests sample), Triton X-100 (surfactant, breaks viscosity), methanol (antifoaming agent), and ammonium phosphate (GFAAS modifier) in water.

Sample Analysis: Blood

Blood samples are analyzed using Graphite Furnace Atomic Absorption Spectrometry (GFAAS). In GFAAS, a 20 microliter volume of sample + modifier is injected into a small graphite tube. This tube is electromagnetically heated to dry, char, and atomize the sample. A hollow cathode lamp (HCL) shines light corresponding to the atomic emission of Pb (283.3 nm) through the tube. As free Pb atoms are generated by the sample atomization, they absorb the radiation, attenuating the amount of signal from the HCL. The degree of attenuation of the HCL radiation is proportional to the concentration of Pb in the sample. Calibration standards of 10, 20 ,and 30 ng/mL are prepared by serial dilution of a 1000 ug/mL stock. Each blood sample is analyzed twice. Each sample and duplicate is then spiked with 10ng Pb and reanalyzed to check for recovery. If recovery is less than 90% or greater than 110%, a one point standard addition calculation is

performed to determine the amount of Pb in the sample. Each result is the average of the two replicates.

Sample Preparation: Aqueous Samples

Samples that are primarily aqueous are prepared by acidifying 10.00 mL of sample with 2.00 mL concentrated HNO3. The sample is then diluted to a final volume of 20.00 mL and analyzed by ICP-AES.

Sample Preparation: Solid Samples

Solid Samples are prepared by microwave assisted digestion. 0.1 - 0.2 g of sample are digested with 5.00 mL concentrated HNO3 in a Teflon lined pressure vessel. The vessels are placed in a microwave digestion apparatus and cooked for 10 minutes at 300@W power. The samples are allowed to cool and are then filtered into a 50 mL volumetric flask and diluted to volume with deionized water. Samples are analyzed for Pb content by ICP-AES.

Sample Analysis : Aqueous and Solid Samples

These samples are analyzed by Inductively Coupled Plasma - Atomic Emission Spectrometry (ICP-AES). The prepared samples are pumped into an sample introduction system where a fine spray of aerosol is generated. This sample aerosol flows along a stream of argon gas into the plasma. The plasma is a highly energetic discharge sustained in argon by applied radio-frequency power in excess of 1000 W. This discharge is energetic enough to desolvate, atomize, and excite the sample species to radiative emission. The intensity of the characteristic emission wavelength of Pb (220.35 nm) is proportional to the amount of Pb in the sample.

Results

The results for all samples submitted under MR9660 are listed immediately following this page.

Comprehensive Blood Report
PB_BL_CP.XLS

Sample	Lead		Sample	Lead		Sample	Lead		Sample	L
	ng/mL			ug/mL			ug/mL			u
a-101	10		102-0.5	39.8		104-12	6.2		A101-144	
a-102	<5		106-0.5	68.9		107-12	8.5		A104-144	
a-103	<5		110-0.5	24.3		111-12	0.8		A107-144	
a-104	<5		116-0.5	54.6		115-12	1.97		A111-144	
a-105	6		122-0.5	49.2					A113-144	
a-106	<5					A108-24	6.4			
a-107	6		108-1	18.6		A109-24	3.3		A103-192	
a-108	9		109-1	24.6		A112-24	6.6		A117-192	
a-109	44		112-1	60.5		A123-24	5.2		A118-192	
a-110	10		123-1	22.8		A124-24	2.7		A119-192	
a-111	50		124-1	34.1					A125-192	
a-112	13					A103-48	0.3			
a-113	13		101-2	15		A117-48	10.3		A105-240	
a-114	10		104-2	15.1		A118-48	13.2		A113-240	
a-115	15		107-2	19.1		A119-48	4.1		A114-240	
a-116	10		111-2	16.6		A125-48	0.4		A120-240	
a-117	6		115-2	4.1					A121-240	
a-118	12					A105-72	3.8			
a-119	12		105-4	11		A113-72	1.8		STABILITY	
a-120	5		113-4	7.7		A114-72	3.1		A105-240s	
a-121	16		114-4	6.6		A120-72	1.6		A113-240s	
a-122	12		120-4	2.6		A121-72	0.5		A114-240s	
a-123	15		121-4	20.3					A120-240s	
a-124	<5					A102-96	2.4		A121-240s	
a-125	<5		103-8	3.1		A106-96	1.4			
			117-8	4.5		A110-96	0.1		REPEATS	
			118-8	6.5		A116-96	1.3		A107-12	
			119-8	3.6		A122-96	1.4		A117-48	
			125-8	0.3					A118-48	
						A108-120	2			
						A109-120	1.1		A121-240(6/2)	
						A112-120	2.3		A121-240(6/7)	
						A123-120	0.9			
						A124-120	0.7			

Comprehensive Blood Report
PB_BL_CP.XLS

Sample	Pb, ng/mL		Sample	Pb, ng/mL		Sample	Pb, ng/mL		Sample	P
B201-PRE	34		B221-0.5	15		B202-24	125		B202-144	
B202-PRE	105		B220-0.5	21		B206-24	55		B206-144	
B203-PRE	23		B214-0.5	24		B210-24	59		B210-144	
B204-PRE	26		B213-0.5	31		B216-24	30		B216-144	
B205-PRE	7		B205-0.5	29		B222-24	43		B222-144	
B206-PRE	32									
B207-PRE	42		B216-1	22		B209-48	54		B201-192	
B208-PRE	16		B202-1	23		B208-48	59		B204-192	
B209-PRE	23		B222-1	20		B223-48	41		B207-192	
B210-PRE	16		B206-1	29		B224-48	39		B211-192	
B211-PRE	23		B210-1	18		B212-48	39		B215-192	
B212-PRE	16									
B213-PRE	12		B208-2	59		B201-72	36		B208-240	
B214-PRE	13		B224-2	53		B204-72	34		B209-240	
B215-PRE	24		B209-2	57		B207-72	30		B212-240	<
B216-PRE	20		B212-2	47		B211-72	35		B223-240	<
B217-PRE	26		B223-2	31		B215-72	15		B224-240	
B218-PRE	15									
B219-PRE	21		B204-4	71		B203-96	50		II-1-7400pre	
B220-PRE	16		B215-4	68		B217-96	82		II-1-7401pre	<
B221-PRE	24		B211-4	197		B218-96	336		II-1-7402pre	<
B222-PRE	8		B207-4	71		B219-96	64		II-1-7403pre	<
B223-PRE	<5		B201-4	155		B225-96	74		II-1-7404pre	
B224-PRE	<5									
B225-PRE	<5		B203-8	290		B205-120	22		II-1-7400-12	
			B217-8	320		B213-120	32		II-1-7401-12	
			B218-8	530		B214-120	16		II-1-7402-12	
			B219-8	250		B220-120	21		II-1-7403-12	
			B225-8	230		B221-120	17		II-1-7404-12	

