DuPont Central Research and Development
Haskell Laboratory for Toxicology and Industrial Medicine

April 28, 1994

TO: Timothy Bingman
DuPont Chemicals
~~1370 Washington, Pike~~
~~Bridgeville, Pennsylvania~~
Suite 10
4 Beaver Grade Rd.
Coraopolis, PA 15108

PROJECT MR-9905 PART I

TITLE: ABSOLUTE BIOAVAILABILITY OF LEAD SOIL IN MICROSWINE:
Soils Identified as *Fort Madison* and *PLW-1*

Authority is requested to conduct a study to determine the absolute bioavailability of soil lead in microswine. Details are in the study protocol, provided under a separate cover.

The full cost of the study is $47,000.00 and is distributed to cost centers 19, 23, and 25.

The allocated cost of this study is $34,625.00. The allocated costs are to be decrementally billed over 5 months (June through December of 1994) as follows:

Cost Center 19	$ 30,000.00	(86%)
Cost Center 23	3,925.00	(11%)
Cost Center 25	700.00	(02%)

			Forecast month and year
Allocated cost requested:	$ 34,625.00	Experiment to start:	6/94
Previously authorized	$ 0.00	Experiment to end:	7/94
Expended through *[date]*	$ 0.00	Final report to issue:	1/95

General Ledger & Sub-Account 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
Hanan N. Ghantous

Approved by: Matthew S. Bogdanffy
Matthew S. Bogdanffy

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

Authorized by: Timothy S. Bingman 5/25/94
(date)

N 26889

Study Title

ABSOLUTE BIOAVAILABILITY OF LEAD-CONTAMINATED SOILS IN MICROSWINE

Laboratory Project ID

Haskell Laboratory Report No. 581-94

Author

Hanan N. Ghantous, Ph.D.

Study Completed on

October 20, 1994

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 19714-0050

Medical Research Project Nos.

9727-001, 9740-001, and 9905-001

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

Study Director: H. Ghantous 10/20/94
Hanan N. Ghantous, Ph.D. (date)
Research Toxicologist
Biochemical Toxicology and
Risk Analysis

Company Representative: _____ _____
(date)

GENERAL INFORMATION

Intravenous Administration

Substance Tested:	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

Oral Administration

Substance Tested:	Soils, Remington site, contaminated with lead
Synonyms/Codes:	H-20366
	Lead-contaminated soil AEC-3-3
	Soil, contaminated with lead
Haskell Sample No.:	20366
Purity:	N/A
Composition:	1070 ppm lead in soil
C.A.S. Registry No.:	None Available

Substance Tested:	Soils, Remington site, contaminated with lead
Synonyms/Codes:	H-20367
	Lead-contaminated soil AEC-9-3
	Soil, contaminated with lead
Haskell Sample No.:	20367
Purity:	N/A
Composition:	1120 ppm lead in soil
C.A.S. Registry No.:	None Available

GENERAL INFORMATION (Continued)

Substance Tested:	Soils, Remington site, contaminated with lead
Synonyms/Codes:	H-20366
	Lead-contaminated soil AEC-10-1
	Soil, contaminated with lead
Haskell Sample No.:	20368
Purity:	N/A
Composition	1210 ppm lead in soil
C.A.S. Registry No.:	None Available

Substance Tested:	Soils, Pompton Lakes, contaminated with lead
Synonyms/Codes:	H-20383
	Lead-contaminated soil PLW
	Soil with lead #PLW (Pompton Sample)
Haskell Sample No.:	20383
Purity:	N/A
Composition	803 ppm lead; 205 ppm mercury
C.A.S. Registry No.:	None Available

Substance Tested:	Soils, East Chicago, contaminated with lead
Synonyms/Codes:	H-20384
	Lead-contaminated soil EC1
	Soil with lead #EC1 (East Chicago Sample)
Haskell Sample No.:	20384
Purity:	N/A
Composition:	3000 ppm lead; 235 ppm arsenic
C.A.S. Registry No.:	None Available

Substance Tested:	Soils, Fort Madison, contaminated with lead
Synonyms/Codes:	H-20659
	Lead-contaminated soil Fort Madison (FM)
	Lead-contaminated soil from Fort Madison (FM)
Haskell Sample No.:	20659
Purity:	N/A
Composition	500 ppm lead
C.A.S. Registry No.:	None Available

GENERAL INFORMATION (Continued)

Substance Tested:	Soils, Pompton Lake, contaminated with lead
Synonyms/Codes:	H-20658
	Lead-contaminated soil (PLW-1 or PLW1)
	Lead-contaminated soil (from Pompton Lake)
Haskell Sample No.:	20658
Purity:	N/A
Composition	4000 ppm lead
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.
Study Initiated/Completed:	10-11-93 / Report Issue Date (see cover page)
Experiments Initiated/Completed:	10-19-93 / 11-08-93 (Groups A, B, C, and D) 11-30-93 / 12-17-93 (Groups E, F, and G) 06-14-94 / 07-01-94 (Groups H, I, and J)

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SUMMARY

The objective of this study was to determine the absolute bioavailability of lead in test soil from contaminated sites. The bioavailabilities of lead from contaminated soils were measured by using a single dose administration to juvenile microswine.

Groups of 5 or 6 male juvenile microswine were orally administered (via gavage) test soil (approximately 17 mg lead/microswine). Three groups of 5 or 6 additional male juvenile microswine received an intravenous injection of lead acetate solution containing a lead dose equivalent to that of the oral (gavage) dose.

Blood was drawn from all groups of microswine 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 and intravenous groups.

The absolute bioavailability results were:

Test Soil Administered	Absolute Bioavailability
AEC 3-3	28.52%
AEC 9-3	16.70%
AEC 10-1	24.12%
PLW	30.97%
EC1	31.72%
FM	14.72%
PLW1	9.18%

The apparent differences in oral bioavailability observed in this study could be related to a combination of factors, including the chemical form of lead in the test soil and the intrinsic soil properties.

