Exponent®

Pilot Study Report: Oral Bioavailability of Dioxins/Furans in Midland and Tittabawassee River Flood Plain Soils

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February 24, 2005

Doc. no. 8601636.005 0201 0105 CC38

Contents

List of Figures

List of Tables

Appendix D tables

Acronyms and Abbreviations

CV	coefficient of variability
EROD	ethoxyresorufin O-deethylase
HR-GC/MS	high-resolution gas chromatography/mass spectrometry
MROD	methoxyresorufin O-deethylase
MSU	Michigan State University
NTP	National Toxicology Program
PCDD/F	polychlorinated dibenzo-*p*-dioxin/furan
4-PeCDF	2,3,4,7,8-pentachlorodibenzofuran
RBA	relative bioavailability
RPD	relative percent difference
SOP	standard operating procedure
TCDD	tetrachlorodibenzo-*p*-dioxin

Executive Summary

The overall objective of this pilot study is to evaluate two animal models (Sprague-Dawley rats and juvenile swine) for measuring the oral bioavailability of 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD), 2,3,4,7,8-pentachlorodibenzofuran (4-PeCDF), and the other dioxin/furan congeners of importance in soils from Midland, Michigan, and the Tittabawassee River flood plain. The study design includes a test soil from each of these two areas, because the toxic equivalent (TEQ) for dioxins/furans in Midland soils is dominated by TCDD, while that of the Tittabawassee River flood plain soils is dominated by furans (4-PeCDF in particular). The results from this pilot study will be used to complete the design of a full-scale study of dioxin/furan bioavailability from soil.

Specific objectives of the pilot study include:

- Evaluate the feasibility of detecting dioxins/furans in the tissues of rats and swine dosed with soil from Midland and the Tittabawassee River flood plain
- Evaluate the proposed study design in rats and swine for measuring the relative bioavailability of dioxins/furans in soil
- Evaluate whether five animals per dose group will be an adequate number for the full study (note that for the rats in the pilot study, 10 animals will be used, and the tissues from each pair of rats will be combined to provide 5 samples for analysis).

Each of the two soils was administered to rats in a soil/feed mixture for 30 days. Reference materials (feed and corn oil gavage) were spiked with the five most predominant TEQ-contributing congeners for each soil at concentrations designed to result in administered doses equivalent to those received in the soil/feed mixtures. Soils were administered to swine for 30 days wrapped in dough balls. The reference corn oil materials with matched doses of the five most predominant TEQ contributors for each soil were administered to swine in gelatin capsules wrapped in dough balls. At the conclusion of dosing, liver and adipose tissues were collected from experimental animals, and concentrations of the congeners of interest and EROD/MROD[1] activity in hepatic tissues were measured in all rats and swine. EROD and MROD activity was measured to evaluate whether or not differential enzyme induction (CYP1A1 and CYP1A2) was occurring between the soil and reference groups. Different levels of enzyme induction could result in different rates of metabolism or different distribution patterns between the two groups.

Relative bioavailability was estimated by comparing the fractions of administered dose retained in liver, adipose, and a combination of the two tissues between the soil and reference materials. This method relies on two assumptions. First, this method assumes that the majority of each compound would be distributed to liver and adipose tissues, and that the proportion of material distributed to other tissues would not be different between the soil and reference groups.

[1] Ethoxyresorufin O-deethylase (EROD) and methoxyresorufin O-deethylase (MROD) assays.

Second, the method assumes that the rate of elimination for each congener is the same in the soil and the reference-material group animals.

The concentrations of test compounds in both liver and adipose tissue were consistently above the detection limits in rats for both soils. In swine, tissue concentrations of congeners of interest were not consistently above detection or lower calibration limits for the Midland soil, but were consistently detectable and quantifiable in the group administered the Tittabawassee River flood plain soil, which had higher levels of contaminants.

Hepatic EROD activity was statistically significantly increased in rats in all reference-material groups compared to the respective soil groups. In swine, no statistically significant difference in EROD/MROD activity was observed between soil and reference groups for either soil.

The two animal models produced statistically significantly different estimates of relative bioavailability (RBA) for all of the congeners in the Tittabawasse River flood plain soil and for two of the congeners in the Midland soil (Figures 10 and 11). These differences may be due in substantial part to the differential induction in the rat soil and reference-material groups. Increased enzyme induction in the reference groups could result in increased metabolism rates in these groups compared to the soil groups, violating the assumption of equal elimination rates between the soil and reference groups. Increased EROD activity in the reference groups, as a marker for the CYP1A1 enzyme, would result in increased metabolism of TCDF in the reference groups compared to the soil groups, with accompanying lower retained fractions of administered dose. This would result in a false elevation of the estimated RBA in the soil groups compared to the reference groups.

Issues associated with differential enzyme induction in rats for both soils, and achieving detectable tissue concentrations in swine for the Midland soil, render most of the RBA estimates resulting from this pilot study unreliable. The swine-based RBA estimates for the Tittabawassee River flood plain soil do not suffer from either of these limitations and may provide a reliable estimate of the RBA values for this soil.

Several design modifications are recommended for future studies, in order to reduce costs, achieve detectable compound concentrations, and reduce the likelihood of differential enzyme induction between soil and reference groups. In summary, the following changes are recommended:

1. Omit the feed reference group, as results in this study confirm the general conclusion that feed has a relative bioavailability compared to corn oil gavage of about 70%. Further demonstration of this is unnecessary.

2. For purposes of reducing costs, it would be desirable to use a single animal model. Based on the results of this pilot study, either animal could be used in experiments going forward, with modifications to the study design. Pros and cons of each model are discussed in more detail in the report below, but specific considerations apply to either model:

   – If rats are used, reference material dose levels will need to be matched more closely to anticipated *absorbed* doses in the soil groups in order

to avoid differential induction of enzyme activity between soil and reference groups.

- If swine are used, the administered doses of soils with lower TEQ concentrations (for instance, Midland-area soils with TEQ concentrations at or below the levels in the soil tested in this study) will need to be increased in order to achieve reliably detectable and quantifiable tissue concentrations.

3. For purposes of reducing costs, it would be desirable to analyze only a single tissue (liver or adipose) from each test animal. Data on compound distribution from this study support use of a single tissue for either animal model, with the most consistent measures resulting from liver tissue in the rat and adipose tissue in the swine.

4. Retain hepatic EROD/MROD measurements as part of the study design, as a means of ensuring that differential induction of hepatic enzymes is not occurring in subsequent tests.

Introduction

The overall objective of this pilot study is to evaluate two animal models (Sprague-Dawley rats and juvenile swine) for measuring the oral bioavailability of 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD), 2,3,4,7,8-pentachlorodibenzofuran (4-PeCDF), and the other dioxin/furan congeners of importance in soils from Midland, Michigan, and the Tittabawassee River flood plain. The study design includes a test soil from each of these two areas, because the toxic equivalent (TEQ) for dioxins/furans in Midland soils is dominated by TCDD, while that of the Tittabawassee River flood plain soils is dominated by furans (4-PeCDF in particular). Because the TCDD and 4-PeCDF may behave differently in these two animal models, a soil from each of these two areas was chosen for evaluation in the pilot study. The results from this pilot study will be used to complete the design of a full-scale study of dioxin/furan bioavailability from soil.

Specific objectives of the pilot study include:

- Evaluate the feasibility of detecting dioxins/furans in the tissues of rats and swine dosed with soil from Midland and the Tittabawassee River flood plain
- Evaluate the proposed study design in rats and swine for measuring the relative bioavailability of dioxins/furans in soil
- Evaluate whether five animals per dose group will be an adequate number for the full study (note that for the rats in the pilot study, 10 animals will be used, and the tissues from each pair of rats will be combined to provide 5 samples for analysis).

The study in the rat model will be used to assess the oral bioavailability of dioxins/furans from soil relative to that from both rat feed and oral gavage doses. This is warranted because relevant toxicology studies underlying estimates of cancer slope and serving as possible sources for reference doses have used both corn oil gavage and feed for administration of compounds. Thus, if dioxins/furans in soil are less bioavailable than those in rat feed, an adjustment in the risk assessment is warranted to account for this difference. In addition, the rat studies will allow for comparison to the recent National Toxicology Program (NTP) chronic carcinogenesis bioassays, in which the rats were dosed by corn oil gavage.

The swine study will be conducted to evaluate the oral bioavailability of dioxins/furans from two Midland soils in an *in vivo* model that is more similar to humans than the rat. The results of the swine and rat studies using corn oil as a vehicle will provide a basis for comparison of results across species.

Methods and Materials

Soil Selection

In preparation for the pilot study, six candidate test soils were collected by CH2M Hill in June 2004. The soils were collected as described in the *Sampling and Analysis Plan – Soil Sampling for the Pilot Bioavailability Study* (provided in Appendix A). These soil samples (approximately 3 gallons each) were shipped to Exponent's Boulder, Colorado, laboratory, where they were air-dried and homogenized, and approximately 500 g was sieved to <250 μm (60 mesh). A 50-g aliquot of each sieved sample was then shipped to Alta Analytical Laboratory (Alta) in El Dorado Hills, California for analysis of polychlorinated dibenzo-*p*-dioxin and furans (PCDD/Fs) by high-resolution gas chromatography/mass spectrometry (HR-GC/MS; EPA Method 8290). Results from these analyses are presented in Table 1. Neither of the Midland soils (TCDD concentrations of 15.2 and 59.5 pg/g TCDD, respectively; Table 1) had TCDD concentrations as high as those in a soil that had been collected previously in bulk from Midland (CC-S-27, which contains 163 pg/g TCDD [Table 1] as reported in Exponent 2003; collected from the southeast portion of the Dow Corporate Center lawn in May 2002 and archived dry in Exponent's Boulder laboratory). Because the CC-S-27 soil exhibits a congener profile consistent with Midland soils (TEQ dominated by TCDD and 1-PeCDD) this soil was selected for the pilot study. Sample THT02769 (from location Imerman Park 2) was selected as the Tittabawassee River soil for use in the pilot study, because it exhibited a congener profile consistent with the flood plain sediments (TEQ dominated by 4-PeCDF and TCDF) and had a total TEQ concentration close to 1,000 pg/g (Table 1).

The remainder of soil THT02769 was sieved to <250 μm, and the entire sieved soil mass was homogenized. Triplicate splits of soils CC-S-27 and THT02769 (collected using a soil splitter, as were all soil aliquots used in this study) were sent to Alta to test for homogeneity of the soil batches. Results from these analyses are presented in Table 2. Coefficients of variability (CVs) for the five congeners that contribute the most to total TEQ in soil CC-S-27 ranged from 1.9% to 5.6% for the triplicate analysis. CVs for the triplicate analysis of soil THT02769 ranged from 16.1% to 19.7%, and resulted from one of the triplicate samples contributing greater concentrations of PCDD/Fs than the other two (Table 2). Soil THT02769 was subsequently rehomogenized and used for the study. Co-planar PCB concentrations in each of the two study soils were also analyzed in one of the triplicate samples (EPA Method 1668); these data are also presented in Table 2.

Methods used to perform the pilot bioavailability study are described in the document titled, *Pilot Study Design: Oral Bioavailability of Dioxins/Furans in Midland and Tittabawassee River Flood Plain Soils* (provided in Appendix B).