Comprehensive Blood Report
PB_BL_CP.XLS

Sample	Pb, ng/mL	Sample	Pb, ng/mL	Sample	Pb, ng/mL	Sample	Pb, ng/mL	Sample	Pb, ng/mL
C301	56			C301 6/3	95	C301 6/10	54	C301 6/17	100
C303	8	C303 5/27	237	C303 6/3	122	C303 6/10	96	C303 6/17	106
C304	19	C304 5/27	132	C304 6/3	83	C304 6/10	99	C304 6/17	95
C305	9	C305 5/27	108	C305 6/3	94	C305 6/10	59	C305 6/17	68
C306	5			C306 6/3	138	C306 6/10	81	C306 6/17	80
C307	6	C307 5/27	77	C307 6/3	112	C307 6/10	55	C307 6/17	79
C308	5	C308 5/27	90	C308 6/3	175	C308 6/10	78	C308 6/17	66
C309	11			C309 6/3	195	C309 6/10	64	C309 6/17	98
C310	14	C310 5/27	58	C310 6/3	122	C310 6/10	70	C310 6/17	65
		C311 5/27	124	C311 6/3	92	C311 6/10	84	C311 6/17	57
		C312 5/27	65	C312 6/3	26	C312 6/10	128	C312 6/17	77
C313	7	C313 5/27	48	C313 6/3	46	C313 6/10	56	C313 6/17	82
C314	5	C314 5/27	70	C314 6/3	77	C314 6/10	44	C314 6/17	42
C315	34	C315 5/27	62	C315 6/3	54	C315 6/10	51	C315 6/17	46
C316	7	C316 5/27	100	C316 6/3	45	C316 6/10	38	C316 6/17	69
C317	11	C317 5/27	39	C317 6/3	54	C317 6/10	56	C317 6/17	66
C319	4	C319 5/27	103	C319 6/3	63	C319 6/10	64	C319 6/17	87
C320	6	C320 5/27	51	C320 6/3	89	C320 6/10	56	C320 6/17	92
C321	6	C321 5/27	121	C321 6/3	51	C321 6/10	59	C321 6/17	53
C322	5	C322 5/27	59	C322 6/3	48	C322 6/10	31	C322 6/17	37
C323	3	C323 5/27	132	C323 6/3	36	C323 6/10	26	C323 6/17	51
C324	<2	C324 5/27	46	C324 6/3	38	C324 6/10	48	C324 6/17	69
C325	24	C325 5/27	64	C325 6/3	12	C325 6/10	78	C325 6/17	81

Comprehensive Blood Report
PB_BL_CP.XLS

Sample	Pb,ug/mL		Sample	Pb,ug/mL		Sample	Pb,ug/mL		
C321 0.5	14.2		C306-24	0.4		C301-192	0.74		
C320 0.5	11.9		C310-24	2.1		C304-192	0.53		
C314 0.5	19.4		C316-24	1.9		C307-192	0.43		
C313 0.5	32.5		C322-24	0.7		C311-192	0.45		
C305 0.5	15.1					C315-192	0.28		
			C308-48	2.2					
C316 1.0	20.7		C309-48	1.2		C308-240	0.41		
C322 1.0	13		C312-48	7.9		C309-240	0.64		
C306 1.0	18.3		C323-48	1.7		C312-240	0.37		
C310 1.0	17.5		C324-48	6.9		C323-240	0.58		
						C324-240	1.72		
C308 2.0	7.5		C301-72	2.8					
C324 2.0	19.1		C304-72	1.4					
C309 2.0	4.3		C307-72	0.7					
C312 2.0	12.2		C311-72	1.3					
C323 2.0	9.7		C315-72	1					
C304 4.0	6.6		C303-96	0.7					
C301 4.0	12.2		C317-96	0.4					
C307 4.0	4.4		C319-96	0.1					
C311 4.0	4.6		C325-96	0.9					
C315 4.0	7.1								
			C305-120	0.6					
C303-8	6.1		C313-120	1.6					
C317-8	3.2		C314-120	0.5					
C319-8	0.8		C320-120	0.7					
C325-8	3.9		C321-120	0.8					
C305-12	4.5		C306-144	0.7					
C313-12	5.3		C310-144	0.6					
C314-12	5.8		C316-144	1.2					
C320-12	4.2		C322-144	0.5					
C321-12	6.8								

Comprehensive Blood Report
PB_BL_CP.XLS

Sample	Pb, ug/mL		Sample	Pb, ug/mL		Sample	Pb, mg/kg		
Rat Gavage DOSING SOLUTION			SUCROSE DIET SAMPLE			#1 top of mixer 5/17/93			
GROUP B	311.9			<5		1a	41		
GROUP B	310.9					2b	46		
			OREO COOKIES			3c	40		
DEIONIZED WATER				<5					
	<5					#1 middle of mixer 5/17/93			
			TEKLAD BASAL			4a	46		
1000 PPM Pb SOIL			SWINE	<5		5b	42		
	840.9					6c	47		
			PURINA RAT CHOW						
SUCROSE				<5		#1 bottom mixer 5/17/93			
	<5					7a	37		
			SWINE BEDDING			8b	36		
				<5		9c	38		
Swine IV Single Dose Sampl									
1a	116.6mg/g		DI H2O	<1		top mixer 6/17/93			
2a	126.8mg/g		Pb ACETATE (RAT IV SOLN)			1a	43.6		
			1.27%			2a	46.6		
			(400mg/20mL)			3a	44		
swine gavage									
7/16/93 30 Day			Swine IV 30 Day Solution			middle mixer 6/17/93			
10a	0.425 mg/g		118.5 mg/g			4a	42.3		
11a	0.348 mg/g					5a	45.7		
KAM 12/6/93			Swine single gavage 8/93			6a	43.1		
			3a	0.339mg/g					
			4a	0.347mg/g		bottom mixer 6/17/93			
						7a	45.6		
						8a	51		
						9a	44.5		