MEDICAL RESEARCH PROJECT NO. 9727-001

Authored by: _____

Hanan N. Ghantous, Ph.D.
Research Toxicologist
Biochemical Toxicology and
Risk Analysis

Approved by: _____

Matthew S. Bogdanffy, Ph.D., D.A.B.T.
Manager
Biochemical Toxicology and
Risk Analysis

Reviewed and
Approved for Issue
by Study Director: _____

Hanan N. Ghantous, Ph.D.
Research Toxicologist
Biochemical Toxicology and
Risk Analysis

Date Report Issued: 10/20/94

QUALITY ASSURANCE DOCUMENTATION

(Haskell Numbers: 20202, 20366, 20367, 20368, 20383, 20384, 20558, and 20659)

Date(s) of Inspection:

Conduct - 10/21,22,25,26/93, 11/5,9/93, 12/2,3,6,7,10/93, 1/11/94, 6/13,14,17,21,24/94, 7/1/94

Records, Report(s) - 10/14/93, 5/23/94, 6/9,11,13,24/94, 8/15,18,19/94, 9/13-16,20-22,29/94, 10/6-8,10-13/94

Date(s) Findings Reported to:

Study Director - 8/1,10,19/94, 9/22,28,29/94, 10/13/94

Management - 8/1,10/94, 9/28/94, 10/3,5,12,20/94

Reported by: Robert C. Rhea 10/20/94

Robert C. Rhea (date)
Quality Assurance Auditor

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:	Benjamin Robinson, B.A.
Assisting Technicians:	Steven C. Carpenter Gerald L. King
Technical Assistants:	A. Richard Conrad, Jr. Victor L. Cruz Roger B. Walls
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 Robert Eyberger (MR 9905-001 only) 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.
Deborah A. Vick, A.A.S.

The health status of the animals on study was assessed by the attending Laboratory Veterinarian, Charles E. Cover, V.M.D.

INTRODUCTION

In a recent study conducted at Haskell Laboratory,[1] the absolute bioavailability of lead from a site's soil was measured in both juvenile rats and microsowine using either *single-doses* or *subchronic-dosing followed by single-dose exposures*. Both rats and microswine were used in order to choose the correct animal model in the conduct of experimental investigations relevant to humans.

Based on the results of the above study, microswine appeared to provide a more conservative estimation of bioavailability than rats. In addition, because of difficulties encountered interpreting blood lead data resulting from a bioaccumulative effect of subchronic exposure, single-dose exposures provided the most meaningful results for measuring lead bioavailability.

Thus, microswine given a single-dose exposure are used in this study to measure the bioavailability of lead from contaminated soils from different sites.

OBJECTIVE

The objective of the study was to determine the absolute oral bioavailability of lead from soil in microswine following single oral (gavage) doses. These results will assist in the development of site-specific clean-up goals by providing soil-specific data on gastrointestinal absorption.

MATERIALS AND METHODS

The original protocols and protocol amendments are in Appendix A.

A. Test Substance

Lead acetate trihydrate was supplied by Aldrich, Milwaukee, Wisconsin; the lead-contaminated soils were supplied by the sponsor. See the *General Information* section of this report for more information about lead acetate trihydrate and the lead-contaminated soils. Characterization and mineralogy data of the lead-contaminated soils are maintained by the sponsor.

B. Test Species

Groups of juvenile male Yucatan microswine were received from Charles River Laboratories, Inc., Windham, Maine or Pittsfield, New Hampshire. Twenty were

received on 10/05/93 (born August or September of 1993), 18 were received on 11/12/93 (born September or October of 1993), and 15 were received on 6/02/94 (born April or May of 1994). The day after arrival, all microswine were weighed. Body weights ranged from 2884 to 7813 g. The male Yucatan microswine was selected on the bases of its low body weight and its reported suitability as a model of soil-lead ingestion by children.

Groups of female Yucatan microswine were received during the pretest periods and used for oral gavage practice. These microswine were housed in the same room with the male microswine, but were not considered part of the study; the females were sacrificed before the males were dosed.

C. Animal Husbandry

During the pretest period, microswine were housed according to the *Guide for Care and Use of Laboratory Animals.*[2] After assignment to groups, microswine were housed in stainless steel cages bedded with biodegradable shredded paper (Enviro Dri). To improve housing conditions, microswine were provided with recreational equipment (balls) in their cages. Occasionally, microswine were rewarded with Oreo® cookies (to calm the animals after blood samples were collected).

The animal room was targeted at a temperature of 25±2°C and a relative humidity of 50±10%. Sporadically, the temperature and relative humidity of the animal room were slightly below these ranges, but did not affect the study. The animal room was 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. The following procedures were performed periodically:

- Water samples were analyzed for total bacterial counts and the presence of coliforms, lead, and other contaminants.
- Feed samples were analyzed for the presence of bacteria and fungi.
- Samples from freshly washed cages and cage racks were analyzed to assure adequate sanitation by the cagewashers.

Haskell Laboratory uses certified animal feed. The feed is guaranteed by the manufacturer to meet specified nutritional requirements and to be free of a list of specified contaminants.

The animal health monitoring program is administered by the laboratory animal veterinarian. Data are maintained separately from study records and are not included in this report. Evaluation of these data did not indicate any conditions that affected the validity of the study.

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

Microswine were quarantined for at least 13 days. During the pretest period, 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%). Transfer to Teklad basal mix and sucrose was completed before study start. Microswine were provided deionized water *ad libitum*.

All microswine were weighed at least 4 times during the quarantine period and observed daily with respect to eating habits, weight gain, and any signs of disease or injury.

E. Assignment to Groups and Study Start

Microswine were divided by computerized, stratified randomization into groups as follows:

Date of Arrival	Experiment Number	Group ID[a]	Number Per Group	Method of Administration	Test Substance Administered
10/05/93	1	A	5	Oral Gavage	Soil AEC 3-3
10/05/93	1	B	5	Oral Gavage	Soil AEC 9-3
10/05/93	1	C	5	Intravenous	Lead Acetate
10/05/93	1	D	5	Oral Gavage	Soil AEC 10-1
11/12/93	2	E	6	Oral Gavage	Soil PLW
11/12/93	2	F	6	Intravenous[b]	Lead Acetate
11/12/93	2	G	6	Oral Gavage	Soil EC1
06/02/94	3	H	5	Oral Gavage	Soil FM
06/02/94	3	I	5	Intravenous	Lead Acetate
06/02/94	3	J	5	Oral Gavage	Soil PLW1

[a] Group identification labels are for the purpose of reporting and do not correspond to the group identification in the study records.