Dose Preparation and Administration

Rat Study

Each of the test soils (<250-μm size fraction) was blended with PMI Nutrition International, Rodent LabDiet® 5001 (meal) (5% w/w) at WIL Research Laboratories, Inc. (WIL) in Ashland, Ohio. The WIL report describing the diet blending is provided in Appendix C, and results for PCDD/Fs in the Rodent LabDiet® batches used in this study are provided in Table 3. To accomplish the blending of soil into the rat diet, soil (475 g) and diet (1,000 g) were blended in a Hobart mixer for 5 minutes to create a diet pre-mixture. The pre-mixture was then blended with 8,025 g of diet in a V-blender to create the final 9,500-g diet batch. Diet homogeneity samples (25 g) were collected from the initial, middle, and final material that emerged from the V-blender; these samples (three samples per blended diet) were sent to Alta for analysis of PCDD/F concentrations. Results for the pre-dosing soil/diet mixtures (Table 4) indicate that for the CC-S-27/diet blend (Test Article #1), the five congeners that contributed most greatly to TEQ were recovered at 79%–131% of expected concentrations (based on concentrations measured in the test soil), and CVs for the pre-dosing triplicate analyses ranged from 2.3% to 12%. For the THT02769/diet blend (Test Article #2), the five most important congeners were recovered at 76%–100% of expected concentrations, with CVs ranging from 4.5% to 14%. These measurements of blended diet PCDD/F concentrations and homogeneity were considered acceptable to proceed with the study.

For the reference material in diet (matched to soil CC-S-27), TCDD, 1-PeCDD, 1,6-HxCDD, 1,4,6-HpCDD, and 4-PcCDF (the five dioxin/furan congeners contributing most greatly to TEQ for this soil) were spiked into 200 mL acetone (B&J Brand®, High Purity Solvent; previously analyzed for dioxins/furans and determined to be below detection limits for all congeners) at concentrations that, once blended with feed, would deliver the same dose of these five congeners as the CC-S-27/diet blend. Analytical results for the reference mixture in acetone are provided in Table 5. At WIL, the acetone (100 mL) and diet (1,000 g) were blended in a Hobart mixer for 5 minutes to create a diet pre-mixture. The pre-mixture was then blended with 8,500 g of diet in a V-blender to create the final 9,500 g diet batch (Test Article #3). Diet homogeneity samples (25 g) were collected from the initial, middle, and final material that emerged from the V-blender; these samples were sent to Alta for analysis of PCDD/F concentrations (Table 4). For Test Article #3, the five spiked congeners were recovered at 83%–118% of expected concentrations in the pre-dosing diet samples, with CVs ranging from 1.0% to 3.0%. Based on these results, the concentrations and homogeneity of PCDD/Fs in Test Article #3 were considered acceptable to proceed with the study.

The two gavage reference materials for the rat study were prepared in corn oil/acetone (99:1), and were designed to deliver the same dioxin/furan doses as the soil/diet blends. To create these reference mixtures, the five dioxin/furan congeners that contribute most greatly to TEQ in each soil were spiked into acetone (20 mL), and the concentrations of the five congeners in the spiked acetone was measured to confirm that analytical concentrations were close to target concentrations. Subsequently, 8.26 mL of this acetone was added to 817.7 mL corn oil (Spectrum Chemicals & Laboratory Products, National Formulary [NF] grade; analysis of the corn oil indicated negligible dioxin/furan concentrations [Table 3]). The two corn oil/acetone

reference materials were then assayed for concentrations of the five target congeners (Table 5). Relative percent differences (RPDs) between target and pre-dosing measured concentrations were generally in the range of 3%–13%, except for 1,2,3,6,7,8-HxCDD, which was present at a concentration approximately 40% greater than the target concentration. Because this compound contributed less than 5% of the total TEQ of the soil and reference oils, this variation was considered acceptable for use in the study. The gavage reference mixtures were stored in amber glass bottles sealed with Teflon-lined lids, and were used within 60 days of preparation.

Swine Study

For the swine pilot study, the test-soil doses were delivered by placing 1 g of the soil (either CC-S-27 or THT02769 in the center of a 10-g moistened dough ball (Zeigler Bros. Swine Diet) and offering it to the swine. The swine were fasted for two hours prior to dosing, because previous studies conducted in this animal model have indicated that a 2-hour fast will ensure eager acceptance of the 10-g dough ball containing the dose. Soil-containing dough balls were prepared every 3–4 days. Five dough balls (containing a total of 5 g of test soil) were given twice daily, at 9 a.m. and 4 p.m., for a total dose of 10 g soil/day. Immediately after dosing, the animals were given one-half of their standard ration of swine feed. The two dose groups receiving the soil doses (Groups 3 and 4) had their feed rations reduced by 80 g/day to compensate for the greater number of feed balls given these animals during dosing, relative to the corn oil–dosed animals. Dosing and feeding continued twice daily for 30 consecutive days.

The dosing materials for the two reference groups were prepared in corn oil/acetone (99:1), and were designed such that 2 mL of the corn oil/acetone mixture would deliver an equivalent dose to 5 g of the test soil to which it was matched. To create these reference mixtures, the five dioxin/furan congeners that contribute most greatly to TEQ in each soil were spiked into acetone (20 mL), and the concentrations of the five congeners in the spiked acetone were measured to confirm that analytical concentrations were close to target concentrations. Subsequently, 10 mL of this acetone was added to 990 mL corn oil (Spectrum Chemicals & Laboratory Products, National Formulary [NF] grade; analysis of the corn oil indicated negligible dioxin/furan concentrations [Table 3]). The two corn oil/acetone reference materials were then assayed for concentrations of the five target congeners (Table 6). Relative percent differences (RPDs) between target and measured concentrations were in the range of 1%–21%, which was considered acceptable for use in the study. The swine reference mixtures were stored in amber glass bottles sealed with Teflon-lined lids, and were used within 60 days of preparation.

For dosing, 1 mL of corn oil/acetone mixture was placed in each gel capsule (Torpac, 1.2 mL volume), and these were embedded in the center of a 10-g ball of moistened swine feed. The oil-filled gel capsules were inserted in dough balls immediately prior to dosing. Two dough balls (containing a total of 2 mL of reference mixture) were given twice daily, at 9 a.m. and 4 p.m., for a total dose of 4 mL reference mixture/day. Immediately after dosing, the animals were given one-half of their standard ration of swine feed. Dosing and feeding continued twice daily for 30 consecutive days.

Animal Handling and Dosing

Rat Study

Animal handling and dosing during the rat study were performed as described in the pilot study design document (see Appendix B), a brief summary of which follows.

Fifty 4-month-old female Sprague-Dawley rats, weighing between 210 and 240 g, were obtained from Harlan (Indianapolis, Indiana) and placed in individual stainless steel cages. Each rat was weighed on arrival (Day –6), then on Day –2 (during the quarantine period) and Day 3 of the dosing period, and then weekly until study termination. The rats were provided with PMI Nutrition International Rodent LabDiet® 5001 (meal) and de-ionized water *ad libitum* during the one-week quarantine period, and their health status was monitored. All LabDiet® 5001 fed to the rats (including during the quarantine period and to the gavage dose groups during the dosing period) was from the same two batches of LabDiet® 5001 that were used by WIL Research to prepare the blended rat diets (Table 3). Two days prior to the start of dosing, healthy animals were randomly assigned to five dose groups (10 rats/group; dose groups are identified in Table 7).

During the 30-day dosing period, each rat received 50 g of feed every 2 days (background feed for Groups 1 and 2, and dosed feed for Groups 3, 4, and 5). The weight of any unconsumed feed at the end of each 2-day period was measured, and an estimate was made of the weight of any spilled feed. Dose groups 1 and 2 were gavaged daily at 11 a.m. with 1 mL of the corn oil/acetone reference mixtures.

Twenty-four hours after the last dose was administered, the rats were weighed and terminated under CO_2 anesthesia. Their livers were excised, blotted dry, weighed and wrapped in foil. The liver samples for the ethoxyresorufin O-deethylase (EROD) and methoxyresorufin O-deethylase (MROD) assays were collected (1-g samples) from the livers of each pair of rats (i.e., 0.5 g collected from each individual liver). The sample was minced, placed in a 2-mL cryovial, immediately frozen in liquid nitrogen, and sent to Michigan State University (MSU) for analysis. The remainder of the pair of livers was then frozen and shipped to Alta, where they were homogenized together to create a sample of sufficient mass for the analytical work. As much fatty tissue as possible (3–6 g) was collected from within the abdominal cavity of each rat, weighed, and wrapped in foil. The fat samples were frozen and shipped to Alta, where the fat samples from each pair of rats were homogenized together to create a sample of sufficient mass for the analytical work.

Triplicate 25-g post-dosing subsamples of each blended rodent diet were collected and shipped to Alta for analysis of dioxins/furans, to evaluate the stability of the blended diets during the 30-day dosing period, and to confirm the doses of dioxins/furans delivered to the rats (Table 4). The CV between all six samples of the blended rodent diet (three pre-dosing and three post-dosing) was no greater than 22% for any congener, indicating that the diets were stable during the study. In addition, the gavage reference mixtures were shipped to Alta for post-dosing analysis (Table 5). The CV between the pre- and post-dosing gavage reference mixtures was no

greater than 21%, indicating that the reference mixtures were also stable during the study period.

Only two rats, both from Group 2, did not complete the 30-day dosing period. Rats #29 and #24 were sacrificed after 15 and 20 days of dosing, respectively, due to persistent problems with administering the gavage dose. On necropsy, it appeared that there was a stricture immediately prior to the stomach of the first rat, and it was found that the esophagus of the second rat had been perforated.

Rat carcasses from the pilot study were wrapped in foil, placed in individual labeled zipper-sealed freezer bags, and archived (–80 °C) for possible further analysis.

Swine Study

Animal handling and dosing during the swine study were performed as described in the pilot study design document (see Appendix B), a brief summary of which follows.

Twenty intact male swine weighing between 8.4 and 10.7 kg were obtained from Chinn Farms (Clarence, Mississippi) and were fed a specially formulated diet (Ziegler Bros. Inc., Gardners, Pennsylvania). Swine were weighed on arrival (Day –8), on Days –4 and –1 during the quarantine week, and then every three days until study termination. Feed was given at 4% of body weight per day, and was adjusted every three days to maintain a constant feed rate during the study. The swine were housed in stainless steel cages, and their health status was monitored during the 1-week quarantine period. Two days prior to the start of dosing, healthy animals were randomly assigned to four dose groups (five swine/group; dose groups described in Table 8).

Three swine were culled prior to the start of the dosing period (e.g., 23 animals were obtained from Chinn Farms, but only 20 were dosed during the study), and these animals were maintained on the weighing/feeding schedule described above, but were not given any doses. At the end of the study, these three animals were necropsied, and body composition of skin, fat, and muscle, as a proportion of body weight, was determined for each animal.

All doses were delivered twice daily in purified feed dough balls, as described in the dose administration section, at 9:00 a.m. (immediately prior to the morning feeding) and at 4:00 p.m. (immediately prior to the afternoon feeding) for 30 days. Twelve hours after the final dose, the animals were weighed and humanely sacrificed, and liver and fat samples were collected for analysis.

Only one animal, from Group 4, did not complete the 30-day dosing period. This animal was found dead in his pen on the morning of the 25th day of the study (he had been ill with what appeared to be a systemic infection, and had been given the antibiotic Naxcel [sodium ceftiofur] for the 9 days prior to his death).

The whole liver of each animal was excised, blotted dry, and weighed. Three 1-gram samples were collected for EROD and MROD assays (for each sample, subsamples from three sections of the liver were collected and diced), placed in 5-mL cryovials, and immediately frozen in

liquid nitrogen. These samples were shipped in liquid nitrogen to MSU for EROD/MROD analysis. The remainder of the liver was wrapped in foil, placed in a zipper-sealed freezer bag, and frozen at –80 °C. Fatty tissue from the abdominal wall, plus a small amount from the abdominal cavity (40–65 g, total) was collected, wrapped in foil, and frozen at –80 °C. The liver and fat were shipped (frozen) to Alta. The residual reference mixtures were shipped to Alta for analysis. The CV between the pre- and post-dosing reference mixtures ranged from 9% to 28%, indicating that the reference mixtures were stable during the study period (Table 6).

All swine carcasses were were double-bagged in heavy black plastic trash bags and stored at –20 °C, in case additional samples were needed.

Tissue Sample Homogenization and Analysis

At MSU, liver microsomes were prepared from each liver sample, and the protein levels and enzymatic activities were measured according to the MSU Standard Operating Procedure (SOP) No. 250 (v 1.1), titled *Protocol for Liver Microsome Preparation, and Microsomal Protein Measurement and AROD Assays in the same 96-Well Plate*. EROD/MROD activities and protein concentrations were measured fluorometrically at the end of the assay, using a Cytofluor multiplate reader.