Comprehensive Blood Report
PB_BL_CP.XLS

Sample	Pb, ng/mL	Sample	Pb, ng/mL	Sample	Pb, ng/mL	Sample	Pb, ng/mL	Sample	Pb, ng/mL
C401-PRE	9	D401 6/17	103	D401 6/24	78	D401 7/1	120	D401 7/8	101
C402-PRE	<5	D402 6/17	102	D402 6/24	86	D402 7/1	55	D402 7/8	61
C403-PRE	<5	D403 6/17	147	D403 6/24	88	D403 7/1	89	D403 7/8	86
C404-PRE	<5	D404 6/17	95	D404 6/24	54	D404 7/1	57	D404 7/8	63
C405-PRE	<5	D405 6/17	71	D405 6/24	56	D405 7/1	120	D405 7/8	133
C406-PRE	8	D406 6/17	116	D406 6/24	124	D406 7/1	126	D406 7/8	88
C407-PRE	6	D407 6/17	145	D407 6/24	113	D407 7/1	109	D407 7/8	103
C408-PRE	<5	D408 6/17	83	D408 6/24	67	D408 7/1	64	D408 7/8	76
C409-PRE	<5	D409 6/17	135	D409 6/24	117	D409 7/1	157	D409 7/8	155
C410-PRE	<5	D410 6/17	59	D410 6/24	101	D410 7/1	80	D410 7/8	62
C411-PRE	<5	D411 6/17	44	D411 6/24	52	D411 7/1	96	D411 7/8	75
C412-PRE	<5	D412 6/17	72	D412 6/24	32	D412 7/1	50	D412 7/8	89
C413-PRE	<5	D413 6/17	67	D413 6/24	57	D413 7/1	36	D413 7/8	55
C414-PRE	7	D414 6/17	67	D414 6/24	37	D414 7/1	53	D414 7/8	66
C415-PRE	6	D415 6/17	78	D415 6/24	31	D415 7/1	34	D415 7/8	61
C416-PRE	5	D416 6/17	99	D416 6/24	44	D416 7/1	129	D416 7/8	63
C417-PRE	5	D417 6/17	123	D417 6/24	84	D417 7/1	108	D417 7/8	79
C418-PRE	<5	D418 6/17	18	D418 6/24	56	D418 7/1	45	D418 7/8	52
C419-PRE	<5	D419 6/17	15	D419 6/24	27	D419 7/1	45	D419 7/8	41
C420-PRE	<5	D420 6/17	55	D420 6/24	37	D420 7/1	105	D420 7/8	60
C421-PRE	7	D421 6/17	16	D421 6/24	16	D421 7/1	96	D421 7/8	59
C422-PRE	<5	D422 6/17	58	D422 6/24	24	D422 7/1	67	D422 7/8	41
C423-PRE	<5	D423 6/17	51	D423 6/24	101	D423 7/1	82	D423 7/8	124
C424-PRE	<5	D424 6/17	22	D424 6/24	62	D424 7/1	135	D424 7/8	89
C425-PRE	<5	D425 6/17	7	D425 6/24	18	D425 7/1	67	D425 7/8	52

Comprehensive Blood Report
PB_BL_CP.XLS

Sample	Pb,ng/mL	Sample	Pb,ng/mL	Sample	Pb,ng/mL				
D405-0.5	108	D405-12	101	D405-120	<5				
D413-0.5	45	D413-12	32	D413-120	<5				
D414-0.5	40	D414-12	58	D414-120	78				
D420-0.5	69	D420-12	62	D420-120	<5				
D421-0.5	47	D421-12	72	D421-120	38				
D402-1.0	89	D402-24	50	D401-144	60				
D406-1.0	48	D406-24	102	D404-144	24				
D410-1.0	115	D410-24	123	D407-144	64				
D416-1.0	79	D416-24	110	D411-144	42				
D422-1.0	37	D422-24	92	D415-144	33				
D408-2	84	D408-48	96	D402-192	19				
D409-2	53	D409-48	83	D406-192	45				
D412-2	36	D412-48	83	D410-192	29				
D423-2	26	D423-48	103	D416-192	39				
D424-2	45	D424-48	469	D422-192	25				
D401-4	120	D401-72	16	D408-240	38				
D404-4	158	D404-72	14	D409-240	167				
D407-4	114	D407-72	74	D412-240	65				
D411-4	109	D411-72	25	D423-240	86				
D415-4	65	D415-72	44	D424-240	45				
D403-8	124	D403-96	82						
D417-8	190	D417-96	51						
D418-8	160	D418-96	<5						
D419-8	121	D419-96	<5						
D425-8	133	D425-96	<5						

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Sample	Pb, ng/mL	Sample	Pb, ng/mL	Sample	Pb, ug/mL	Sample	Pb, ug/mL	Sample	Pb, ng/mL	S
G501-PRE(a)	30	G501 7/7	139	G501-0.5	7.35	G501-8	0.31	G501-72	122	G
G502-PRE(a)	8	G502 7/7	152	G502-0.5	1.4	G502-8	0.18	G502-72	240	G
G503-PRE(a)	20	G503 7/7	225	G503-0.5	2.73	G503-8	0.88	G503-72	201	G
G504-PRE(a)	34	G504 7/7	135	G504-0.5	0.99	G504-8	0.13	G504-72	69	G
G505-PRE(a)	8	G505 7/7	173	G505-0.5	2.62	G505-8	0.34	G505-72	376	G
G506-PRE(a)	17	G506 7/7	195	G506-0.5	7.88	G506-8	1.12	G506-72	460	G
G501 6/16	129	G501 PRE(b)	203	G501-1	3.04	G501-12	0.27	G501-96	313	G
G502 6/16	257	G502 PRE(b)	201	G502-1	0.17	G502-12	0.12	G502-96	227	G
G503 6/16	323	duplicate	185	G503-1	0.98	G503-12	0.86	G503-96	305	G
G504 6/16	150	G503 PRE(b)	194	G504-1	0.31	G504-12	0.19	G504-96	110	G
G505 6/16	195	G504 PRE(b)	145	G505-1	2.33	G505-12	0.31	G505-96	225	G
G506 6/16	160	G505PRE(b)	90	G506-1	1.03	G506-12	1.95	G506-96	554	G
		G506 PRE(b)	143							
G501 6/23	100			G501-2	1.17	G501-24	0.24	G501-120	239	F
G502 6/23	190			G502-2	0.32	G502-24	0.22	G502-120	147	G
G503 6/23	150			G503-2	1.25	G503-24	0.24	G503-120	372	G
G504 6/23	50			G504-2	0.28	G504-24	0.11	G504-120	112	G
G505 6/23	140			G505-2	1.34	G505-24	0.24	G505-120	193	G
G506 6/23	100			G506-2	3.19	G506-24	0.82	G506-120	438	G
										G
G501 6/30	258			G501-4	0.76	G501-48	0.36	G501-144	225	G
G502 6/30	347			G502-4	0.39	G502-48	0.25	G502-144	57	G
G503 6/30	266			G503-4	0.37	G503-48	0.65	G503-144	206	G
G504 6/30	155			G504-4	0.29	G504-48	0.07	G504-144	115	*
G505 6/30	147			G505-4	1.22	G505-48	0.16	G505-144	369	G
G506 6/30	193			G506-4	1.93	G506-48	0.52	G506-144	372	
										G
										G
(a) denotes predose prior to weekly testing										G
(b) denotes predose prior to hourly testing										G
										G
										G