[b] Due to technical error, the dosing solution was incorrectly prepared. Lead concentration data from this group will not be used in the report.

F. Diet Preparation, Administration, and Sampling

Diets were prepared by combining Teklad basal mix (95%) with sucrose (5%). These ingredients were mixed in a high-speed mixer for 3 minutes. Diets were refrigerated until used or discarded.

Each microswine received 340 g diet per day. Samples of the Teklad basal mix and sucrose diet were sent to DuPont Chambers Works, Deepwater, New Jersey and analyzed for presence of lead.

G. Dose Preparation, Administration, and Sampling

Oral Dose. Microswine designated for oral (gavage) administration received a 30.0 or 40.0 mL suspension of test soil and deionized water. Each microswine was targeted to receive approximately 17 mg lead within the 30.0 or 40 mL dose. The suspensions were constantly stirred until all animals were dosed. The dose of some soils was diluted due to the consistency of the soil; however, the dilution was taken into consideration in calculating the dose of lead administered.

Oral dosing suspensions were analyzed for lead concentration (Table 1) and the measured values were used for calculating lead bioavailability.

Intravenous Dose. Microswine designated for intravenous administration received a 0.1 mL solution of lead acetate and sterile water via the ear vein. The solution was prepared to contain a lead dose equivalent to that contained in the oral (gavage) dose.

Dose group F is not included in this report because the dosing solution was not prepared correctly.

Intravenous dosing solutions were analyzed for lead concentration (Table 1) and the measured values were used for calculating lead bioavailability.

H. Blood Sampling

Approximately 0.2-0.5 mL of blood were drawn from the *vena cava* at 0.5, 1, 2, 4, 8, 12, 24, 48, 72, 96, 120, 144, 192, and 240 hours after dosing. All blood samples were stored frozen at -20°C, then sent to DuPont Chambers Works for analysis.

I. Body Weights, Clinical Observations, and Mortality

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

On test day 1, animal #1094 (Group B) was accidentally killed as a result of a dosing accident. In addition, it was noticed at the 4-hour blood collection time point that microswine #984 (Group A) was experiencing labored breathing. At the 24-hour blood collection time point, microswine #984 was still having difficulty breathing; it was sacrificed *in extremis*, and a necropsy was performed. Samples collected from these microswine were not evaluated or further used in the study.

J. Sacrifice of Animals

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 were collected, weighed, frozen at approximately -20°C, and stored for potential future analysis.

K. 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.

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 1-7).

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 4).

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

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 DuPont Records Management Center, E. I. du Pont de Nemours and Company, Wilmington, Delaware.

RESULTS AND DISCUSSION

A. Diet Samples

Stability testing of the soils was not performed. U.S. EPA Publication SW-846, *Methods for the Analysis of Solid Waste*, specifies a holding time for solid material samples of 6 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 and sucrose diets, Oreo® cookies, swine bedding, and deionized water were also analyzed. Results are provided in Table 2. Results from these analyses demonstrated that no lead was present in any of the samples except the lead-contaminated soils.

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 Table 3. Individual blood lead concentrations are listed in Appendix C.

Individual animal AUCs were determined for the microswine and are reported in Table 5. The mean AUC for each group is as follows:

Experiment Number	Group ID	Substance Administered	Mean µg(hr)/mL	Standard Deviation
1	A	Soil AEC 3-3	50.45	5.418
1	B	Soil AEC 9-3	40.72	21.183
1	C	Lead Acetate	107.20	70.647
1	D	Soil AEC 10-1	38.61	6.726
2	E	Soil PLW	16.29*	7.126
2	F	Lead Acetate	(not used)	
2	G	Soil EC1	52.89	10.868
3	H	Soil FM	25.86**	6.594
3	I	Lead Acetate	28.06	8.674
3	J	Soil PLW1	49.83**	21.217

* Statistically significant difference from lead acetate (Group C) by Student t-test ($p \leq 0.05$).

** Statistically significant difference from lead acetate (Group I) by Student t-test ($p \leq 0.05$).

In Experiment #1 (Groups A, B, and D), the mean AUC of soils AEC 3-3, AEC 9-3, and AEC 10-1 were compared to the mean AUC of the intravenous dose (Group C). There were no statistically significant differences (Student t-test) between the AUCs of the oral (soils) and intravenous doses. The absolute oral bioavailability was 28.52% for AEC 3-3, 16.70% for AEC 9-3, and 24.12% for AEC 10-1.

In Experiment #2 (Groups E and G), the mean AUC of soils PLW and EC were also compared to the mean AUC of the intravenous dose from Group C. Group F, which received an intravenous dose, was not used in the calculations because the dose was prepared incorrectly. The Group C intravenous dose was chosen because that experiment was conducted just 1 month earlier; Experiment #3 was not conducted until 7 months after Experiment #2. There was a significant difference (Student t-test) between the AUCs of the oral PLW soil and the intravenous dose (Group C). The absolute oral bioavailability was 30.97% for PLW and 31.72% for EC1.

In Experiment #3 (Groups H and J), the mean AUC of soils FM and PLW1 were compared to the mean AUC of the intravenous dose (Group I). There were statistically significant differences (Student t-test) between the AUCs of the oral (soils) and intravenous dose. The absolute oral bioavailability was 14.72% for FM and 9.18% for PLW1.

D. Body Weights and Clinical Observations

Exposure to lead produced no effects on body weights (Appendix D) or food consumption. No deaths or compound-related clinical observations of toxicity were observed in any group; however, two microswine died from dosing accidents (Appendix E).

CONCLUSION

The bioavailability of lead in soils following single-dose oral gavage administration to microswine were:

Test Soil	Absolute Bioavailability
AEC 3-3	28.52%
AEC 9-3	16.70%
AEC 10-1	24.12%
PLW	30.97%
EC1	31.72%
FM	14.72%
PLW1	9.18%

The observed differences in oral bioavailabilities support the necessity to determine these values for each unique site and to develop site-specific clean-up goals.

