
Protocol #2: Soils PLW and EC1

Protocol
Absolute Bioavailability of Soil Lead in Microswine: Soils PLW and EC1

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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 food and water analyses for contaminants and sampling freshly washed cages and cage racks for bacteria for pathogens.

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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 $25 \pm 2^\circ\text{C}$ and a relative humidity of $50 \pm 10\%$.

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 this species. The diets will contain $< 0.1 \text{ ug Pb/g}$.

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. 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 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 0.2-0.5 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.
Consultant
(412) 257-7761

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

B. Study Dates

Proposed Experiment Start: November, 1993

Proposed Sponsor Approval: Date the Sponsor signed the MR Project

Proposed Experiment Completion: December, 1993

Proposed Report Issue Date: June, 1994

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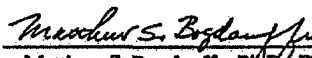
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MEDICAL RESEARCH PROJECT NO. 9740-001

Approved:


Hanan N. Ghantous, Ph.D.
Study Director

Nov. 23/1993
(date)


Matthew S. Bogdanffy, Ph.D., D.A.B.T.
Manager

11/29/93
(date)

Biochemical Toxicology and Risk Analysis

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

Protocol #2 (Amendment): Soils PLW and EC1

Protocol Amendment No. 1
Absolute Bioavailability of Soil Lead in Microswine: Soils PLW and EC1

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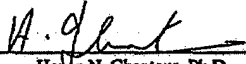
TO: MEDICAL RESEARCH PROJECT NO. 9740-001

October 18, 1994

The original protocol is amended as follows:

1. Add the following to *Materials and Methods*, section B, *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 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.
3. The intravenous dose of Pb Acetate administered in this study was intended to be used in comparison with the test soils labeled PLW and EC1. 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. Due to technical error, the Pb Acetate dose was incorrectly prepared. However, the Pb Acetate dose group from MR 9727 was designed to accomplish the same task and at the same dose of lead per animal. Therefore, the Pb Acetate dose group from MR 9727 will be used in comparison with the groups orally dosed with test soils in MR 9740. This change is made in order to correct a mistake in a cost-effective manner.

Approved by:


Halim N. Ghanous, Ph.D.
Study Director

10/19/94
(date)


Matthew S. Bogdanffy, Ph.D.
Manager

10/19/94
(date)

Biochemical Toxicology and Risk Analysis

cc: T.S. Bingman
K. McCleary
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