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Sample	Pb, ng/mL	Sample	Pb, ng/mL	Sample	Pb, ng/mL	Sample	Pb, ng/mL	Sample	Pb, ng/mL
H701-PRE	7	H701-PRE	169	H701-4	158	H701-48	263	H701-144	144
H702-PRE	23	H702-PRE	119	H702-4	142	H702-48	302	H702-144	97
H703-PRE	5	H703-PRE	145	H703-4	317	H703-48	200	H703-144	128
H704-PRE	57	H704-PRE	168	H704-4	366	H704-48	137	H704-144	172
H705-PRE	10	H705-PRE	224	H705-4	275	H705-48	164	H705-144	163
H706-PRE	7	H706-PRE	255	H706-4	304	H706-48	271	H706-144	132
H701 6/23	190	H701-0.5	255	H701-8	340	H701-72	159	H701-192	116
H702 6/23	130	H702-0.5	177	H702-8	268	H702-72	224	H702-192	101
H703 6/23	80	H703-0.5	180	H703-8	322	H703-72	114	H703-192	97
H704 6/23	170	H704-0.5	200	H704-8	268	H704-72	204	H704-192	127
H705 6/23	180	H705-0.5	130	H705-8	160	H705-72	181	H705-192	130
H706 6/23	290	H706-0.5	203	H706-8	133	H706-72	254	H706-192	157
H701 6/30	206	H701-1	288	H701-12	267	H701-96	172	H701-240	100
H702 6/30	206	H702-1	112	H702-12	316	H702-96	189	H702-240	91
H703 6/30	217	H703-1	282	H703-12	234	H703-96	104	H703-240	95
H704 6/30	261	H704-1	210	H704-12	187	H704-96	176	H704-240	111
H705 6/30	258	H705-1	190	H705-12	255	H705-96	199	H705-240	118
H706 6/30	201	H706-1	242	H706-12	143	H706-96	168	H706-240	160
H701 7/7	193	H701-2	222	H701-24	318	H701-120	122		
H702 7/7	169	H702-2	207	H702-24	220	H702-120	146		
H703 7/7	137	H703-2	142	H703-24	216	H703-120	161		
H704 7/7	209	H704-2	170	H704-24	224	H704-120	See Note		
H705 7/7	227	H705-2	258	H705-24	203	H705-120	183		
H706 7/7	173	H706-2	347	H706-24	131	H706-120	181		

Comprehensive Blood Report
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Sample	Pb, ng/mL	Sample	Pb, ng/mL	Sample	Pb, ng/mL	Repeat	Pb, ng/mL
F801 PRE	7	F801-12	133	F801-144	206	F803-48	65.1
F802 PRE	5	F802-12	73	F802-144	162	F801-144	14.4
F803 PRE	<5	F803-12	118	F803-144	147	F802-144	21.2
F804 PRE	<5	F804-12	96	F804-144	727	F803-144	9.9
F805 PRE	<5	F805-12	115	F805-144	33	F804-144	21.9
						F805-144	38.5
F801 0.5	7	F801-24	74	F801-192	81		
F802 0.5	<5	F802-24	15	F802-192	26		
F803 0.5	<5	F803-24	71	F803-192	23		
F804 0.5	7	F804-24	74	F804-192	33		
F805 0.5	6	F805-24	105	F805-192	25		
F801-1	19	F801-48	31	F801-240	22		
F802-1	7	F802-48	36	F802-240	13		
F803-1	23	F803-48	229	F803-240	9		
F804-1	23	F804-48	25	F804-240	13		
F805-1	16	F805-48	79	F805-240	22		
F801-2	27	F801-72	26				
F802-2	10	F802-72	40				
F803-2	33	F803-72	44				
F804-2	59	F804-72	23				
F805-2	28	F805-72	41				
F801-4	96	F801-96	20				
F802-4	49	F802-96	36				
F803-4	85	F803-96	15				
F804-4	57	F804-96	10				
F805-4	52	F805-96	22				
F801-8	79	F801-120	31				
F802-8	78	F802-120	55				
F803-8	65	F803-120	38				
F804-8	56	F804-120	30				
F805-8	74	F805-120	98				

Comprehensive Blood Report
PB_BL_CP.XLS

Sample	Pb, ng/mL	Sample	Pb, ug/mL	Sample	Pb, ug/mL	Sample	Pb, ng/mL	REPEATS	Pb, ug/mL
E601-PRE	6	E601-4	9.8	E601-48	0.39	E601-144	232	E601-0.5	22.35
E602-PRE	27	E602-4	1.9	E602-48	0.23	E602-144	267	E601-1	21.51
E603-PRE	<5	E603-4	6.3	E603-48	0.32	E603-144	136	E601-2	16.7
E604-PRE	14	E604-4	4.1	E604-48	0.25	E604-144	165		
E605-PRE	16	E605-4	clotted	E605-48	0.19	E605-144	32	REPEATS	Pb, ug/mL
E606-PRE	7	E606-4	5.3	E606-48	0.09	E606-144	101	E601-4	2.6
								E602-4	0.3
Sample	Pb, ug/mL	E601-8	1.6	E601-72	0.44	E601-192	148	E603-4	0.4
E601-0.5	22.3	E602-8	0.27	E602-72	1.98	E602-192	118	E604-4	0.46
E602-0.5	1.85	E603-8	0.47	E603-72	0.48	E603-192	88	E605-4	0.2
E603-0.5	1.28	E604-8	0.39	E604-72	0.32	E604-192	102	E606-4	0.43
E604-0.5	2.07	E605-8	0.24	E605-72	0.1	E605-192	32		
E605-0.5	0.41	E606-8	0.34	E606-72	0.32	E606-192	106		
E606-0.5	2.02								
		E601-12	0.91	E601-96	0.27	E601-240	195		
E601-1	12.6	E602-12	0.31	E602-96	0.37	E602-240	86		
E602-1	1.14	E603-12	0.37	E603-96	0.18	E603-240	109		
E603-1	1.85	E604-12	0.47	E604-96	0.23	E604-240	125		
E604-1	2.23	E605-12	0.26	E605-96	0.02	E605-240	16		
E605-1	0.3	E606-12	0.35	E606-96	0.18	E606-240	69		
E606-1	1.28								
		E601-24	0.66	E601-120	0.34				
E601-2	11.2	E602-24	0.45	E602-120	0.19				
E602-2	0.53	E603-24	0.35	E603-120	0.14				
E603-2	0.29	E604-24	0.42	E604-120	0.14				
E604-2	1.16	E605-24	0.11	E605-120	0.05				
E605-2	0.32	E606-24	0.2	E606-120	0.09				
E606-2	0.47								

ABSOLUTE BIOAVAILABILITY OF SOIL LEAD IN RATS AND MICROSWINE

MEDICAL RESEARCH PROJECT NO. 9660

PROTOCOL

Stine-Haskell Animal Welfare Committee No. CBT51-P

ABSOLUTE BIOAVAILABILITY OF SOIL LEAD IN RATS AND MICROSWINE

MEDICAL RESEARCH PROJECT NO. 9660

OBJECTIVE

The objective of this study is to determine the absolute bioavailability of lead from soil in rats and microswine to aid in developing a site specific clean-up goal.

SPONSOR AND TEST FACILITY

This study is sponsored by the Corporate Remediation Group of DuPont Chemicals, Bellevue Corporate Center, Wilmington, DE 19809. The study will be conducted at Haskell Laboratory for Toxicology and Industrial Medicine, E. I. du Pont de Nemours and Company, Newark, Delaware, in accordance with all applicable Good Laboratory Practices. The sponsor's approval was effective the date the sponsor signed the Medical Research (MR) project.

STUDY DESIGN

A. Single Dose Administration

- o Fifty male rats and ten male microswine will be used.
- o A predosing blood sample will be drawn from each animal for levels of background lead.
- o Twenty-five rats and five microswine will be dosed orally (gavage) with test soil containing 1,000 ppm lead (approximately 1 g soil/rat and 17 g soil/microswine).
- o Twenty-five rats and five microswine will be given a single intravenous injection of lead acetate solution containing lead-dose equivalent to that contained in the gavage dose.