REFERENCES

1. Haskell Laboratory Report No. 671-93. Unpublished data.

2. U.S. Department of Health and Human Services, Public Health Service, National Institute of Health. Revised 1985. *Guide for the Use and Care of Laboratory Animals.*

TABLES

TABLE 1. Measured concentration of lead in intravenous and oral gavage dosing solutions.

Experimental Group	Route of Administration	Substance Administered	Amount of Lead[a]	Amount of Water
A	Oral Gavage	AEC 3-3	31.56 mg	300 mL (deionized)
B	Oral Gavage	AEC 9-3	43.51 mg	500 mL (deionized)
C	Oral Gavage	AEC 10-1	28.55 mg	300 mL (deionized)
D	Intravenous	Pb Acetate	19.12 mg	10 mL (sterile)
E	Oral Gavage	PLW	9.38 mg	400 mL (deionized)
F	Intravenous	(not used)		
G	Oral Gavage	EC1	29.74 mg	300 mL (deionized)
H	Oral Gavage	FM	11.01 mg	400 mL (deionized)
I	Oral Gavage	PLW1	34.05 mg	400 mL (deionized)
J	Intravenous	Pb Acetate	1.76 mg	10 mL (sterile)

[a] The amount of lead was calculated from the values of analyzed dosing solutions. See Appendix F for a sample calculation.

TABLE 2. Miscellaneous lead concentrations.

Type of Sample	Concentration of Lead (µg/mL)[a]
Teklad Basal Chow + Sucrose	<1
	<1
Cage Bedding	<1
	73
	15
Nitric Acid[a]	<1
	<1
Deionized Water	<1
	0.071
Oreo® Cookies	<15

[a] Nitric acid was used in the process to determine the concentration of lead in samples.

TABLE 3. Mean blood lead concentration (µg/mL).

Collection Time (hrs)	Group A AEC 3-3 Mean	S.D.	Group B AEC 9-3 Mean	S.D.	Group C Lead Acetate Mean	S.D.
predose	0.02	0.03	0.01	0.00	0.01	0.00
0.5	0.23	0.15	0.08	0.07	3.75	4.95
1	0.31	0.17	0.13	0.09	3.57	4.71
2	0.51	0.22	0.43	0.18	1.86	1.50
4	0.49	0.11	0.31	0.15	0.87	0.75
8	0.20	0.11	0.32	0.13	0.95	0.47
12	0.22	0.08	0.26	0.08	0.65	0.41
24	0.42	0.13	0.20	0.08	0.54	0.33
48	0.33	0.15	0.13	0.03	0.33	0.26
72	0.14	0.07	0.12	0.05	0.29	0.16
96	0.12	0.05	0.09	0.03	0.29	0.15
120	0.07	0.02	0.08	0.02	0.23	0.13
144	0.12	0.05	0.12	0.04	0.18	0.07
192	0.07	0.02	0.04	0.03	0.16	0.10
240	0.05	0.00	0.06	0.04	0.14	0.10

Collection Time (hrs)	Group D AEC 10-1 Mean	S.D.	Group E PLW Mean	S.D.	Group G EC1 Mean	S.D.
predose	0.01	0.02	0.01	0.00	0.04	0.01
0.5	0.06	0.06	0.01	0.00	0.05	0.01
1	0.12	0.10	0.01	0.00	0.05	0.01
2	0.15	0.13	0.01	0.00	0.05	0.01
4	0.42	0.29	0.05	0.04	0.06	0.01
8	0.27	0.10	0.08	0.03	0.06	0.02
12	0.24	0.08	0.06	0.02	0.10	0.02
24	0.26	0.08	0.07	0.03	0.15	0.04
48	0.19	0.03	0.02	0.01	0.10	0.02
72	0.16	0.05	0.04	0.02	0.07	0.02
96	0.13	0.03	0.03	0.01	0.07	0.01
120	0.08	0.03	0.02	0.01	0.07	0.02
144	0.07	0.03	0.02	0.01	0.05	0.02
192	0.06	0.02	0.02	0.01	0.06	0.01
240	0.05	0.01	0.02	0.01	0.07	0.01

TABLE 3 (continued). Mean blood lead concentration (µg/mL).

Collection Time (hrs)	Group H FM Mean	Group H FM S.D.	Group I Lead Acetate Mean	Group I Lead Acetate S.D.	Group J PLW1 Mean	Group J PLW1 S.D.
predose	0.01	0.00	0.01	0.00	0.01	0.00
0.5	0.01	0.00	0.28	0.20	0.31	0.31
1	0.02	0.01	0.27	0.21	0.63	0.79
2	0.03	0.03	0.32	0.12	0.69	0.53
4	0.12	0.09	0.17	0.10	0.34	0.19
8	0.10	0.05	0.17	0.07	0.38	0.18
12	0.11	0.04	0.13	0.07	0.29	0.11
24	0.08	0.04	0.11	0.04	0.41	0.14
48	0.04	0.02	0.08	0.03	0.54	0.21
72	0.02	0.02	0.08	0.03	0.13	0.05
96	0.03	0.02	0.06	0.02	0.06	0.01
120	0.02	0.01	0.05	0.01	0.04	0.01
144	0.03	0.01	0.04	0.02	0.05	0.02
192	0.02	0.01	0.04	0.01	0.04	0.02
240	0.02	0.01	0.05	0.02	0.04	0.03

TABLE 4. Absolute bioavailability of soil lead in microswine.

Substance Administered	Absolute Bioavailability[a]
AEC 3-3	28.52%
AEC 9-3	16.70%
AEC 10-1	24.12%
PLW	30.97%
EC1	31.72%
FM	14.72%
PLW1	9.18%

[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.

TABLE 5. Area under the blood concentration-time curve [AUC] (μg(hr)/mL).