At Alta, the rat liver samples were homogenized using a Cuisinart mini-prep processor. The processor was run on the "high" setting until the sample was liquefied (for the liver samples) or thoroughly homogenized (for the fat samples). The sample was then poured into separate 40-mL amber glass VOA vials for extraction. After homogenization of each sample, all parts of the processor that were in contact with sample material were washed with soap and hot water, rinsed with de-ionized water, and then rinsed with ultra-high-purity solvents (hexane followed by dichloromethane).

The swine liver samples were homogenized using a Villaware model 5265-05 power grinder. The grinder was fitted with a 4-mm-diameter mesh gate for all grinding. Samples were collected directly from the grinder into labeled amber glass jars. Between samples, all parts of the grinder that were in contact with sample material were washed with soap and hot water, rinsed with de-ionized water, and then serially rinsed with ultra-high-purity solvents (acetone, toluene, hexane, and dichloromethane).

The rat and swine fat samples were homogenized with a Sumeet Multi-Grind Model 964, a small-volume grinder suitable for small sample sizes. Samples were collected directly from the grinder into labeled amber glass jars. Between samples, all stainless steel parts of the grinder that were in contact with sample material were washed with soap and hot water, rinsed with de-ionized water, and then serially rinsed with ultra-high-purity solvents (acetone, toluene, hexane, and dichloromethane). The polycarbonate grinder lid was washed with soap and hot water, rinsed with de-ionized water, and then serially rinsed with ultra-high-purity methanol followed by hexane.

Subsamples of the liver and fat homogenates were extracted in methylene chloride/hexane and analyzed for lipid content (EPA Method 1613), and PCDD/F concentrations by HR-GC/MS

(EPA Method 1613). Selected samples were also analyzed for co-planar PCBs (EPA Method 1668).

Estimation of Relative Bioavailability

Relative bioavailability was estimated by comparing the fraction of administered dose retained in the tissues of animals in the groups dosed with soil with the fraction of administered dose retained by animals given the reference vehicle(s) (oil or feed), similar to the method used by Wittsiepe et al. (2004). Several assumptions were made in this estimation process:

1. *The whole-body elimination rate for each compound would be the same in the reference-dosed animals as in the soil-dosed animals, and can be approximated by a first-order model.* Diliberto et al. (2001) demonstrated that, in mice exposed subchronically to TCDD, the fraction of administered dose retained in the animal tissues decreased as the body burden increased, indicating an increase in elimination rate with increasing body burden. To account for this issue, reference dosing materials for each group were formulated to try to match the anticipated administered soil doses for that group. In addition, measurements of hepatic EROD and MROD activity were made for each group to assess whether enzyme induction (and the associated increase in hepatic metabolism) was occurring, and if so, whether it was occurring to a different extent in soil-dosed groups than in reference groups. EROD activity is a marker for the CYP1A1 enzyme, while MROD activity is a marker for CYP1A2 activity. CYP1A1 is the enzyme that mediates metabolism of several PCDD/F compounds, while the CYP1A2 protein in the liver serves as a binding protein for many PCDD/F compounds. When CYP1A2 is induced, hepatic sequestration of these compounds occurs. For some compounds, this hepatic sequestration may result in either a greater or lesser elimination rate, depending on the compound, its binding affinity for CYP1A2, and the mechanism of metabolism. If either enzyme is induced to a different extent in the soil-group animals compared to the reference-group animals, the assumption of equivalent whole-body elimination rates between groups would likely be violated.

2. *The majority of retained administered dose would be distributed in liver and adipose tissues, and the proportion of retained dose distributed to* ***tissues other than liver and adipose*** *would not be different in soil-dosed groups compared to reference-dosed groups.* Distribution studies following subchronic administration of TCDD in mice and rats demonstrate that, at the lowest doses tested, liver and adipose account for 70% to 80% of retained body burden; this percentage increases to approximately 90% at higher tested doses (Diliberto et al. 2001; Hurst et al. 2000). The remainder of the retained compound in these studies was found in skin and muscle, and concentrations were consistent with simple lipid-based partitioning of compound in these tissues.

The relative bioavailability (RBA) of a compound from soil administration, compared to administration of a reference material ($RBA_{soil:ref}$), is the ratio of the absolute absorption fractions (f_{abs}) of the compound from the two media:

$$RBA_{soil:ref} = \frac{f_{abs,soil}}{f_{abs,ref}}$$

In general, after daily administration of a compound, the amount of compound in the body at the end of 30 days is a function of both the administered dose rate and the elimination rate. Using the assumption of first-order elimination, the whole-body amount of compound as a function of time can be estimated as follows:

$$Q_{body} = \frac{D * f_{abs}}{k}(1 - e^{-kt})$$

where:

Q_{body} = mass of compound in body, ng

D = daily administered dose, ng/d

k = elimination rate, d^{-1}

t = duration of dosing, d

Solving for f_{abs},

$$f_{abs} = \frac{Q_{body}\ k}{D\,(1 - e^{-kt})}$$

Solving for the RBA,

$$RBA = \frac{Q_{body,soil}\,k \,/\, D_{soil}\,(1 - e^{-kt})}{Q_{body,ref}\,k \,/\, D_{ref}\,(1 - e^{-kt})}$$

Because the elimination rate, k, is assumed to be equal between the two groups, and because the time of administration, t, is the same, this simplifies to:

$$RBA = \frac{Q_{body,soil} \,/\, D_{soil}}{Q_{body,ref} \,/\, D_{ref}}$$

Again, the time of administration is the same for both groups, 30 days, so the daily doses for the two groups can be converted to the total administered dose:

$$RBA = \frac{Q_{body,soil} / Q_{ad\,\min,soil}}{Q_{body,ref} / Q_{ad\,\min,ref}}$$

where:

Q_{admin} = total mass of compound administered

The ratio of Q_{body}/Q_{admin} for a given dose group is the fraction of administered dose retained in the body (*FR*). Thus, the RBA evaluation for soil compared to a reference group simplifies to:

$$RBA = \frac{FR_{soil}}{FR_{ref}}$$

As discussed above in assumption 2, distribution studies for dioxin demonstrate that liver and adipose tissue account for the majority of dioxin retained in the body (70% to 90%, depending on the species and dose range tested; Diliberto et al. 2001; Hurst et al. 2000). Thus,

$$Q_{body} = Q_{liver} + Q_{adipose} + Q_{other}$$

where Q_{tissue} is the product of the concentration of compound in the tissue, C_{tissue}, and the weight of the tissue, w_{tissue}. Then, the fraction of administered dose retained in a given tissue is:

$$FR_{tissue} = \frac{Q_{tissue}}{Q_{ad\,\min}}$$

If the proportional distribution of compound among tissues is the same among dose groups, then an RBA value can be calculated on the basis of a single tissue or on the basis of a combination of tissues. As discussed above, for this effort, liver and adipose tissues serve as the basis for the RBA calculation. Liver weights were measured at sacrifice for rats and swine. Adipose tissue weights for the rats were estimated as a function of body weight at sacrifice using the relationship from Brown et al. (1997) based on data for male Sprague-Dawley rats developed by Bailey et al. (1980; as cited by Brown et al. 1997).

$$w_a = (0.0199*BW + 1.644) / 100$$

Adipose tissue weights for the swine were estimated as a percentage of body weight using the results of the total fat dissection for the three control swine described above.

Results

Rat Study

As discussed in the Animal Handling and Dosing section, two rats from the Tittabawassee River gavage oil reference group (Group 2) were sacrificed before the end of the study (after 15 and 20 days of dosing) due to persistent problems with administering the gavage dose. Results from this rat pair were not included in the data analysis discussed below.

Feed Intake

Details of feed intake for all groups are presented in Table D-1, and the feed intake is illustrated in Figure 1. The mean daily feed intake for all dosing groups was approximately 16 g/day. The mean daily intakes for the two oil reference groups were 14 g/day and 13 g/day, for the Midland oil and Tittabawassee River oil reference groups, respectively. The mean daily feed intake for the Midland soil group was 17 g/day (Group 3) and 19 g/day for the Tittabawassee River soil group. The mean daily feed intake for the Midland feed reference group (Group 5) was 16 g/day. The lower feed consumption in the oil gavage groups compared to the soil/feed and reference feed groups is consistent with the expectation that these groups might consume less feed due to caloric intake from the oil gavage vehicle (9 kcal per g, or about 8 kcal per mL; USDA National Nutrient Database for Standard Reference, Release 17, 2004). This is approximately 15% of the caloric intake from feed observed in the soil groups, so the lower feed intake in the oil gavage groups is consistent with an adjustment of feed intake by the animals, reflecting the caloric intake from corn oil gavage.

The doses and reference materials had been prepared assuming that the rats would consume 23 g/day, based on a literature value (Freeman et al. 1992), so the observed daily feed intake was less than anticipated. The feed was administered in a loose meal form rather than pellets, and this may have influenced feed intake rates. This lower feed consumption resulted in the administered doses of study compounds for the gavage oil groups being higher than the soil groups (see below in Administered Dose section).

Body and Liver Weights

Rat body weights for all five dosing groups averaged 238 g at study initiation (study day –2), and 259 g at study termination (Figure 2; detailed data for all animals are presented in Table D-2), a gain of 9% over the 30-day study period. This weight gain was similar to the 10% gain observed in the background study, and reflects the fact that female Sprague-Dawley rats have already reached adult body weight at 4 months of age. Rat liver weights at study termination ranged from 7.3 to 11.4 g (average of 9.0 g) over all dosing groups, approximately 3.5% of body weight (Table D-3).

Administered Doses

The average daily doses of contaminants in each group are summarized in Table 9. Doses received by the rats in the oil and feed reference groups were generally somewhat higher than the doses received in the soil group. This is due primarily to two factors: lower feed consumption rates for the soil/feed-dosed animals than expected based on literature values, and deviations from the targeted concentrations in both the soil/feed mixture and in the reference materials. The literature-based feed consumption values were used to establish the target corn oil concentrations.

EROD and MROD Activity

Mean EROD and MROD activities in rat liver tissue from all dose groups are reported in Table 10, and the complete data set is presented in Table D-4. EROD activity was statistically significantly elevated in both reference material groups compared to the paired soil groups. MROD activity was elevated in reference groups compared to soil groups, but the difference was not statistically significant. This result is consistent with the difference in dosing rates between the reference and soil groups, and indicates that the dosing rates in the reference groups were sufficiently greater than the soil groups to result in increased enzyme induction.

RBA Estimates

Concentrations of contaminants in liver and adipose tissues from each pair of rats are reported in Tables D-5 and D-6. Tissue concentrations of the contaminants of interest were all above detection limits for all dose groups and compounds and were also greater than the instrument calibration limits in nearly all samples (Table 11). Figure 3 illustrates the fraction of administered dose present in liver and adipose tissues, and in the summed tissues, for all dose groups. A larger proportion of administered dose was retained in liver than in adipose tissue for all dose groups. The coefficient of variability was generally in the range of 10% to 15%, with one exception (Table 12). In the Tittabawassee River flood plain soil group, the liver concentration in one rat pair of 1,2,3,6,7,8-HxCDD was approximately four times greater than the concentrations in the other rats in this group, and corresponded to a retained dose in liver greater than the total administered dose of this compound. The adipose tissue concentration for this rat pair was not significantly different from the others in the group. This data point qualifies as an outlier at the 1% level using Dixon's extreme value test, and was omitted from further calculations of relative bioavailability.

Estimates of average relative bioavailability of the two soils in rats, based on comparisons of fraction of dose retained in liver, adipose, or the sum of liver and adipose tissues in reference materials, are presented in Table 12 and Figure 4 (calculated as described in the section on Estimation of Relative Bioavailability). For the Midland soil, comparison to the reference feed produces higher relative bioavailability estimates than comparison to the reference oil gavage. This is expected due to the lower absolute bioavailability of contaminants from feed compared to corn oil.