Single Dose Administration (continued)

- o Approximately 0.1-0.3 ml of blood will be drawn from the rats (each rat will be used to collect three blood samples only) and approximately 0.5 ml of blood will be drawn from each microswine at 0.5, 1, 2, 4, 8, 12, 24, 48, 72, 96, 120, 144, 192, and 240 hours after dosing.

- o Blood samples will be collected in lead-free tubes containing EDTA and stored at 4°C until analysis.

- o At sacrifice, liver and bone (both cortical and trabecular as well as whole femur) will be collected, weighed, and frozen at -20°C.

B. Single Dose Administration After Feeding For 30 Consecutive Days

- o Fifty male rats and ten male microswine will be used.

- o A predosing blood sample will be drawn from each animal for levels of background lead, followed by one sample/week for the duration of 30 days.

- o All animals will be provided with optimized diet of approximately 21.5 g/rat/day or 340 g/microswine/day of a special purified diet mixed with test soil containing 1,000 ppm lead, 5% w/w, for 30 days.

- o After 30 days, blood samples will be drawn from each animal for levels of background lead and the animals will be divided into two groups.

- o Twenty-five rats and five microswine will be dosed orally (gavage) with test soil containing 1,000 ppm lead (approximately 1 g soil/rat and 17 g soil/microswine).

- o Twenty-five rats and five microswine will be given a single intravenous injection of lead acetate solution containing lead-dose equivalent to that contained in the gavage dose.

- o Approximately 0.1-0.3 ml of blood will be drawn from the rats (each rat will be used to collect three blood samples only) and approximately 0.5 ml of blood will be drawn from microswine at 0.5, 1, 2, 4, 8, 12, 24, 48, 72, 96, 120, 144, 192, and 240 hours after dosing.

- o Blood samples will be collected in lead-free tubes containing EDTA and stored at 4°C until analysis.

- o At sacrifice, liver, kidney, brain, spleen, and bone (both cortical and trabecular as well as whole femur) will be collected, weighed, and frozen at -20°C.

MATERIALS AND METHODS

A. Test Material

The test materials will be supplied by Aldrich, Milwaukee, WI 53733, U.S.A. The lead acetate trihydrate will be assigned Haskell Laboratory No. 20202. The lead/soil will be assigned Haskell Laboratory No. 20204. Characterization and mineralogy data of the test soils will be maintained by the sponsor.

B. Test Species

Male Crl:CD®BR rats, approximately 21 days of age, with body weights in the range of approximately 40 to 65 grams will be acquired from Charles River Laboratories, Inc., Kingston, New York. The Crl:CD®BR rat has been selected on the bases of extensive experience with this strain and its suitability with respect to hardiness, longevity, sensitivity, and low incidence of spontaneous disease.

Male Yucatan microswine, approximately 30 days of age, with body weights in the range of approximately 3 to 6 kilograms will be acquired from Charles River Laboratories, Inc., Wilmington, Massachusetts. The weanling Charles River microswine has been selected on the bases of its low body weight, its suitability as a model of soil-lead ingestion by children, and its suitability with respect to longevity, hardiness, sensitivity, and low incidence of spontaneous disease.

C. Pretest Procedures

Rats and microswine will be housed according to the Guide for Care and Use of Laboratory Animals. (NIH Publications No. 86-23, Revised 1985)

The quarantine period will be approximately one week, during which rats will be fed optimized diet (once per day) of irradiated Purina® Certified

Rodent Chow (PCRC) #5002 meal and transferred gradually to Teklad basal mix (95%) for rats. Microswine will be fed optimized diet (twice per day) of microswine diet (Charles River) and transferred gradually to Teklad basal mix (95%) for microswine. Rats and microswine will be provided deionized water ad libitum, weighed three times, and observed with respect to eating habits, weight gain, and any signs of disease or injury.

During the test period, all rats and microswine will be fed the diet of their respective treatment groups.

Animals that die or are sacrificed in extremis during the quarantine period will be necropsied to check for the presence of disease. Animals will be released by the laboratory veterinarian at the end of the quarantine period.

Haskell Laboratory has an animal health monitoring program which consists of periodic food and water analyses for contaminants, sampling freshly washed cages and cage racks for bacteria, and sampling non-study animals housed in the study room for pathogens. The program is monitored and administered by the laboratory veterinarian; data are maintained separately from study records.

D. Assignment to Groups

Healthy animals will be assigned to groups by computerized, stratified randomization. Rats will be housed individually while microswine will be housed two per cage. Each animal will be assigned its own Haskell animal number, and permanently identified by a number marked on the rat's tail or the microswine's ear.

E. Animal Husbandry

After assignment to groups, animals will be identified with a cage card and housed in stainless steel cages. All rats and microswine will be provided optimized test diets of approximately 21.5 g/rat/day and 340 g/microswine/day. Deionized water will be provided ad libitum.

Animal rooms will be targeted at a temperature of 23 ± 2°C and a relative humidity of 50 ± 10%.

F. Diet Preparation and Analysis

During the test period, rats and microswine in each group will be fed the following purified diets:

- o Rat Basal mix (95%) (TD 92242)
- o Swine Basal mix (95%) (TD 93034)

These diets will be custom formulated by their manufacturers so that each diet contains complete normal nutrition for each species when the diet is diluted (weight/weight) with test soil containing 1,000 ppm lead or with sucrose. These diets are sucrose-free so that the soil added will replace the sucrose rather than diluting the complete mixed diet. The diets will contain < 0.1 ug Pb/g.

Lead/soil or sucrose will be added to the diets and thoroughly mixed for a period of time that is adequate to assure homogeneous distribution in the diet. Once prepared, diets will be refrigerated until used.

Sucrose mixed diets will be given to all animals during quarantine and the single dose administration group (Group A in Study Design). The lead/soil mixed diet will be given to the other group for 30 days (Group B in Study Design). After 30 days, Group B animals will be given sucrose-mixed diets for the rest of the study period.

Before the beginning of the study, samples will be collected from the diet prepared with lead/soil. These samples will be analyzed to verify concentration and homogeneity in the test diets.

Homogeneity samples will be collected from the top, middle, and bottom of the diet mixer. Samples will be frozen until analyzed.

Once during the study, samples will be collected from randomly selected feed jars to verify concentration of lead in the feeders. Samples will be collected from each species. Backup samples will be taken on the same day from other feeders in the same species and analyzed if needed. Feeder samples and backup samples will be frozen on the day of collection.

G. Body Weights

All rats and microswine will be weighed twice per week unless experimental findings warrant a more infrequent weighing schedule.

H. Food Consumption and Intake of Test Material

The amount of food consumed by each microswine will be determined twice per week throughout the study. The amount of food consumed by each rat will be determined weekly throughout the study. From these determinations and body weight data, average test animal daily food consumption, and intake of test material will be calculated.

I. Dosing

Rats will be dosed intravenously via the tail vein, while microswine will be dosed via the ear vein. Sterile water will be used as a vehicle for intravenous administration. Oral doses to both species will be administered by gavage. Deionized water will be used as a vehicle.

