Diana Lead Data Entry Coding Form:											
								DecControl#	281		
BatesBegin:	DUP040005095	BatesEnd:		TotalPages	12	Attmnt?#	2				
Doc Type:	Protocol (amendment)	Year Date:	1993	Month Date:	10	Day Date:	27				
Title Reference:	Protocol Amendment No. 1 - Absolute Bioavailability of Soil Lead in Microswine: Soils AEC 3-3, AEC 9-3, AEC 10-1										
Primary Author Last Name:	Ghantous	First Name, Middle Initial:	Hanan N.								
Secondary Authors:		Publication:									
Primary Recipient (s):	MEDICAL RESEARCH PROJECT NO. 9727-001										
CC's:	P.E. Ross, T.S. Bingman, K. McCleary, Quality Assurance										
Names Stated:	Matthew S. Bogdanffy, Ph.D.										
Doc Problems:											
Case Produced In	Steven Thomas	Produced By:	DPT	Source:	Responsive to Plaintiff's 1st Re						
Dup/Cross Ref #'s:											
Doc Control Comments:	Disk, Box 1, January 2002, SH001001 - SH001002										
Obj. Coder Nam	D. Green	Obj. Data Entry Nam	D. Green	ObjDataDate	8/22/2002						

N 26928

Diana Lead Data Entry Coding Form:

DecControl#: 281.01

BatesBegin:	DUP040005096
BatesEnd:	DUP040005097
TotalPages:	2
Attmnt?#:	0
Doc Type:	Protocol (Amendment)
Year Date:	1994
Month Date:	10
Day Date:	18
Title Reference:	Protocol Amendment No. 2 - Absolute Bioavailability of Soil Lead in Microswine: Soils AEC 3-3, AEC 9-3, AEC 10-1
Primary Author Last Name:	Ghantous
First Name, Middle Initial:	Hanan N.
Secondary Authors:	
Publication:	
Primary Recipient (s):	MEDICAL RESEARCH PROJECT NO. 9727-001
CC's:	T.S. Bingman, K. McCleary, Quality Assurance, P.E. Ross
Names Stated:	Matthew S. Bogdanffy
Doc Problems:	
Case Produced In:	Steven Thomas
Produced By:	DPT
Source:	Responsive to Plaintiff's 1st Re
Dup/Cross Ref #'s:	
Doc Control Comments:	Disk, Box 1, January 2002, SH001001 - SH001002
Obj. Coder Nam:	D. Green
Obj. Data Entry Nam:	D. Green
ObjDataDate:	8/22/2002

N 26928.01

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.

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

Diana Lead Data Entry Coding Form:

DecControl#: 281.02

BatesBegin:	DUP040005098	BatesEnd:	DUP040005106	TotalPages	9	Attmnt?#	0
Doc Type:	Protocol	Year Date:	1993	Month Date:	10	Day Date:	11

Title Reference:	Protocol - Absolute Bioavailability of Soil Lead in Microswine: Soils AEC 3-3, AEC 9-3, AEC 10-1
Primary Author Last Name:	Ghantous
First Name, Middle Initial:	Hanan N.
Secondary Authors:	
Publication:	
Primary Recipient (s):	Medical Research Project 9727-001
CC's:	T.S. Bingman, K. McCleary, R.C. Rhea, P.E. Ross
Names Stated:	Stine-Haskell Animal Welfare Committee, Corporate Remediation Group of DuPont Chemicals, Bellevue Corporate Center, Wilmington, Delaware, Haskell Laboratory, Newark, Delaware, Aldrich, Milwaukee, Wisconsin, Yucatan Microswine, Charles River Laboratories, Inc., Windham, Maine, Guide for Care and
Doc Problems:	

Case Produced In	Steven Thomas	Produced By:	DPT	Source:	Responsive to Plaintiff's 1st Re
Dup/Cross Ref #'s:					
Doc Control Comments:	Disk, Box 1, January 2002, SH001001 - SH001002				
Obj. Coder Nam	D. Green	Obj. Data Entry Nam	D. Green	ObjDataDate	8/22/2002

N 26928.02

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

Stine-Haskell Animal Welfare Committee No. CBT51-P

TABLE OF CONTENTS

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

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.

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.

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.

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.

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

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

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