The relative bioavailability of the feed reference mixture compared to the corn oil reference mixture for the Midland soil congener pattern is shown in Figure 5. As expected, congeners in feed were somewhat less bioavailable than congeners in the reference corn oil, with RBA (reference feed compared to reference oil) ranging from about 60% to 80%.

Swine Study

One animal from the Tittabawassee River soil group (Group 4) became ill during the study and was found dead on day 25. Results from this animal were not included in the data analysis discussed below.

Body and Liver Weights

Swine weights for all dosing groups averaged 11.3 kg at study initiation (Study Day –1), and 28.0 kg at study termination (Figure 6; see Table D-7 for detailed individual animal data), a gain of 149% over the 30-day maintenance on the Ziegler Bros. swine diet. This rapid weight gain is typical of juvenile swine. For each dosing group, the initial group mean body weights ranged from 10.8 kg to 11.7 kg, and at study termination, group mean body weights ranged from 27.2 kg to 28.6 kg. The group mean weight gains ranged from 145% to 155%, with consistent weight gains for all four groups throughout the 30-day study. Swine liver weights for all four groups ranged from 501 to 796 g (average of 653 g, or 2.3% of bodyweight). The group mean liver weights ranged from 585 g to 731 g (Table D-8).

Swine Necropsy and Body Fat Dissection Results

As described earlier, three additional swine were maintained on the weighing and feeding schedule, but were not dosed. These three swine were analyzed to determine the body composition of muscle, skin, and fat as a percentage of body weight (Table D-9). The percent of body weight that was muscle ranged from 52.9% to 57.6% (average 55.2%), and the percent of body that was skin ranged from 7.25% to 7.50% (average 7.41%). The body fat as a percent of body weight ranged from 6.22% to 7.22%, with an average of 6.74%. This average value was used to determine the weight of adipose tissue based on body weight in the RBA calculations.

Administered Doses

The average daily doses over the 30-day study for all swine study groups are summarized in Table 13. The administered dose for the reference oil groups matched those for the soil groups much more closely than in the rat study. This is due primarily to the mode of administration of soils in the swine study, in which weighed amounts of soil were wrapped in dough balls and fed directly to the swine, rather than mixed with loose feed material. Administered doses on a ng/kg bw/day basis were much lower than in the rat study, due to the larger animal size and limitations in how much soil can be effectively administered to the animals.

EROD and MROD Activity

Mean EROD and MROD activities in swine liver tissue from all dose groups are reported in Table 10, and the complete data set is presented in Table D-10. In contrast to the rat study, no statistically significant differences in EROD or MROD activity between soil and corresponding reference oil groups were observed. This is consistent with the better matching of doses between soil and reference oil groups in the swine study compared to the rat study.

RBA Estimates

Concentrations of contaminants in liver and adipose tissues from each animal are reported in Tables D-11 and D-12. In contrast to the rat study, tissue concentrations of the contaminants of interest did not always exceed the limits of detection, particularly for the Midland soil group. Table 14 summarizes the numbers of non-detected results per tissue and dose groups for the swine study. The prevalence of non-detected results in the swine studies necessitates consideration of appropriate handling of non-detects in the analysis of the data. Dual data analyses were conducted for all swine data, assuming either one-half the detection limit or the detection limit for all non-detects in the data set. There were also a number of results that were below the lower calibration limit of the lab equipment (qualified with a "J"). These were identified and handled as detected values with the reported concentrations used in calculations.

Figure 7 illustrates the fraction of administered dose present in liver and adipose tissues, and in the summed tissues, for all dose groups, assuming either one-half the detection limit or the detection limit for all non-detected results. The fraction of administered dose retained in adipose is greater than in liver in the swine, in contrast to the pattern observed in rats. The inter-animal variability in tissue concentrations and fractions retained is greater in the Midland soil and corresponding oil reference group compared to the Tittabawassee River flood plain groups. This is consistent with the lower doses in the Midland soil groups, which resulted in tissue concentrations near or below the detection limits in many cases, resulting in greater variability. However, the variability among animals in the Tittabawassee River flood plain soil group and corresponding oil reference group was comparable to the variability observed in the rat data.

Estimates of average relative bioavailability of the two soils in swine based on comparisons of fraction of dose retained in liver, adipose, or the sum of liver and adipose tissues in reference materials, are presented in Tables 15a and 15b and Figure 8. The RBA values across tissues are generally consistent with one another. No reliable RBA values for 1-PeCDF and TCDF for the Tittabawassee River flood plain soil using liver tissue only could be calculated. Liver tissue concentrations for these compounds were undetectable in all of the soil group animals. In addition, in the corn oil reference group, 1-PeCDF was undetectable for four of the five liver samples, and below the instrument calibration limit in the fifth sample. Given the lack of detectable liver concentrations in the soil group for these compounds, RBA estimates based on swine ***liver*** tissue for these two compounds cannot be made. The RBA estimates for these compounds based on adipose tissue are based on detectable results, and the combined fraction retained in liver and adipose tissue is dominated by the adipose tissue results, so the RBAs based on adipose tissue and the combined tissue are reliable.

Discussion

Sensitivity of Models

Tissue concentrations achieved in rats after 30 days of administration of soils and reference compounds were consistently above analytical detection limits for both liver and adipose tissue (Table 11). In contrast, in swine dosed with the Midland soil, a substantial fraction of both adipose tissue and liver samples displayed specific congener concentrations below detection or analytical lower calibration limits. In swine dosed with Tittabawassee River flood plain soil, adipose tissue levels were generally detectable. In liver tissue, TCDF and 1-PeCDF were not detected in any of the soil group animals, but the remaining compounds were generally detectable in swine liver (Table 14).

For animal tissues and compounds in which the analytes were generally detectable, the results were generally consistent from one animal (or pairs of animals, in the case of the rats) to another, resulting in coefficients of variation (CVs) on the estimated mean RBA values in the range of 10% to 25% (Tables 12 and 15). The CVs were larger for specific congeners in the swine study of Midland soil for which a substantial number of non-detects were obtained. The use of fraction of dose retained in liver plus adipose tissue as the basis for the RBA calculations produced generally stable results, although, as discussed further below, the rats and swine showed different patterns of distribution between liver and adipose tissue. Increasing the number of animals per dose group might decrease the CVs observed, but the variation observed in this study is probably sufficiently small to be acceptable.

Consistency of Models

Distribution Patterns

The retention and distribution of test compounds between liver and adipose tissues in the rats and swine are summarized in Figure 3 and 7. In general, rats retained higher percentages of the total administered dose at the end of 30 days than did swine for both soils. Swine exhibited modest liver sequestration for most compounds, compared to substantial liver sequestration for most of the tested compounds in rats (Figure 9). This may reflect, in part, physiological differences between swine and rats, or it may be a result of the lower liver tissue concentrations resulting from the lower administered dose and large swine growth rate compared to the rats. At the higher dose rates used in the rat study, the relatively high hepatic retention compared to adipose tissue suggests that some induction of CYP1A2 protein is likely occurring in all groups, even though differences in MROD activity between groups were not significant. CYP1A2 protein in liver binds several of the PCDD/PCDF compounds effectively, resulting in hepatic sequestration. In the swine, lower doses on a body-weight basis were used, resulting in lower hepatic TEQ concentrations. The concentrations in swine tissue may be low enough that substantial induction of CYP1A2 protein did not occur, and thus, less marked hepatic sequestration occurred.

RBA Estimates

The RBA estimates obtained in swine were statistically significantly lower than those obtained in rats for all of the congeners tested in the Tittabawassee River flood plain soil and for TCDD in the Midland soil (Figures 10 and 11). In contrast, the RBA obtained in swine for 1,2,3,4,6,7,8-HpCDD in the Midland soil was statistically significantly higher than in rats (mean RBA estimates of 0.55 in swine and 0.34 in rats, $p<0.05$). The EROD and MROD enzyme activity data may shed light on some of these differences. The EROD data suggest differential enzyme induction in the rats between the reference and soil groups for both soils, with significantly greater EROD activity in the reference groups compared to the soil groups (Table 10). As discussed above, EROD activity is a marker for induction of CYP1A1. CYP1A1 is responsible for the metabolism of 2,3,7,8-TCDF in rats (Tai et al. 1993), and induction of CYP1A1 has been shown to strongly increase the hepatic metabolism rate for TCDF in rats (McKinley et al. 1993; Olson et al. 1994). 4-PeCDF also can induce its own metabolism due to induction of CYP1A enzymes (Brewster and Birnbaum 1987). Other compounds, including TCDD and 1-PeCDF, show decreased retention of administered dose with increasing dose in subchronic studies, suggesting autoinduction of metabolism, although the specific metabolic pathways have not been identified (DeVito et al. 1998; Diliberto et al. 2001; Jackson et al. 1998). The metabolic pathways for the other compounds that contribute substantially to the total TEQ in the Midland and Tittabawassee River flood plain soils have not been examined to date, but may be influenced by CYP1A1 induction.

The statistically significant increase in EROD activity in rats treated with the reference corn oil and reference feed materials corresponds to the increased doses of these compounds received by the reference groups compared to the soil groups. This was due to lower-than-targeted concentrations of key contaminants in the soil/feed mixtures, as well as lower feed intake in the soil/feed rat groups than estimated prior to the experiment (although growth and body weight were not affected), resulting in lower administered dose in the rat soil groups than initially targeted (Table 9). In addition, if the relative bioavailability of the TCDF or other congeners in soil was low, the actual differential in absorbed dose of furan compounds between the two groups may have been much higher. The RBA estimates developed in swine for the Tittabawassee River flood plain soil PCDF congeners indicate that these congeners were approximately one-fourth as bioavailable as in corn oil. This indicates that, even if the administered doses of compounds in the soils and reference corn oil mixtures were equal, the absorbed doses may have differed by nearly a factor of four.

Increased EROD activity in reference-group rats compared to soil-group rats could result in an increase in hepatic metabolism rates in the reference-group rats, especially for TCDF. Such a differential in metabolism rates would violate the assumption (discussed above in the methods section) that rates of elimination in the soil and reference groups are the same. A greater elimination rate in the reference groups compared to the soil groups would result in an apparently greater relative bioavailability for the soil group. That is, a larger percentage of the *absorbed* dose would be retained in the soil groups compared to the induced reference groups that would be eliminating absorbed compound more rapidly. Thus, the high relative bioavailability estimate obtained in rats for TCDF in the Tittabawassee River flood plain soil may be in part due to elevated elimination rates in the reference groups, consistent with the elevated EROD activity observed in these groups. The statistically significant increase in

EROD activity in reference-group rats compared to soil-group rats may have resulted in higher metabolic rates in the reference-group rats for compounds of interest other than TCDF as well.

In contrast with the rats, the swine did not exhibit a statistically significant difference in EROD activity between the soil and reference material groups (Table 10). This is consistent with the better control of soil dosing rates in this model and could account for at least some of the apparent inconsistency in estimated relative bioavailability between the rats and swine in this study.

The EROD and MROD activities for all of the animals in the study are plotted in Figure 12. For rats, EROD activity is strongly correlated with hepatic TEQ, while MROD shows a weaker relationship. In swine, EROD and MROD activity are also correlated with hepatic TEQ, but MROD shows a stronger relationship. The positive dose-response for EROD and MROD, even at the low doses used in these studies, indicates that in future studies, in order to avoid differential EROD and MROD induction and activity among groups, soil and reference administered doses will need to be matched more closely. In fact, administered doses should probably be adjusted to reflect expected differences in relative bioavailability. That is, if the relative bioavailability is expected to be in the range of 25% to 75 percent for soil compared to reference corn oil materials, the administered dose of compounds in the reference corn oil material could be reduced by 25% to 50% compared to the soil dose, to try to ensure similar absorbed doses between the two groups. This approach should minimize any differences in enzyme induction between soil and reference groups.