The route of administration of the lead-contaminated soil will be by oral gavage and/or feeding since ingestion of soil lead activity presents one of the principle direct pathways for exposure to children. Intravenous administration of lead acetate will be performed in order to calculate area under the curve (AUC) and absolute bioavailability of lead.

J. Blood Sampling

Approximately 0.1-0.3 ml of rat venous blood and 0.5-1 ml of microswine venous blood will be drawn from each designated animal at 0, 0.5, 1, 2, 4, 8, 12, 24, 48, 72, 96, 120, 144, 192, and 240 hours after dosing. Blood will be collected into a lead-free tube containing EDTA and refrigerated until the time of preparation for analysis.

K. Sacrifice of Animals

At the end of the experiment, the animals will be humanely euthanized. Animals will be necropsied, liver and bone will be collected from the single dose administered animals (Group A in Study Design) and liver, bone, kidney, spleen, and brain will be collected from the animals fed lead/soil for 30 days (Group B in Study Design). All tissues will be weighed, frozen at approximately -20°C, and stored for potential future analyses.

L. Blood Analysis

The blood lead concentration will be analyzed by graphite furnace atomic absorption spectroscopy (GFAA), at DuPont Chambers Works. Dosing solutions, diets, and drinking water will also be analyzed.

M. Data Analysis

The blood lead concentrations determined by atomic absorption will be used to calculate AUC (area under blood concentration versus time curve) and absolute bioavailability of lead. Individual animal data as well as the mean for five animals in each group will be reported.

N. Statistical Methods

Body weights, body weight gain, and food consumption will be analyzed by one-way analysis of variance. Appropriate statistical analysis will be performed on mean AUC values between oral and intravenous administrations.

SAFETY AND HOUSEKEEPING

Good housekeeping practices will be used to avoid contamination of work areas and potential health hazards. Gloves will be worn when handling formulation, blood, animals, animal tissues, or analytical standards. Animal carcasses, feces, and diet will be incinerated.

RECORDS AND SAMPLE RETENTION

All original records will be retained at Haskell Laboratory or at the Records Management Center, E. I. du Pont de Nemours and Company, Wilmington, Delaware.

PROTOCOL APPENDIX I

MEDICAL RESEARCH PROJECT NO. 9660

Study Personnel and Study Dates

STUDY PERSONNEL

Study Sponsor: Corporate Remediation Group of DuPont Chemicals
E. I. du Pont de Nemours and Company
Bellevue Corporate Center
Wilmington, Delaware 19809

Contact: Timothy S. Bingman, D.A.B.T.
Consultant
(302)571-7761

Study Director: Hanan N. Ghantous, Ph.D.
Research Toxicologist

STUDY DATES

Proposed Experiment Start:	May, 1993
Proposed Sponsor Approval:	Date the Sponsor signed the MR Project
Proposed Experiment Completion:	August, 1993
Proposed Report Issue Date:	February, 1994

ABSOLUTE BIOAVAILABILITY OF SOIL LEAD IN RATS AND MICROSWINE

MEDICAL RESEARCH PROJECT NO. 9660

PROTOCOL

Date

Approved: H. Ghantous 5/17/93

Hanan N. Ghantous
Study Director

Matthew S Bogdanffy 5/18/93

Matthew S. Bogdanffy
Manager
Biochemical Toxicology

cc: P. E. Ross
G. S. Elliott
T. S. Bingman (REO-PIT)
K. Mc Cleary
M. S. Bogdanffy
R. C. Rhea

DUPONT CENTRAL RESEARCH AND DEVELOPMENT
HASKELL LABORATORY FOR TOXICOLOGY
AND INDUSTRIAL MEDICINE

June 28, 1993

TO: MEDICAL RESEARCH PROJECT NO. MR 9660-001

ABSOLUTE BIOAVAILABILITY OF SOIL LEAD IN RATS AND MICROSWINE

PROTOCOL AMENDMENT #1

The protocol is amended as follows:

1. Page 2, Study Design, Section A. Single Dose Administration, replace "Fifty male rats and ten male microswine will be used." with "Fifty male rats and twelve male microswine will be used."

Reason: To obtain better statistical analysis of results.

2. Page 2, Study Design, Section A. Single Dose Administration, replace "Twenty-five rats and five microswine will be dosed orally..." with "Twenty-five rats and six microswine will be dosed orally...".

Reason: To obtain better statistical analysis of results.

3. Page 2, Study Design, Section A. Single Dose Administration, replace "Twenty-five rats and five microswine will be given..." with "Twenty-five rats and six microswine will be given...".

Reason: To obtain better statistical analysis of results.

4. Page 3, Section B. Single Dose Administration After Feeding For 30 Consecutive Days, replace "Fifty male rats and ten male microswine..." with "Fifty male rats and twelve male microswine..."

Reason: To obtain better statistical analysis of results.

5. Page 3, Section B. Single Dose Administration After Feeding For 30 Consecutive Days, replace "Twenty-five rats and five microswine will be dosed orally..." with "Twenty-five rats and six microswine will be dosed orally...".

Reason: To obtain better statistical analysis of results.

6. Page 3, Section B. Single Dose Administration After Feeding For 30 Consecutive Days, replace "Twenty-five rats and five microswine will be given..." with "Twenty-five rats and six microswine will be given...".

Reason: To obtain better statistical analysis of results.

7. Page 4, Pretest Procedures, second paragraph, delete "irradiated" from first sentence.

Reason: The animals are not being feed irradiated chow. The study is being conducted in Building 1 and irradiated chow is not received there.

8. Page 5, second sentence, replace "(twice per day)" with "(once a day)".

Reason: More convenient for individual food consumption calculations.

9. Page 5, Section D. Assignment to Groups, second sentence, replace "housed two per cage" with "housed individually".

Reason: Animals will be housed individually to calculate individual food consumption.

10. Page 6, Section E. Animal Husbandry, second paragraph should read "Animal rooms will be targeted at a temperature of 23 $\pm$ 2°C for rats and 25 $\pm$ 2°C for microswine and a relative humidity of 50 $\pm$ 10%."

Reason: Microswine should be kept at a higher temperature than rats.

11. Page 6, Section E. Animal Husbandry, after second paragraph, insert the following paragraph: Animal rooms that will house rats will be artificially illuminated (fluorescent light) on a 12-hour light/dark cycle. Animal rooms that will house microswine will be artificially illuminated (fluorescent light) on a 12-hour light/dark cycle with additional natural light being available through a window at one end of the room.

Reason: Microswine do not need to be on a 12 hour light cycle. The animals should be housed in conditions similar to a barn.

12. Page 2, Section A. Single Dose Administration, "Blood from the single dose administered group of rats was collected in lead-free tubes containing EDTA. Heparin containing tubes were used for collecting blood from the other groups.

Reason: EDTA caused complications in analyzing blood lead by atomic absorption.

13. Page 6, Section E. Animal Husbandry, add the following paragraph "Microswine were provided with recreational equipment (balls) in their cages.

Reason: To improve housing conditions. Balls were added after consulting with Linda Panepinta, Swine Research Consultant.

