# Protocol #3: Soils FM (Fort Madison) and PLW1



## ABSOLUTE BIOAVAILABILITY OF SOIL LEAD IN MICROSWINE: SOILS IDENTIFIED AS *FORT MADISON* AND *PLW-1*

## MEDICAL RESEARCH PROJECT NO. 9905-001

## PROTOCOL

Stine-Haskell Animal Welfare Committee No. CBT51-P

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## 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:

- fifteen male microswine will be used;
- a predosing blood sample will be drawn from each animal for levels of background lead;
- ten microswine will be dosed orally by gavage (five microswine/soil) with test soils containing 500 ppm (Fort Madison) or 4,000 ppm (PLW-1) lead (approximately 17 mg of lead/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 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.

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## MATERIALS AND METHODS

### A. Test Materials

Lead acetate will be supplied by Aldrich, Milwaukee, Wisconsin. The test soils will be supplied by the sponsor. Upon arrival to Haskell Laboratory, each test substance will be identified with a Haskell Laboratory sample number. 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 water analyses for contaminants and sampling freshly washed cages and cage racks for bacteria and for pathogens. The potential effects of dietary contaminants have been considered and, on the basis of the manufacturer's data, are believed to be within acceptable ranges.

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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 selected by computerized stratified-randomization such that there are no statistically significant differences among group body weight means. 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 temperature 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).

This diet will be custom formulated by the manufacturer so that it contains complete normal nutrition for this species. The diet will contain < 0.1 ug Pb/g.

Sucrose will be added to the diet and thoroughly mixed for a period of time adequate to assure homogeneous distribution in the diet. Once prepared, the diet 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. Concurrent with the collection of body weights, microswine will be individually examined and clinical observations will be recorded.

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## 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 by oral gavage since soil ingestion 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 0.2-0.5 ml of 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 frozen (approximately -20°C) 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.

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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 six 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, Jackson Laboratory (DuPont Chambers Works) or at the Records Management Center, E. I. du Pont de Nemours and Company, Wilmington, Delaware.

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

### B. Study Dates

Proposed Experiment Start: June, 1994

Proposed Sponsor Approval: Date the Sponsor signed the MR Project

Proposed Experiment Completion: July, 1994

Proposed Report Issue Date: January, 1995

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MEDICAL RESEARCH PROJECT NO. 9905-001

Approved: _____ 5/19/94
Hanan N. Ghantous, Ph.D. (date)
Study Director

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cc: P.E. Ross
T.S. Bingman (REO-PIT)
K. McCleary
Quality Assurance
Study Technician
Pathology Steward

## Diana Lead Data Entry Coding Form:

| | |
|---|---|
| DecControl#: | 238.01 |
| BatesBegin: | DUP040004298 |
| BatesEnd: | |
| TotalPages: | 1 |
| Attmnt?#: | 0 |
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| First Name, Middle Initial: | Hanan N. |
| Secondary Authors: | Dupont |
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| CC's: | T.S. Bingman, K. McCleary, Quality Assurance, P.E. Ross |
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| Doc Problems: | |
| Case Produced In: | Steven Thomas |
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| 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 |
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# Protocol #3 (Amendment): Soils FM (Fort Madison) and PLW1



TO: MEDICAL RESEARCH PROJECT NO. 9905-001

October 18, 1994

The original protocol is amended as follows:

1. Add the following to *Materials and Methods*, section E, *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 Haskell Sample Nos. assigned to the test substances were 20659, 20658, and 20202 for Fort Madison (FM), PLW-1, and Pb Acetate, respectively. The sample numbers for the two test soils were not available when the original protocol was written.

3. In Materials and Methods, section L, Data Analysis, it is erroneously stated that individual animal data as well as the mean for six animals in each group will be reported. This is changed to state that individual animal data as well as the mean for five animals in each group will be reported. The number of animals in each group was incorrectly stated in the original protocol.

4. 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.

Additional Information:

The intravenous dose of Pb Acetate administered in this study was intended to be used in comparison with the test soils labeled Fort Madison (FM) and PLW-1. The dosage of the Pb Acetate was calculated to be equivalent to the dosage of lead received by animals orally dosed with the lead-containing test soils. The intravenous dose of Pb Acetate used in MRs 9727 and 9740 were designed to accomplish a similar task; however, due to technical error, the Pb Acetate dose for MR 9740 was incorrectly prepared. The Pb Acetate dose group from MR 9727 will be used in comparison with the test soils in MR 9740. This information is being provided to explain why MRs 9727 and MR 9740 were sharing a Pb Acetate dose group, while MR 9905 was not.

Approved by: H. Ghantous 10/19/94
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