Comparative Evaluation of Rat and Swine Models

For reasons of efficiency in a full bioavailability study of a number of soils, it would be desirable to identify a single animal model, rather than continue with two animal models. Swine are the preferred animal model for humans in research on the bioavailability of lead and arsenic from soils for a variety of biological reasons (Weis and Lavelle 1991). Wittsiepe et al. (2004) used minipigs in an evaluation of PCDD/F bioavailability from soils based on an evaluation of their gastrointestinal tract similarity to humans (Swindle and Smith 1998). Young pigs have comparable physiology and have been used successfully as a model for gastrointestinal function of children (Dodds 1982; Miller and Ullrey 1987). However, evaluation of swine as a model for humans in the study of highly lipophilic compounds is much less complete. Kararli (1995) notes that for highly lipophilic compounds, bile fluid plays an important role in absorption and uptake. Rats have no gallbladder, so the patterns of secretion of bile fluid are different from those in animals that do have gallbladders (including humans and pigs). However, there is a lack of comparative studies among swine, rats, and humans for assessing the bioavailability of lipophilic compounds, so there is no clear reason to prefer swine over rats as a model for human bioavilability of PCDD/Fs from soil.

From a practical perspective, additional issues could influence the choice of a single animal model. Arguments in favor of the rat model include:

- In this pilot study, rats were more sensitive than swine based on tissue detection limits, due to the ability to administer a larger dose of soil on a

body-weight basis and smaller relative changes in body weight over the course of the study. The swine dosing regimen would need to be altered to improve the sensitivity of this model for soils with contaminant concentrations in the same range as or lower than the Midland soil tested here.

- The swine growth rate was very large, with body weights more than doubling over the course of the 30-day experiment. In contrast, rat body weights were more consistent. The rapid growth of the swine decreases the sensitivity of the model, because the volume of distribution for the administered compounds more than doubles over the course of the study.

Arguments in favor of the swine model include the following. Control of soil dosing levels was easier to achieve in swine because of the method of administration. For swine, a measured amount of soil was wrapped in a dough ball and fed directly to the animal. For the rats, soil was mixed with rat feed (in a meal form) at the maximum proportion deemed palatable. The daily intake of soil and feed was then estimated by weighing the remaining feed and estimating spilled feed weights. In addition to the possible variability in doses and estimates of dose resulting from this dosing procedure, there is also the possibility of occasional inhomogeneities in the soil/feed mixture, resulting in variable doses.

Soil Bioavailability Evaluations

TEQ Weighting

The two soil samples tested each contained a number of dioxin and/or furan contaminants, but for each soil, the total TEQ of the soil was dominated by two congeners (Table 2). For the Midland soil, the TEQ was dominated by TCDD and PeCDD, accounting together for approximately 75% of the total TEQ concentration. The TEQ concentration of the Tittabawassee soil was dominated by TCDF and 4-PeCDF, again together accounting for 75% of the TEQ.

Table 16 provides estimates of the overall relative bioavailability for the two soils compared to the corn oil reference material based on weighting the RBA estimates for individual congeners in proportion to their contribution to the total soil TEQ. RBA estimates based on the rat model and on the swine model under the two assumptions regarding non-detects are presented.

Absolute Bioavailability Estimates

This pilot study allows direct estimates of relative biovailability from soil compared to corn oil (rats and swine) or, for the Midland soil, compared to diet (rats only). The absolute bioavailability of the congeners may be of of interest for the risk assessment of these soils if soil exposure is compared to established intake targets for humans that rely on absolute estimates of dose or body burden (for example, the WHO/JECFA or ECSCF TDI values). The absolute bioavailability of the tested congeners from soil can be estimated if the absolute bioavailability

from the corn oil reference material is known. Rats and mice absorb between 60% and 90% of TCDD from oral administration in corn oil (Hurst et al. 2000; Diliberto et al. 1996, 2001). Other congeners with 4 to 6 chlorine atoms probably have similar absorption rates from corn oil, although congeners with 7 and 8 chlorine atoms may be much more poorly absorbed from corn oil (Birnbaum and Couture 1988).

Table 16 presents estimates of absolute bioavailability for the tested congeners and soils, assuming that the PCDD/Fs in the corn oil reference material have absolute bioavailability of 80%. The absolute bioavailability estimates of the soils would decrease if the absolute bioavailability of the cornoil–administered compounds is lower than 80%, and would increase if the absolute bioavailability of corn oil–administered compounds is greater than 80%.

Comparison with *In Vitro* Bioaccessibility Data

A sample of the Midland soil tested in rats and swine (CC-S-27) was evaluated previously for dioxin/furan bioaccessibility using an *in vitro* assay (Ruby et al. 2002). This assay measured the ability of a synthetic digestive fluid in an *in vitro* system to disassociate dioxin and furan congeners from soil. Such a test could serve as a predictor of the fraction of contaminant likely to be available for absorption in the gastrointestinal tract. Congener-specific bioaccessibility estimates ranged from about 16% to 26% of the total soil contamination for the Midland soil (Table 16). These estimates are similar to, but slightly lower than, the estimated absolute bioavailability of this soil based on the swine results. No Tittabawassee River flood plain soil was evaluated using the bioaccessibility assay, so no results are available for comparison to the flood plain soil test results presented here.

Conclusions and Recommendations for Final Study Design

The RBA estimates derived in this pilot study based on the rat model cannot be relied upon due to differential enzyme induction between soil and reference groups. To our knowledge, no previous evaluations of relative bioavailability for PCDD/Fs in soil in rats have measured EROD or MROD activity in the study animals. This suggests the possibility that previous bioavailability estimates may have been influenced by this factor as well.

The RBA estimates for the Midland soil based on the swine model also suffer from limitations due to the low tissue concentrations attained and failure to consistently exceed analytical detection limits. However, there are no *a priori* reasons to reject the swine-based RBA estimates for the Tittabawassee River flood plain soil compounds.

The data developed in this pilot study indicate that either of these animal models could potentially be used to assess PCDD/F bioavailability and provide a basis for developing a final study design that can be used to evaluate a selection of soils from both Midland and the Tittabawassee River flood plain.

Following are our recommendations for a final study design.

1. Choose a single animal model for future studies. Based on a variety of considerations, the rat model may be more practical for further studies. The rats are a more sensitive model based on attained tissue concentrations for a given soil concentration, and this will be important in future studies. The Midland soil tested, CC-S-27, is toward the upper end of TCDD and TEQ concentrations for Midland city soils analyzed to date. Even if a higher rate of soil dosing can be achieved with the swine, the swine model still might not be sensitive enough to obtain detectable tissue levels using Midland soils with lower TCDD or TEQ concentrations, which would greatly limit the Midland soil selection for future testing. Although achieving good control over the dosing rate of soil for the rats is more complicated than for the swine, this issue should be surmountable based on the experience gained during the pilot study. In addition, the results of this pilot study exhibited good reproducibility from one rat pair to the next, with relatively low CVs on the mean RBA estimates for all congeners. This indicates good inter-animal reproducibility with the current rat study design. In addition, rats have a long history of use as a dioxin bioavailability model, whereas swine, although widely used for assessing bioavailability of lead and arsenic, have almost no track record as a model for lipophilic compounds. Finally, although the RBA estimates derived in this pilot study are questionable due to the enzyme activity differences among groups, these preliminary data suggest that, for the congeners of greatest concern, the rats are producing greater RBA estimates than the swine. The rats would therefore be a conservative choice for future bioavailability studies.

   If rats are chosen as the model for use in further studies, several specific study design changes should be made:

- Reduce administered doses somewhat for soils with TEQ concentrations above 500 ppt TEQ, to reduce enzyme induction but still maintain detectable, quantifiable tissue levels. The administered dose of Tittabawassee River flood plain soil used in this study was more than sufficient to produce detectable, reproducible tissue concentrations of the compounds of interest. The Midland soil used here consistently produced quantifiable liver concentrations, and adipose tissue concentrations were consistently above detection limits but were sometimes below the analytical lower calibration limit.

- Match oil gavage reference doses to anticipated *absorbed* doses of soil congeners as closely as possible. This involves three adjustments to the current protocol:

    1. Match reference-dose material to mixed soil/feed analysis results, rather than trying to match both materials to the "target" dosing concentrations.

    2. In addition, when establishing target congener concentrations for the reference soil, reduce the expected soil/feed consumption rate to 18 g/day, consistent with what was observed in the pilot study for both soil/feed groups.

    3. Account for the range of likely relative bioavailability in choosing target gavage oil concentrations and doses. That is, if the relative bioavailability is expected to be in the range of 25% to 50% for soil compared to reference corn oil materials, the administered dose of compounds in the reference corn oil material should probably be reduced by 50% to 75% compared to the *administered* soil dose, to try to assure similar *absorbed* doses between the two groups. This approach should minimize any differences in enzyme induction between soil and reference groups.

- Omit the reference feed study group, because the results in this pilot study are consistent with conventional assumptions regarding bioavailability from feed, and two reference groups are unnecessary going forward.

However, if swine are chosen, the following protocol changes should be considered:

- Increase administered dose as much as possible to ensure tissue concentrations above detection limits.

- Consider doing an intravenous comparison group for one soil each from Midland and Tittabawassee to assess the absolute bioavailability of the corn oil-administered compounds.

2. Choose one tissue (either liver or fat) to reduce study costs in the future. The choice of tissue would depend on the choice of animal model.

In the swine model, in the dose ranges used in this pilot study, adipose tissue accumulated a much greater fraction of administered dose and exhibited a greater rate of detectable tissue levels (Figure 7).

However, in rats, the fraction of retained dose of the two predominant congeners, TCDD and PeCDD, was similar between liver and adipose tissue, while the higher chlorinated PCDDs and the 4-PeCDF were found predominantly in the liver (Figure 3). In addition, the RBA estimates derived based on liver tissue alone vs. adipose tissue alone were very consistent in the rat for both soils, so a single tissue could be chosen. The liver tissue is the simplest tissue to collect. In addition, livers can be weighed directly, so the total mass of the tissue compartment can be measured rather than estimated (as was done for the adipose tissue weight). Finally, if liver tissue is the basis for comparison, it will not be necessary to use pairs of rats rather than single animals for the tissue collection, because this was done to facilitate collection of sufficient fat tissue for analysis.