14. Page 6, Section F. Diet Preparation and Analysis, the following should be added "Microswine were given Oreo cookies after blood sampling.

Reason: Linda Panepinta, Swine Research Consultant, suggested the administration of Oreo cookies after blood sampling as a reward.

15. Page 7, first paragraph, the following sentence should be added "Diet samples will be refrigerated, not frozen until analysis.

Reason: Analysis was done within a short period of time after sampling.

16. "Due to technical error, the 12 hr. interval blood collection was not taken for the single dose gavage group of rats. Five male rats from the animals not used on the study were randomly selected, prebled, dosed by gavage, and bled after 12 hrs.

Reason: Technical error.

17. Page 8, Section K. Sacrifice of Animals, Replace the final sentence with "Tissues from rats bled at the 240 hr. time point will be saved at sacrifice.

Reason: Tissues from 5 rats only are needed for statistical analysis.

Date

Approved: H. N. Ghantous July 20/1993

Hanan N. Ghantous
Study Director

Matthew S. Bogdanffy 7/26/93

Matthew S. Bogdanffy
Manager
Biochemical Toxicology

cc: P. E. Ross
G. S. Elliott
T. S. Bingman (REO-PIT)
K. Mc Cleary
M. S. Bogdanffy
R. C. Rhea

DUPONT

CC: H. Ghantous - CR&D, Haskell

DuPont Central Research
and Development

MR-9660-001

June 9, 1995

TO: Tim Bingman - SPEC

FROM: Matthew S. Bogdanffy - CR&D, Haskell 1-1712-A

SUBJECT: Response to Comments from the Washington Department of Health

Dr. Ghantous has reviewed the comments on the lead bioavailability study prepared by the Washington State Department of Health. Seven points were raised in their letter to Mike Blum, Department of Ecology, which we wish to address. A summary of our responses are presented below:

1. *Inaccuracies in calculating the doses given to the study animals.*

In the documentation supporting the DOH memo, it is stated that "the author then mistakenly assumed that the 30 ml suspension weighed 47 g (i.e. 30 g of water plus 17 g soil) in the calculated dose. However, mixing soil with water results in a suspension with a volume greater than 30 ml. Thus, 30 ml of this 47 g suspension weighed less than 47 g, and the actual dose of lead received by each animal was less than the dose used in the calculations by the author." The reviewer then goes on to demonstrate, hypothetically, that such an error would result in a decrease in the final calculated bioavailability.

In the absence of an understanding of the nature of the test materials, the reviewer would have been correct on this point. The volume added to the overall dose suspension contributed by the volume of the admixed soil was not taken into account in the dose calculations and would have, theoretically, resulted in an overestimation of the true bioavailability.

To examine the validity of this theoretical point, Dr. Ghantous returned to the lab and conducted an experiment in which 17 grams of soil (the same soil used in the subject bioavailability study [Haskell Laboratory Report 671-93]) was mixed with 30 mls of water. The methods for weighing and mixing the soil were similar to those used in the bioavailability study except that in the latter, and as correctly noted by DOH, the investigators mixed a bulk amount of soil in water and then withdrew individual doses from this suspension. The volume of the resulting suspension in Dr. Ghantous's experiment was measured. The volume was approximately 30 mls (limit of accuracy was $\pm$ 1 ml). Dr. Ghantous's observations suggested that this negligible volume increase was a result of the ultrafine powder-like consistency of the soil. These soils were sifted to a mesh of approximately 250 microns.

The entire soil/water mixture was then drawn into a tarred syringe, in a manner similar to that used in the study, and weighed. The weight of the mixture was 41.6 grams. This is less than 47 grams and, since mass is conserved, this suggested some loss of material on the walls of the mixing vessel. This was confirmed by observation. Therefore, preparation of the dosage in this manner would have lead to negligible loss of dosage volume, but an 11.5% loss of soil weight (1.96 grams per dosage).

However, as noted above, the investigators prepared the dose mixture in bulk. Therefore the soil loss noted in the paragraph above would only apply to the last dosage removed from the mixing vessel. Thus, given that a) the volume change induced by soil addition to water is negligible, and b) enough dose solution was prepared for 10 animals, but only five dosages were removed, the point made by the DOH regarding inaccuracies in calculating the doses given to the study animals becomes moot. Nevertheless, we thank the DOH reviewer for assisting us in evaluating the data.

Regarding the density of the i.v. dose solution, Dr. Ghantous also looked into this question experimentally in response to the comments of the reviewer. The density of the dose prepared by weighing 3.527 g of lead acetate trihydrate and dissolving it in 10 mls of water (approximately 35% solution) was 1.2 g/ml. Therefore, given that the density of water is 1.0, the difference between 1.2 and 1.0 (i.e. 0.2 g/ml) is due to the added lead acetate. A calculation of proportions can then be made to estimate the density of the i.v. dose solution. Appendix H contains measured values of 116.6 mg/g of water (116.6 mg/ml of water, or 11.7%) and 126.8 mg/g of water (126.8 mg/ml of water, or 12.7%). Using the 11.7% value as an example, 35%/0.2 = 11.7%/X; X = 0.06. Therefore, the density of the i.v. lead acetate solution equals 1.0 + 0.06, or 1.06. This value is very near the assumed density of 1.0 and, therefore, has no material effect on the final calculations.

2, 3. *The small number of microswine used in each experiment.*
The high inter-animal and intra-animal variability in blood lead concentration over time.

The DOH reviewer notes that "It is difficult to have confidence in studies that looked at so few animals." The reviewer goes on to support this with discussion around the variability in the blood lead measures. As a scientist, the term confidence implies statistical interpretations. Large *intra*animal variability is common in pharmacokinetic data. The reviewer is directed to any of a number of texts and literature references on pharmacokinetics and bioavailability for discussions on these points[1].

Clearly, increasing the numbers of microswine per group could reduce the *inter*animal variability. The question is how many microswine per group would have to be added and, importantly, whether the statistical "pay-off" achieved by increasing the group size justifies the ethical issues of increased animal usage. We considered these arguments in the study design. Our study was also subjected to review by our in-house animal welfare committee. Consultation

[1] For a recent article, see Prelusky, D. B., Hartin, K. E., Trenholm, H. L., and Miller, J. D. (1988). Pharmacokinetic fate of ^{14}C-labeled deoxynivalenol in swine. *Fundam. Appl. Toxicol.*, **10**, 276-286.

with our statistician indicated that increasing the numbers of microswine per group by 2 would yield little, if any, increase in statistical power. This suggests that a large increase in group size would be necessary to off-set the *inter*animal variation. It is our opinion that such a large increase could not be justified against the ethical issues. Furthermore, recent swine bioavailability studies conducted by the U. S. EPA used three swine per group[2].

4,5. *The lack of scientifically sound justification for choosing one particular experiment out of all the Phase I and Phase II studies on which to base the "absolute bioavailability" figure.*
The lack of justification for applying a bioavailability figure derived from acute dosing experiment to a chronic exposure scenario.