Test Substance	Animal #1	Animal #2	Animal #3	Animal #4	Animal #5	Animal #6	Mean	S.D.
AEC 3-3	57.63	44.56	49.09	50.53	–	–	50.45	5.42
AEC 9-3	32.55	27.11	30.92	72.31	–	–	40.72	21.18
Pb Acetate (Grp C)	96.13	214.34	128.60	70.84	26.07	–	107.20	70.65
AEC 10-1	32.91	40.88	30.37	46.38	42.50	–	38.61	6.73
PLW	14.75	14.37	9.97	29.99	11.83	16.85	16.29*	7.13
Pb Acetate	(not used)							
EC1	46.87	44.15	60.35	44.76	-98.46[a]	68.32	52.89	10.87
FM	18.31	19.50	29.96	28.35	33.17	–	25.86*	6.59
Pb Acetate (Grp I)	36.31	14.83	35.02	25.13	29.03	–	28.06	8.67
PLW1	23.88	63.09	47.87	36.64	77.64	–	49.83*	21.22

Note: For statistical evaluation, soils AEC 3-3, 9-3, 10-1, PLW and EC1 were compared to Pb Acetate (Grp C). Soils FM and PLW1 were compared to Pb Acetate (Grp I). In addition, AUCs were multiplied by the dose value to determine statistical significance.

* Statistically different from lead acetate ($p \leq 0.05$) by Student t-test.

[a] Value not used in calculation of the mean AUC nor the bioavailability of the test substance.

FIGURES

FIGURES 1 and 2.

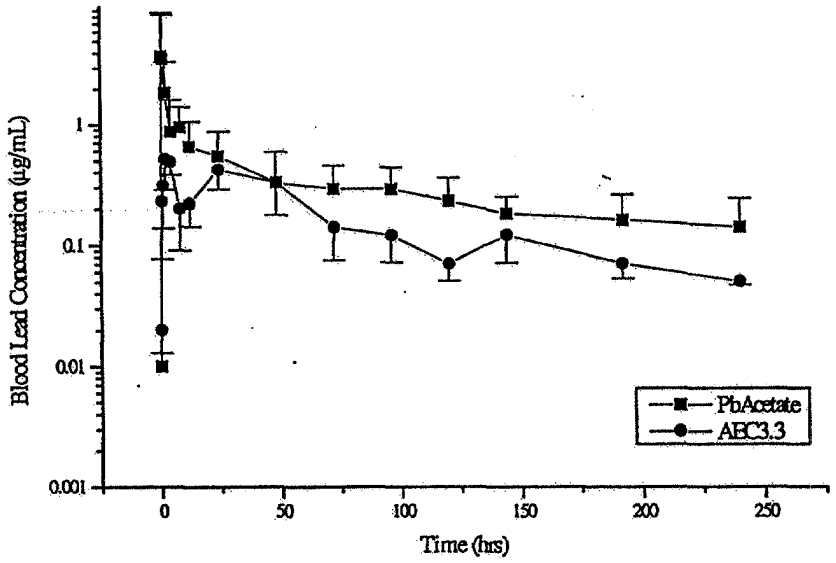

Figure 1. Mean blood lead concentration in male microswine administered Pb Acetate (Group C) or AEC 3-3 (Group A).

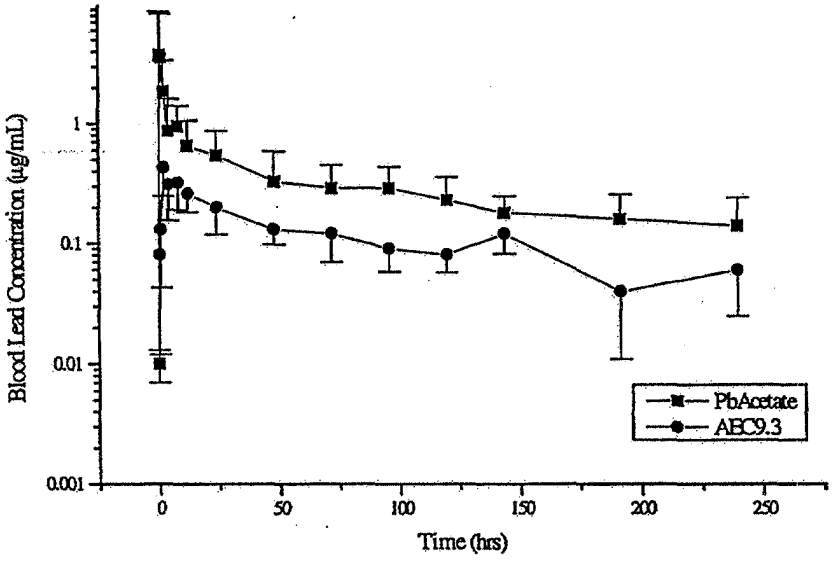

Figure 2. Mean blood lead concentration in male microswine administered Pb Acetate (Group C) or AEC 9-3 (Group B).

FIGURES 3 and 4.

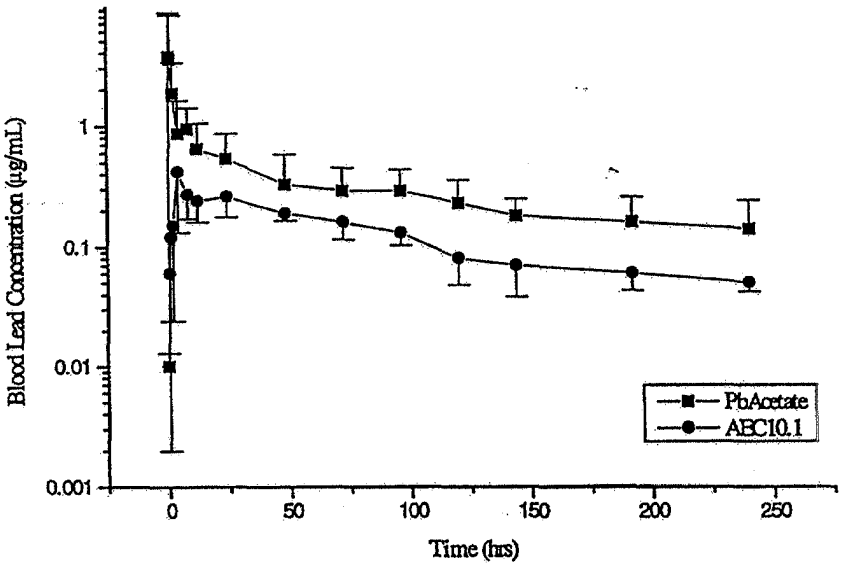

Figure 3. Mean blood lead concentration in male microswine administered Pb Acetate (Group C) or AEC 10-1 (Group D).