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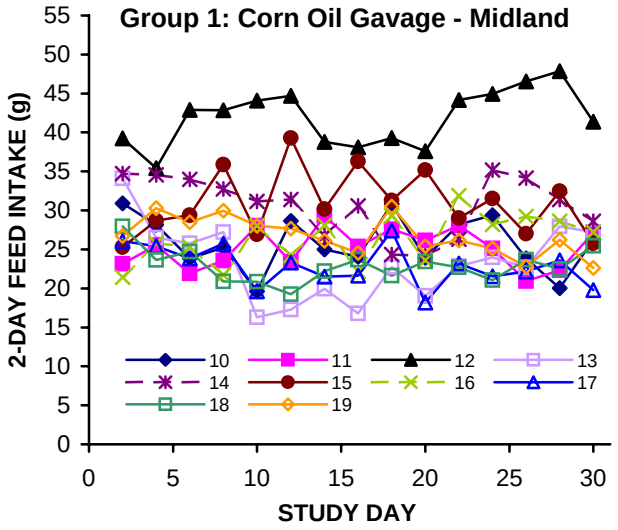
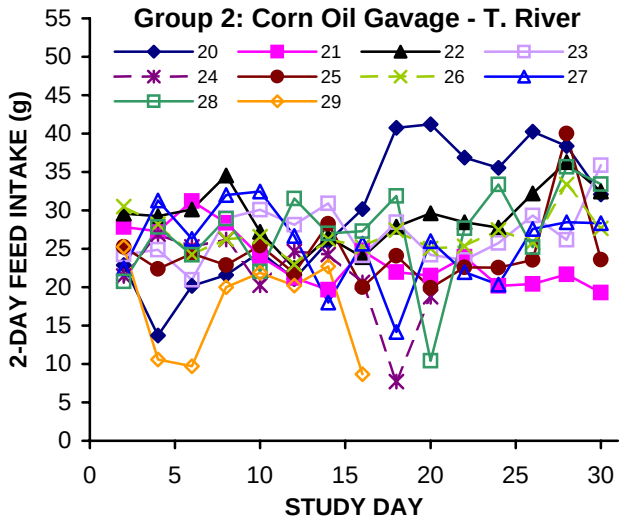
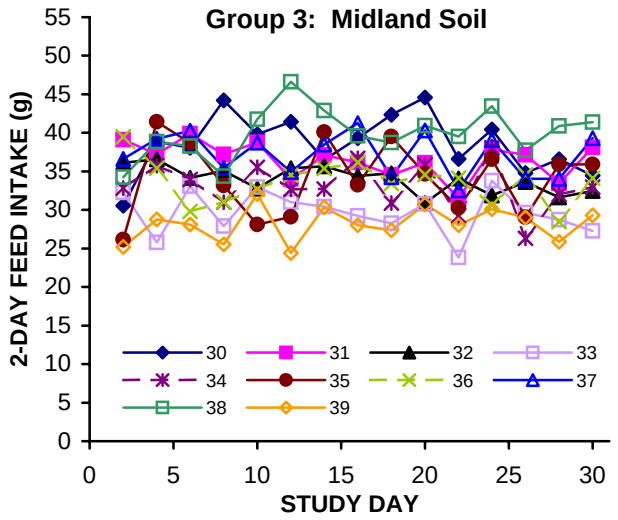
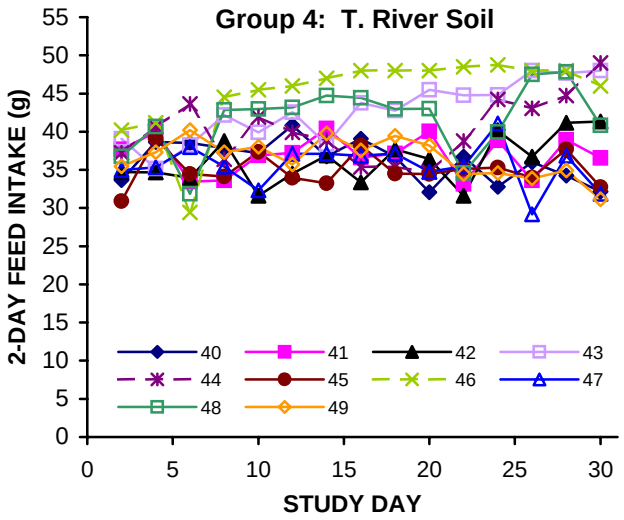
Figures