The choice of the bioavailability value derived from the Single Dose Administration (Acute) experiment using microswine over other bioavailability values derived has been addressed. In brief, the Phase I work was conducted to provide range-finding data for the Phase II work. The Phase I work could only yield relative bioavailability values. The choice of the Single Dose Administration (Acute) bioavailability value was based on a) the pig model is more suitable than rats as a representative of humans for soil bioavailability studies[3], and b) greater statistical confidence and interpretive value was placed on the Single Dose Administration (Acute) microswine.

Regarding the bioavailability calculations, Dr. Ghantous has recalculated the bioavailability of lead using the subchronic feeding study data. Following the guidance contained in "Gudinace for the Integrated Exposure Uptake Biokinetic Model for Lead in Children[4]" the data were re-evaluated by measuring areas under the blood lead curves (AUC) from time zero (just prior to dosing) to 24 hours, rather than extending the AUC calculation to infinity (as was done in the report)[5]. Recalculating the data in this manner avoids the complications observed in the subchronic study that were attributed to remobilization of lead. The mean AUC for the i.v. group was 15.08 and the mean AUC for the oral group was 5.50. Thus the calculated bioavailability is 23.8%.

6. *The lack of evidence to indicate that the soil used in the bioavailability studies is representative of soils throughout the site.*

This comment is best addressed by the remediation program scientists.

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[2] Lorenzana, R. M., Dawson, M., Kettere, M., Simon, J., Lowry, J., and Poppenga, R. (1995). Stable isotope method to determine bioavailability of lead in swine. *Toxicologist*, abstract no. 716.
[3] Weis, C. P., and LaVelle, J. M. (1991). Characteristics to consider when choosing an animal model for the study of lead bioavailability. *Chemical Speciation and Bioavailability* 3(134), 113-119
[4] U. S. EPA. Guidance Manula for the Integrated Exposure Uptake Biokinetic Model for Lead in Children.
[5] Welling, P. G. (1986). Multiple Dose Kinetics. In Pharmacokinetics Process and Mathematics, Chapter 12.

7. *The lack of quality assurance/quality control data for the studies.*

This study was conducted in full compliance with Good Laboratory Practices as stated on page 2 of the report. Minor protocol and GLP deviations are disclosed in Appendix B. The four deviations noted did not, as attested to in the report, affect the integrity of the report.

TEST SUBSTANCE TRACKING FORM

MR # 9660 H # ~~20204~~ 20202 ① Test Code(s) 89

① WRONG H# H#20204 WAS LEAD SOIL BR 10/25/93

Test Substance Name (synonym) LEAD (II) ACETATE (Solid) / Gas / Liquid

Gross Weights Taken In Test Substance Room:

Amount Received 114.792g Balance # NX2845 Date Received 5/13/93 T.I. BR

Amount Returned _____ Balance # _____ Date Returned __/__/__ T.I. _____

Gross Weights Taken In Laboratory:

Amount Received 114.792 g Balance # NX2845 Date Received 5/13/93 T.I. BR

Amount Returned _____ Balance # _____ Date Returned __/__/__ T.I. _____

Study Type (ALC, LC50, etc.)	Balance Calibration			Amount Removed	Gross Weight After Removal	Comments
	I.D.#	Weight	Reading			
LEAD (II) ACETATE	EZ-3	30mg	31.6 mg	* 31.12 mg	114.761 g	METHODS DEVELOPMENT
LEAD (II) ACETATE	NX3474	50mg	51.0 mg	① ~~0.1g~~ 0.2g	114.561g	METHODS DEVELOPMENT
Lead (II) acetate	L-1522	1g ~~50 mg~~ ②	1.002g	4~~8~~00 mg	114.161g ②	IV Injection Dose cf 5/18/93
LEAD (II) ACETATE	L-1522	1g	1.000g	497.92mg	113.663g	IV Dose Swine
Lead (II) acetate	L-1522	1g	1.000g	400 mg	113.263 g	IV Dose Rats
LEAD (II) ACETATE	NX3474	3g	3.0052g	3,113.0mg	110.150 g	IV DOSE SWINE 8/20/93 BR
LEAD (II) ACETATE - MR-9227	EZ-3	5.000g	4.995g	③ 3528.5mg	106.259g	I.V. DOSE SWINE FOR 10/26/93 BR 10/25/93
LEAD (II) ACETATE - MR-9740	NX3474	30mg	30mg	3112mg	106.000g	I.V. DOSE SWINE FOR 12/3/93. BR 12/2/93

Initials	Signatures
BR	Benjamin Robyn
CF	Christine Loeffler

*31.12 mg = 0.03112g

① WRONG OVER. BR 5/14/93

② Entry error cf 5/18/93

③ Entry error cf 8/3/93

③ PLUS SMALL AMOUNT OF WASTE. BR 10/25/93

DUP04000733

This accountability sheet was for practice animals used in support of MR 9660. This record is not considered part of the study (MR 9660) records, but will be maintained in file for MR 9660 John Allgeier 1/11/94

ANIMAL ROOM TASK ACCOUNTABILITY SHEET

Room # 1106 Species Pigs MR # ~~5460~~ 9660 H # 20204 Year 1993

WRONG INFORMATION. CS 1/5/94 WRONG! CS 1/5/94

Date	5/6	5/7	5/8	5/9	5/10	5/11	5/12
a.m. Check							
Temperature (°C)	—	22	28	26	22	22	23
Humidity (40-60%)	—	37	38	36	35	38	38
Time of Readings	—	8:00	7:45	7:00	8:12	10:00	8:30
Recorder's initials	—	CS	JH	JH	CS	CS	CS
Mortality/Moribundity checked	—	CS	JH	JH	CS	CS	CS
Water availability checked/Bowls filled	—	CS	JH	JH	CS	CS	CS
Animals fed Time 8:00	—	CS	7:45	7:00	8:30	10:00	9:00
p.m. Check							
Temperature (°C)	22	28	20	22	22	22	22
Humidity (40-60%)	37	42	34	38	36	38	38
Time of readings	1:00	3:00	1:06	1:00	2:20	2:25	1:30
Recorder's initials	CS	JH	MH	MH	CS	CS	CS
Mortality/Moribundity checked	CS	JH	MH	MH	CS	CS	CS
Water availability checked/Bowls filled	CS	JH	1:06	1:00	CS	CS	CS
Animals fed Time	CS	3:00	1:06	1:00	2:10	2:20	1:30
Tasks (initial each item							
Room cleaned					JH	CS	CS	CS
Cage change					—	—	—	—
Room scrubbed					—	—	—	—
Animals rotated/Animal					—	—	—	—
Feeders cleaned and dri					JH	CS	CS	CS
Water bowls cleaned an					—	CS	—	—
Ceiling tiles checked					N/A	N/A	N/A	N/A

Place into MR file (MR 9660)

o
Rabbit room temperatur 21-25 C).
N/A = Not applicable.
a Hygrothermograph us iits.
b Temperature or humi ciate personnel contacted.

Remarks/Footnotes:

① animals arrived 5/6/93 CS 5/6/93

NOTE: THIS ACCOUNTABILITY SHEET WAS NOT PUT INTO THIS BOOK UNTIL 1/5/94 BY BR ON 1/5/94 BR.

Witnessed by: BR Date: 1/5/94