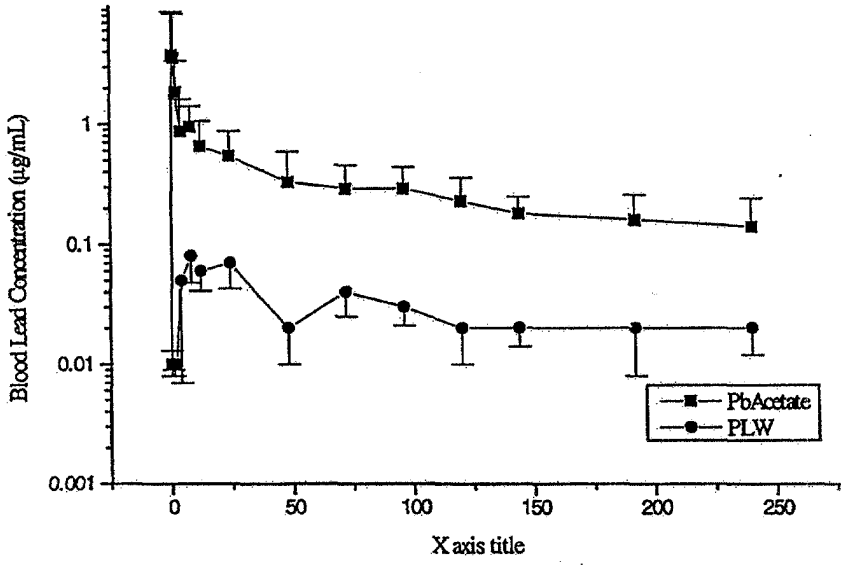

Figure 4. Mean blood lead concentration in male microswine administered Pb Acetate (Group C) or PLW (Group E).

FIGURES 5 AND 6.

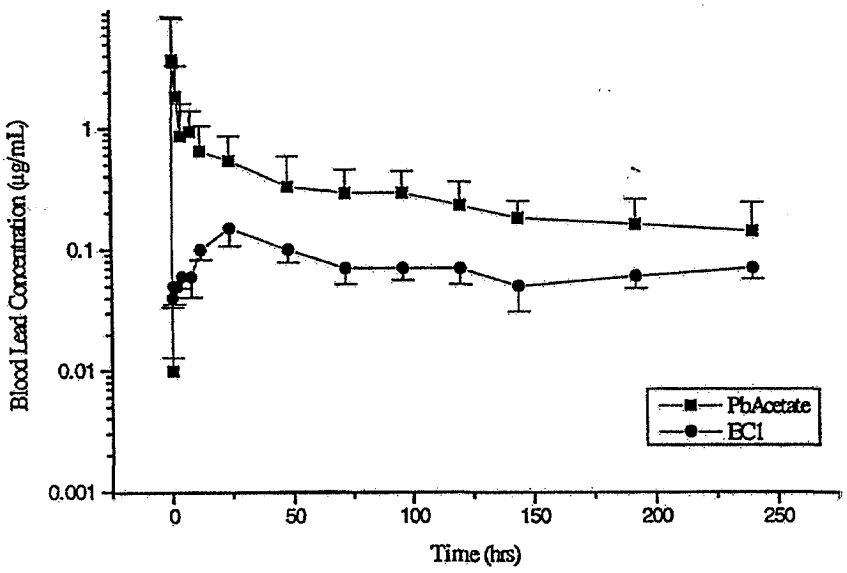

Figure 5. Mean blood lead concentration in male microswine adiministered Pb Acetate (Group C) or EC1 (Group G).

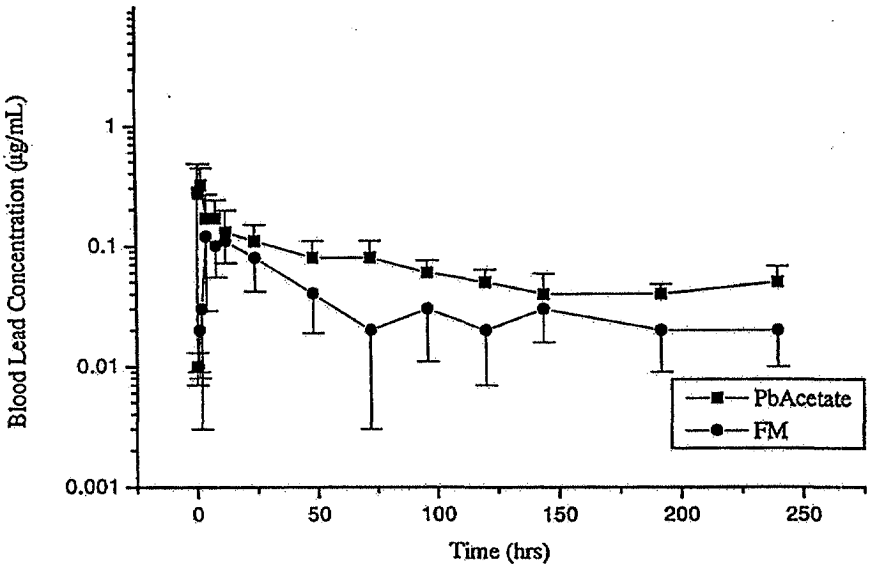

Figure 6. Mean blood lead concentration in male microswine adiministered Pb Acetate (Group I) or FM (Group H).

FIGURE 7.

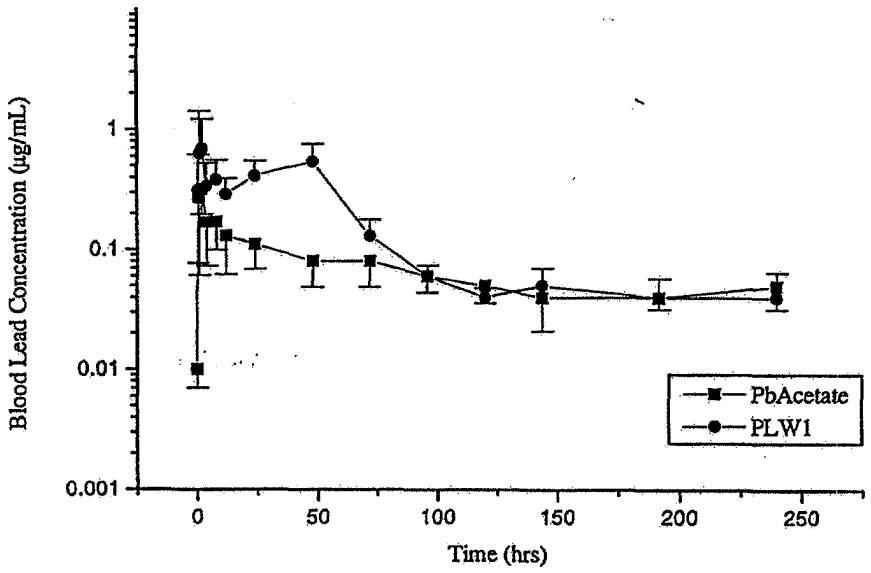

Figure 7. Mean blood lead concentration in male microswine adiministered Pb Acetate (Group I) or PLW1 (Group J).