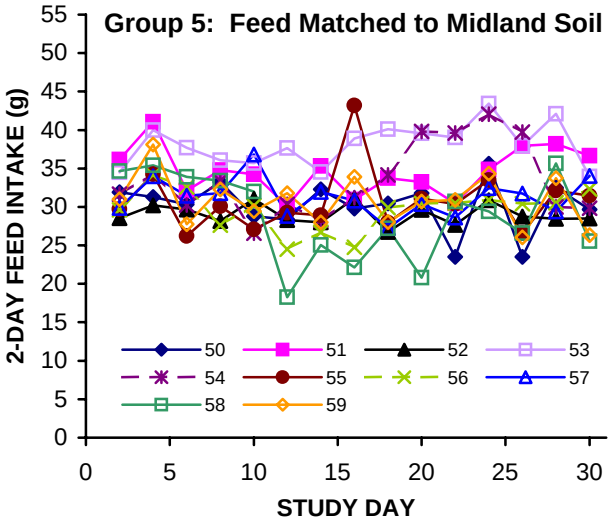


Figure 1. Feed intake for the rat pilot study

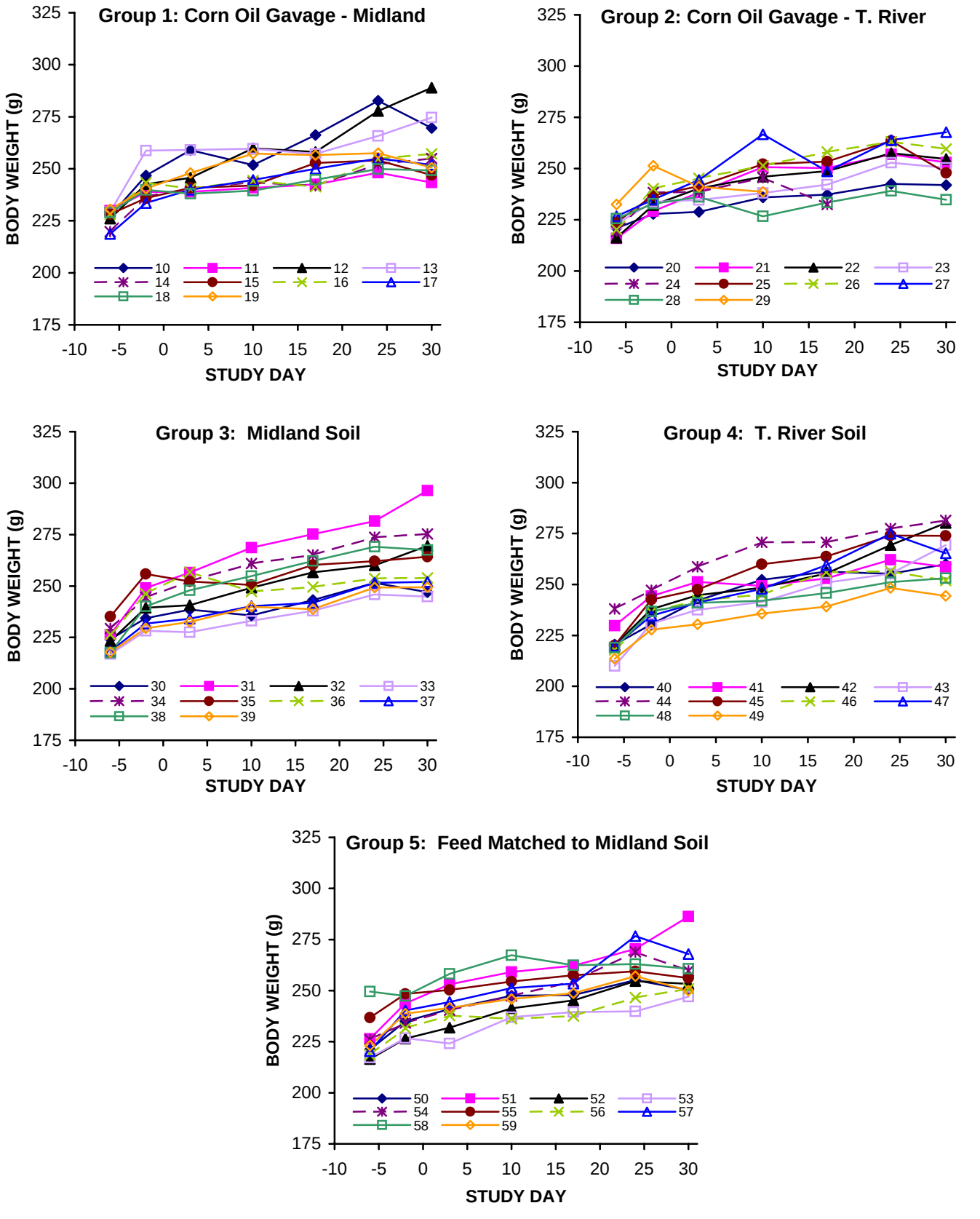


Figure 2. Body weights for the rat pilot study

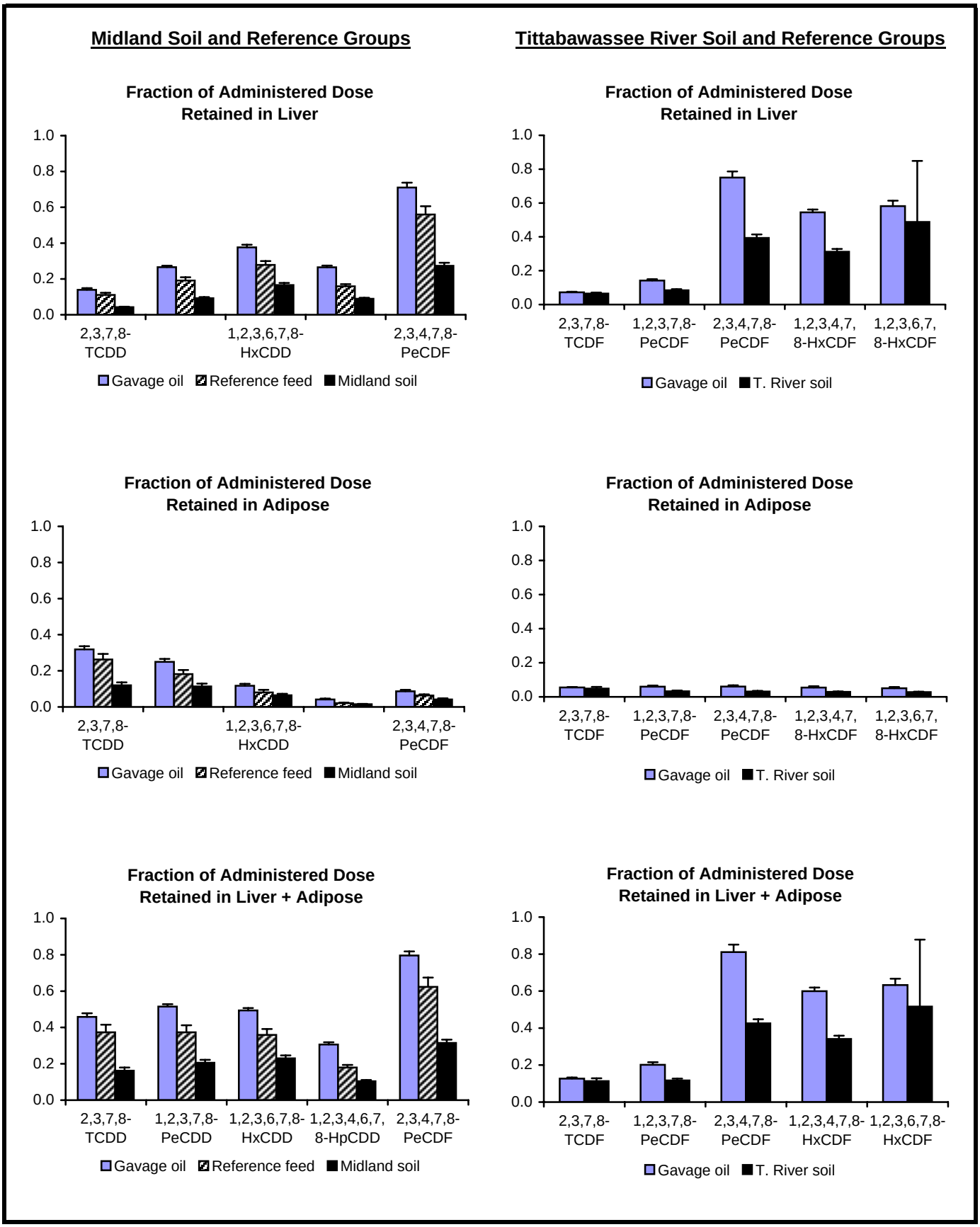
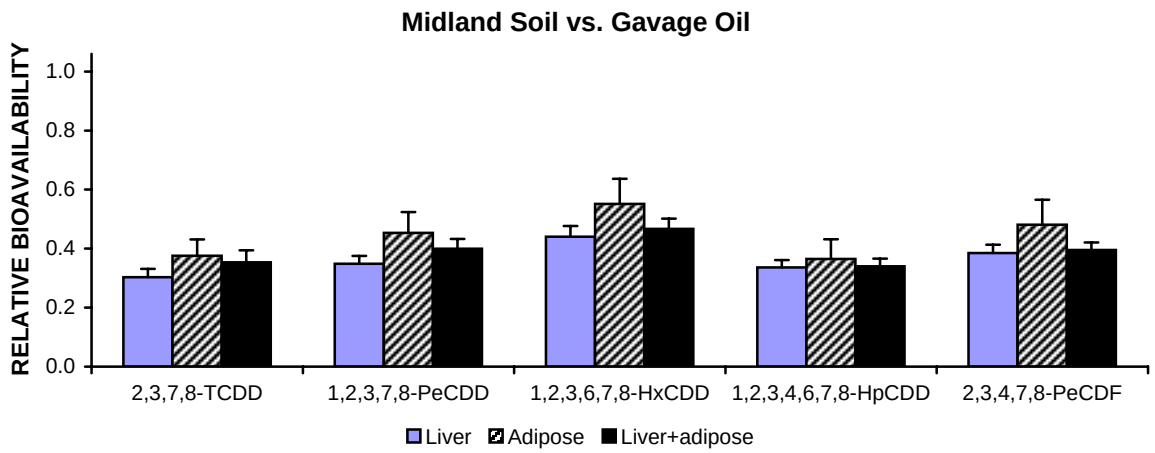
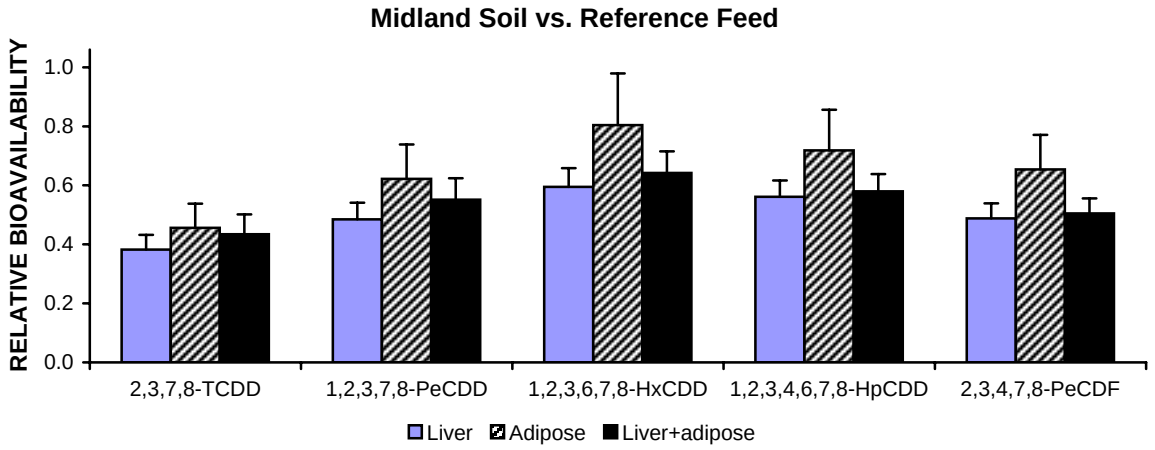
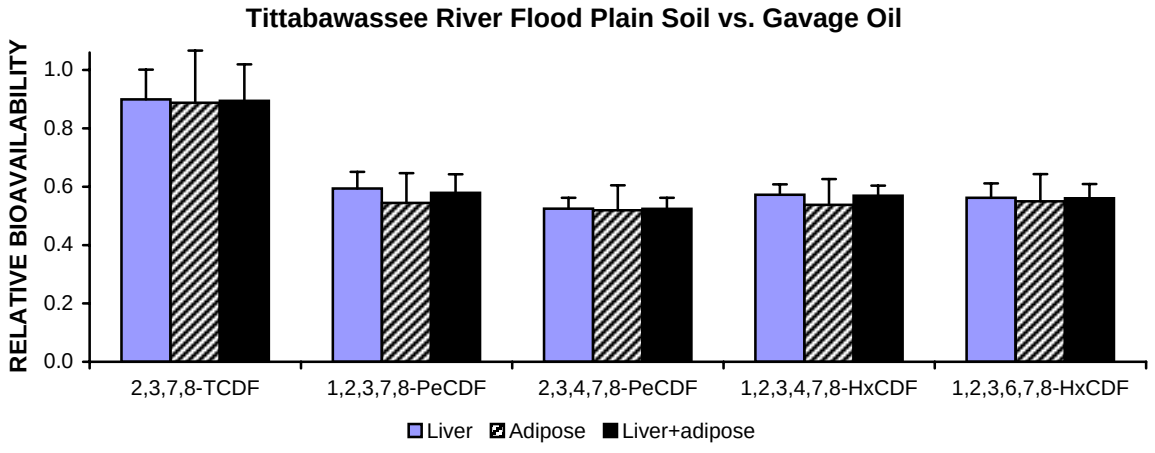


Figure 3. Distribution of administered doses in rat tissues







One outlier excluded for 1,2,3,6,7,8-HxCDF.

Figure 4. Relative bioavailability estimates for the rat pilot study

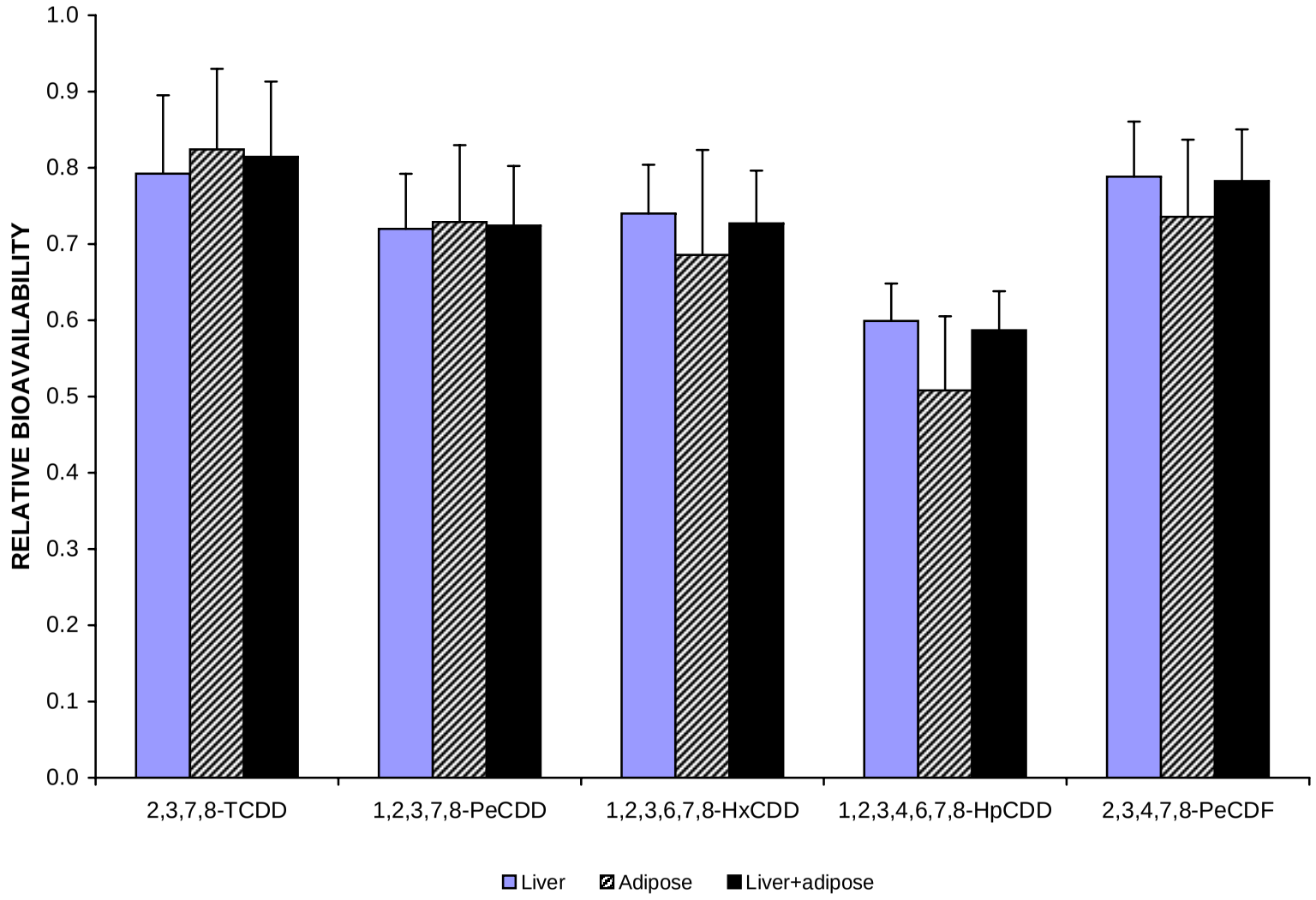
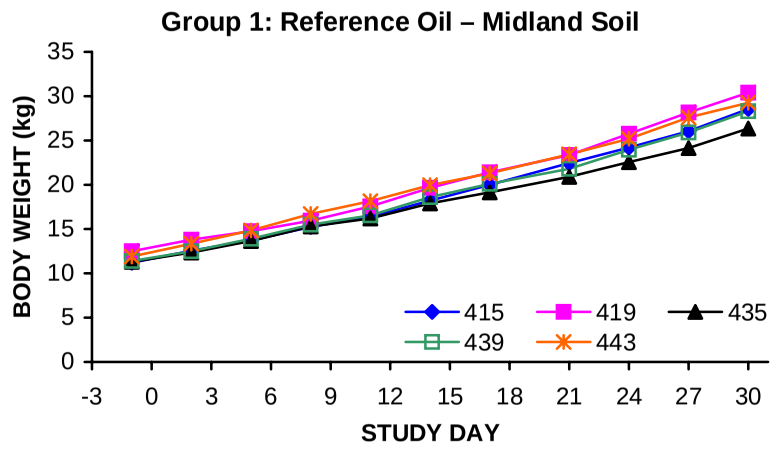
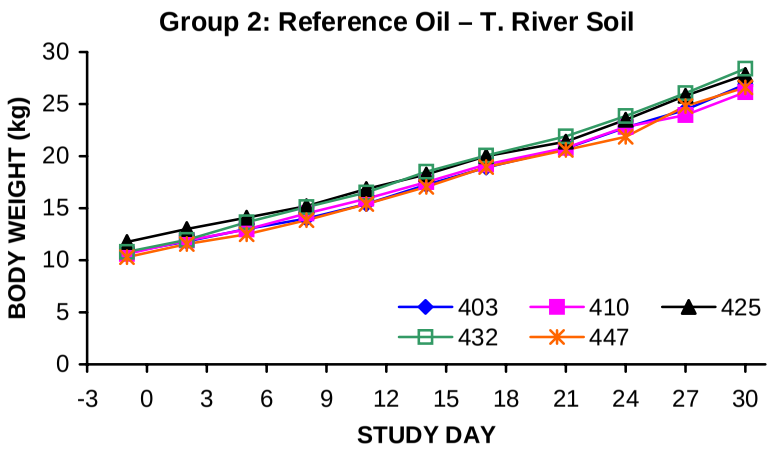
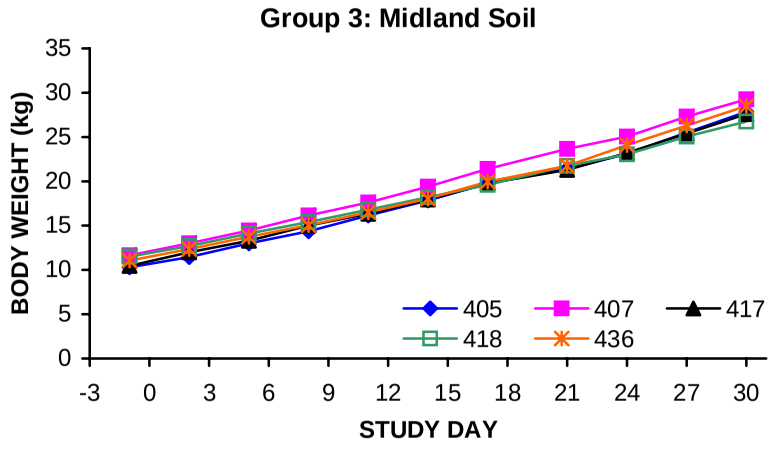