APPENDICES

APPENDIX A. Protocol and protocol amendments.

Protocol #1: Soils AEC 3-3, AEC 9-3, and AEC 10-1

ABSOLUTE BIOAVAILABILITY OF SOIL LEAD IN MICROSWINE:
SOILS AEC 3-3, AEC 9-3, AND AEC 10-1

MEDICAL RESEARCH PROJECT NO. 9727-001

PROTOCOL

N 26889.01

Stine-Haskell Animal Welfare Committee No. CBT51-P

Protocol #1: Soils AEC 3-3, AEC 9-3, and AEC 10-1

TABLE OF CONTENTS

Protocol #1: Soils AEC 3-3, AEC 9-3, and AEC 10-1

OBJECTIVE

The objective of this study is to determine the absolute bioavailability of lead from three different soils in 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, E. I. du Pont de Nemours and Company, Bellevue Corporate Center, Wilmington, Delaware. 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 Practice Standards. The sponsor's approval was effective the date the sponsor signed the Medical Research (MR) project.

STUDY DESIGN

The study design is as follows:

- twenty male microswine will be used
- a predosing blood sample will be drawn from each animal for levels of background lead
- fifteen microswine will be dosed orally by gavage (5 microswine/soil) with test soils containing 520, 570, or 585 ppm lead (approximately 17 grams/microswine)
- five microswine will be given a single intravenous injection of lead acetate solution containing lead-dose equivalent to that contained in the gavage dose
- 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.
- blood will be collected in lead-free tubes containing heparin and stored at 4°C until analysis
- at sacrifice, liver and bone (both cortical and trabecular as well as whole femur) will be collected, weighed, and frozen at -20°C

Protocol #1: Soils AEC 3-3, AEC 9-3, and AEC 10-1

MATERIALS AND METHODS

A. Test Materials

The test materials will be supplied by Aldrich, Milwaukee, Wisconsin. The lead acetate trihydrate will be assigned Haskell Laboratory sample no. 20202. The lead/soils will be assigned Haskell Laboratory sample number as follows: AEC 3-3 will be assigned no. 20366; AEC 9-3 will be assigned no. 20367; and AEC 10-1 will be assigned no. 20368. Characterization and mineralogy data of the test soils will be maintained by the sponsor.

B. Test Species

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., Windham, Maine. 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

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 two weeks, during which microswine will be fed an optimized diet (once per day) of microswine diet (Charles River) and transferred gradually to Teklad basal mix (95%) for microswine. 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.

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.

Protocol #1: Soils AEC 3-3, AEC 9-3, and AEC 10-1

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 according to age. Microswine will be housed individually. Each animal will be assigned its own Haskell animal number and permanently identified by a number marked on 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 microswine will be provided optimized test diets of approximately 340 gram/microswine/day. Deionized water will be provided *ad libitum*.

Animal rooms will be targeted at a tempeature of 25 ± 2°C and a relative humidity of 50 ± 10%.

F. Diet Preparation and Analysis

During the test period, microswine in each group will be fed 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. The diets will contain < 0.1 ug Pb/g.

Sucrose will be added to the diets and thoroughly mixed for a period of time adequate to assure homogeneous distribution in the diet. Once prepared, diets will be refrigerated until used or discarded.

G. Body Weights

Microswine will be weighed twice per week unless experimental findings warrant a more infrequent weighing schedule.

Protocol #1: Soils AEC 3-3, AEC 9-3, and AEC 10-1

H. Dosing

Microswine will be dosed intravenously via the ear vein. Sterile water will be used as a vehicle. Oral doses will be administered by gavage. Deionized water will be used as a vehicle.

The route of administration of the lead-contaminated soil will be used by oral gavage 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.

I. Blood Sampling

Approximately 0.5-1.0 ml of microswine venous blood will be drawn from each designated animal at 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 heparin and refrigerated until the time of preparation for analysis.

J. Sacrifice of Animals

At the end of the experiment, the animals will be humanely euthanatized. Animals will be necropsied, liver and bone will be collected, weighed, frozen at approximately -20°C, and stored for potential future analyses.

K. Blood Analysis

The blood lead concentration will be analyzed by graphite furnace atomic absorption spectroscopy (GFAA), at DuPont Chambers Works, E. I. du Pont de Nemours and Company, Deepwater, New Jersey. Dosing solutions, diets, and drinking water will also be analyzed.

Protocol #1: Soils AEC 3-3, AEC 9-3, and AEC 10-1

L. 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.

M. Statistical Methods

Body weights and body weight gain 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 #1: Soils AEC 3-3, AEC 9-3, and AEC 10-1

APPENDIX I

A. Study Personnel

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

B. Study Dates

Proposed Experiment Start: October, 1993

Proposed Sponsor Approval: Date the Sponsor signed the MR Project

Proposed Experiment Completion: November, 1993

Proposed Report Issue Date: April, 1994

Protocol #1: Soils AEC 3-3, AEC 9-3, and AEC 10-1

MEDICAL RESEARCH PROJECT NO. 9727-001

Approved: _____ 10/11/93
Hanan N. Ghantous, Ph.D. (date)
Study Director

_____ 10/11/93
Matthew S. Bogdanffy, Ph.D. (date)
Manager
Biochemical Toxicology and Risk Analysis

cc: P.E. Ross
T.S. Bingman (REO-PIT)
K. McCleary
R.C. Rhea

Protocol #1 (Amendment): Soils AEC 3-3, AEC 9-3, and AEC 10-1

TO: MEDICAL RESEARCH PROJECT NO. 9727-001

The following changes are made to the original protocol:

page 4 *MATERIALS AND METHODS*
C. Pretest Procedures
paragraph 4, sentence 1

Delete '...and sampling non-study animals housed in the study room for pathogens.' This study will not use non-study animals to test for pathogens.

page 5 *MATERIALS AND METHODS*
D. Assignment to Groups
paragraph 1, sentence 1

Delete sentence 1. Replace with 'Healthy animals will be selected by computerized stratified-randomization such that there are no statistically significant differences among group body weight means.'

page 5 *MATERIALS AND METHODS*
F. Diet Preparation and Analysis
paragraph 2, sentence 1

Change '...normal nutrition for *each* species.' to '...normal nutrition for *this* species.' This was a clerical error.

page 5 *MATERIALS AND METHODS*
G. Body Weights
paragraph 1

Add the following sentence: 'Concurrent with the collection of body weights, microswine will be individually examined and clinical observations will be recorded.'

Approved by: H. Ghantous — Oct. 26/93
Hanan N. Ghantous, Ph.D. (date)
Study Director

Matthew S. Bogdanffy — 10/27/93
Matthew S. Bogdanffy, Ph.D. (date)
Manager
Biochemical Toxicology and Risk Analysis

cc: P.E. Ross K. McCleary
T.S. Bingman Quality Assurance

Protocol #1 (Amendment): Soils AEC 3-3, AEC 9-3, and AEC 10-1

TO: MEDICAL RESEARCH PROJECT NO. 9727-001

October 18, 1994

The original protocol is amended as follows:

1. Add the following to *Materials and Methods*, section F, *Diet Preparation and Analysis*: 'The contaminants, if any, in the sucrose to be used for this study were considered. Because the sucrose chosen for use was originally intended for human consumption, it was felt that any contaminants would be within acceptable ranges. However, the diet (containing sucrose) will be analyzed for lead content and the results from the analysis will be presented in the final report.' This addition, required by Good Laboratory Practices, was erroneously omitted from the original protocol.

2. The original protocol states that blood samples will be refrigerated until the time of preparation for analysis. This is changed to state that blood samples will be frozen (approximately -20°C) until preparation for analysis. This change was made because blood samples will be held until all time points have been collected, then sent to Jackson Labs for analysis.

3. Add the following to *Materials and Methods*, section J, *Sacrifice of Animals*: 'Animals that are sacrificed *in extremis* or accidentally killed will be necropsied and examined for cause of death.' This addition is made to explain the procedure for animals that are sacrificed *in extremis* or accidentally killed.

4. The original protocol stated that each microswine would receive an oral dose of test soil which contained approximately 17 g lead. This is changed to state that each microswine would receive an oral dose of test soil which contained approximately 17 mg lead. This change is made to correct a typing error.

5. The amount of lead contained in each soil is changed from 520, 570, and 585 ppm to 1070, 1120, and 1210 ppm for AEC 3-3, AEC 9-3, and AEC 10-1, respectively. This change was made based on data received after the sponsor sent the soils for reanalysis of lead concentrations.

6. In protocol Amendment #1, reasons for two changes were not presented. Regarding the change describing animal grouping, random animal selection replaced selection by age in order to enhance the study design. Regarding the addition of animal examination and recording of clinical signs concurrent with the collection of body weights, this was always the intent and was erroneously not included in the original protocol.

7. The results of this study will be included in a final report written for Medical Research Projects (MRs) 9727, 9740, and 9905. These three projects were absolute bioavailability studies of soil lead in microswine; each project used different test soils. This change is made in order to present the results more efficiently and in a timelier manner.

Protocol #1 (Amendment): Soils AEC 3-3, AEC 9-3, and AEC 10-1

8. The intravenous dose of Pb Acetate administered in this study was intended to be used in comparison with the test soils labeled AEC 3-3, AEC 9-3, and AEC 10-1. The dose of the Pb Acetate was calculated to be equivalent to the dose of lead received by animals orally gavaged with the lead-containing test soils. The intravenous dose of Pb Acetate used in MR 9740 was designed to accomplish a similar task (at the same dose of lead per animal); however, due to technical error, the Pb Acetate dose was incorrectly prepared. Therefore, the Pb Acetate dose group from MR 9727 will be used in comparison with the test soils in MR 9740. This change is made in order to correct a mistake in a cost-effective manner.

Approved by: H. Ghantous 10/19/94
Hanan N. Ghantous, Ph.D. (date)
Study Director

Matthew S. Bogdanffy 10/19/94
Matthew S. Bogdanffy, Ph.D. (date)
Manager
Biochemical Toxicology and Risk Analysis

cc: T.S. Bingman
K. McCleary
Quality Assurance
P.E. Ross

Protocol #2: Soils PLW and EC1

ABSOLUTE BIOAVAILABILITY OF SOIL LEAD IN MICROSWINE: SOILS PLW and EC1

MEDICAL RESEARCH PROJECT NO. 9740-001

PROTOCOL

Stine-Haskell Animal Welfare Committee No. CBT51-P

Protocol #2: Soils PLW and EC1

TABLE OF CONTENTS

Protocol #2: Soils PLW and EC1

OBJECTIVE

The objective of this study is to determine the absolute bioavailability of lead from two different soils in 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, E. I. du Pont de Nemours and Company, Bellevue Corporate Center, Wilmington, Delaware. 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 Practice Standards. The sponsor's approval was effective the date the sponsor signed the Medical Research (MR) project.

STUDY DESIGN

The study design is as follows:

- eighteen male microswine will be used;
- a predosing blood sample will be drawn from each animal for levels of background lead;
- twelve microswine will be dosed orally by gavage (6 microswine/soil) with test soils containing 803 or 3000 ppm lead (approximately 17 mg of lead/microswine);
- six microswine will be given a single intravenous injection of lead acetate solution containing lead-dose equivalent to that contained in the gavage dose;
- 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;
- blood will be collected in lead-free tubes containing heparin and stored at approximately -20°C until analysis; and
- at sacrifice, liver and bone (both cortical and trabecular as well as whole femur) will be collected, weighed, and frozen at approximately -20°C.