Figure 5. Relative bioavailability of the feed reference mixture compared to the corn oil reference mixture for the Midland soil







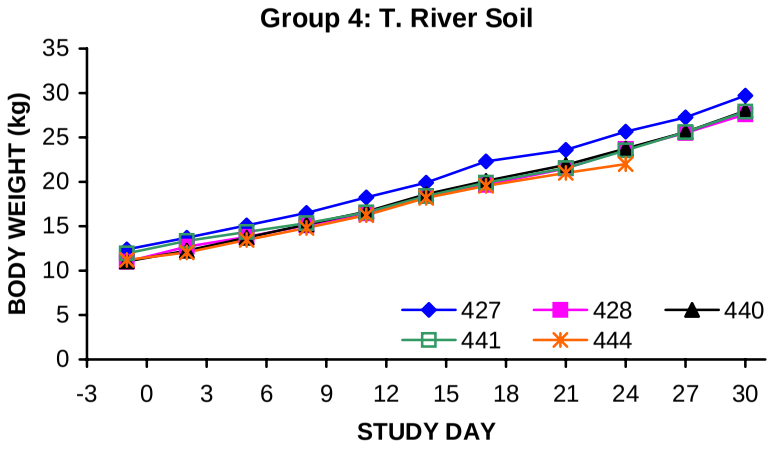


Figure 6. Body weights for the swine pilot study

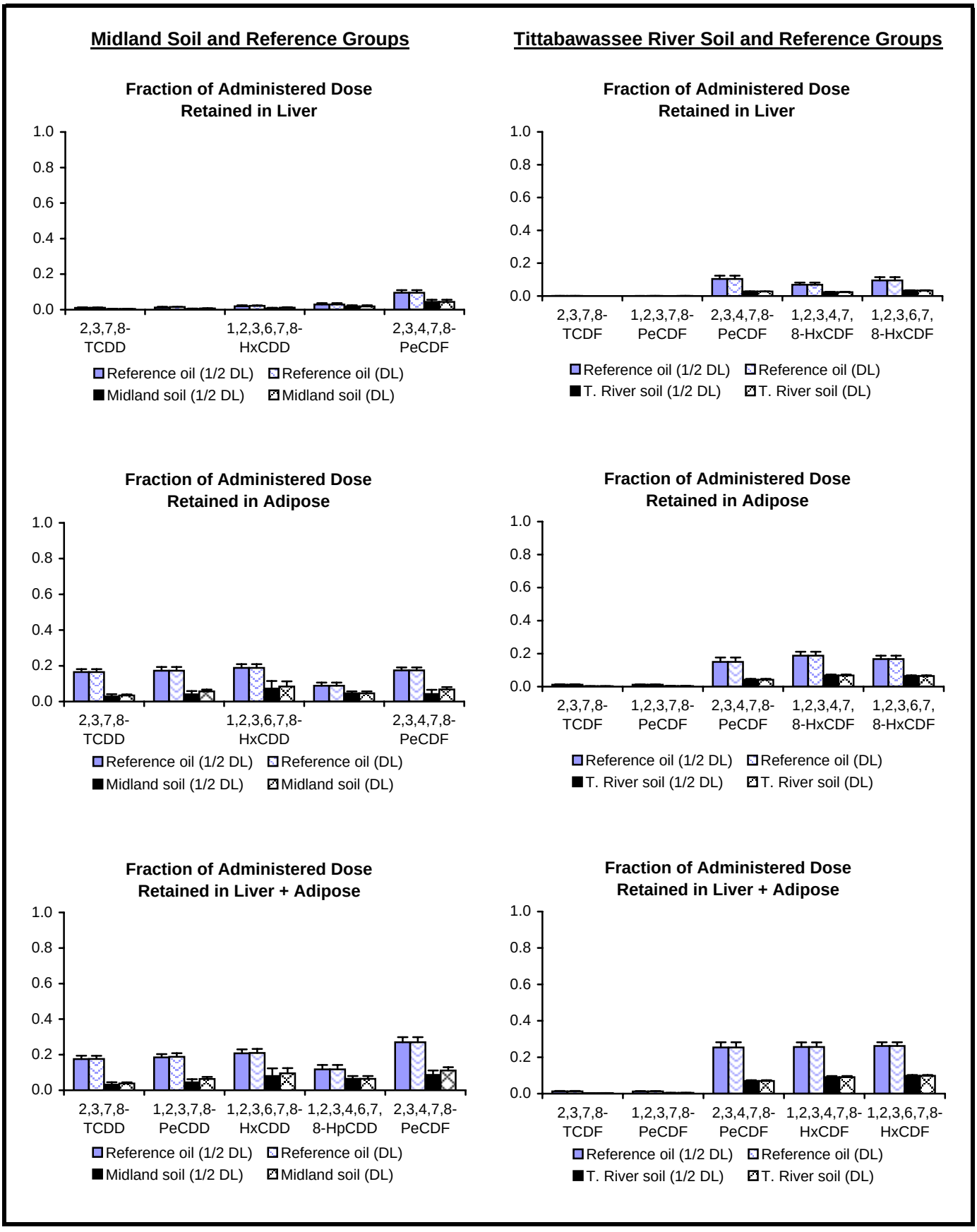
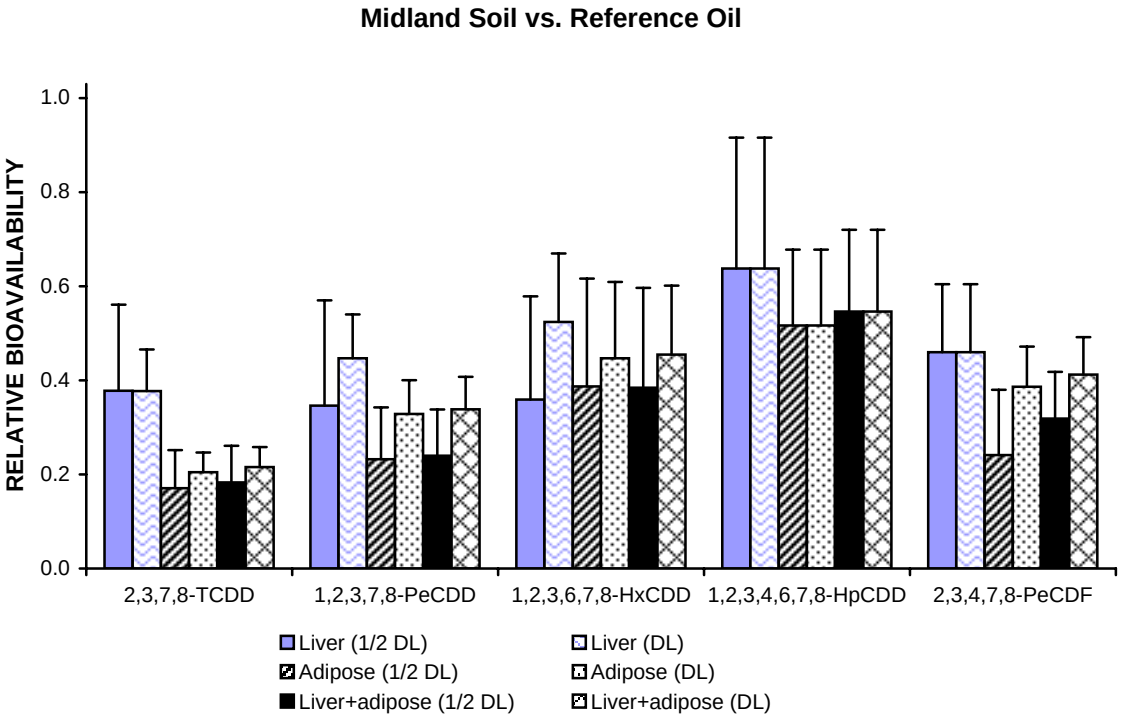
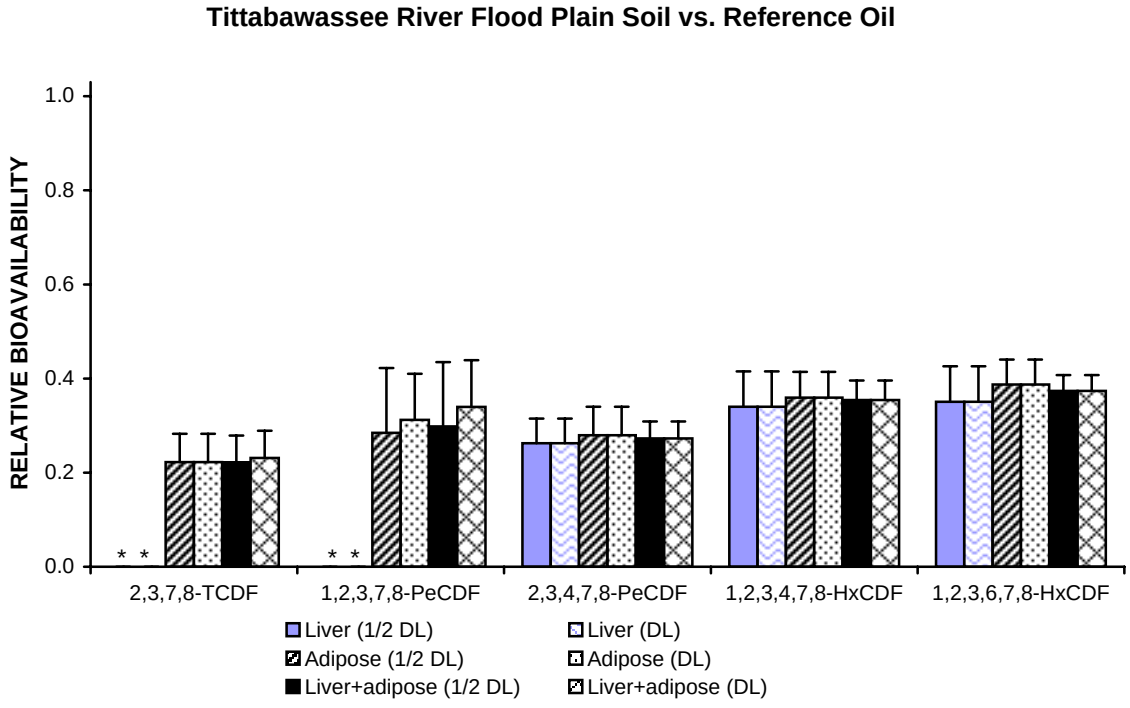


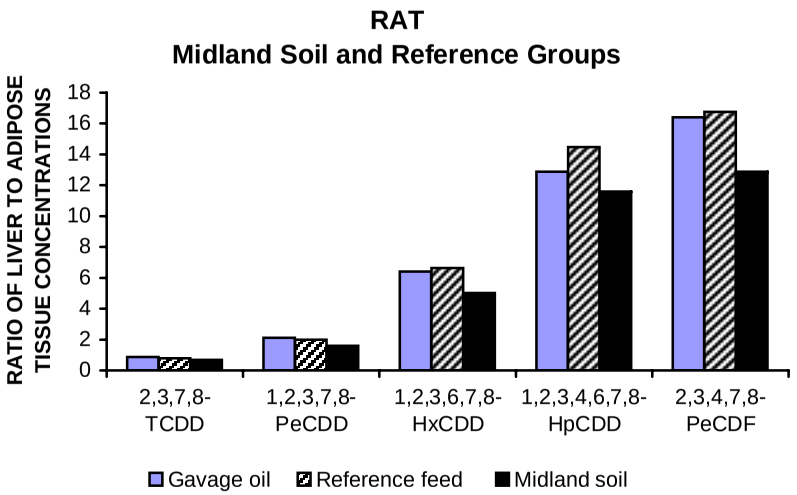
Figure 7. Distribution of administered doses in swine tissues

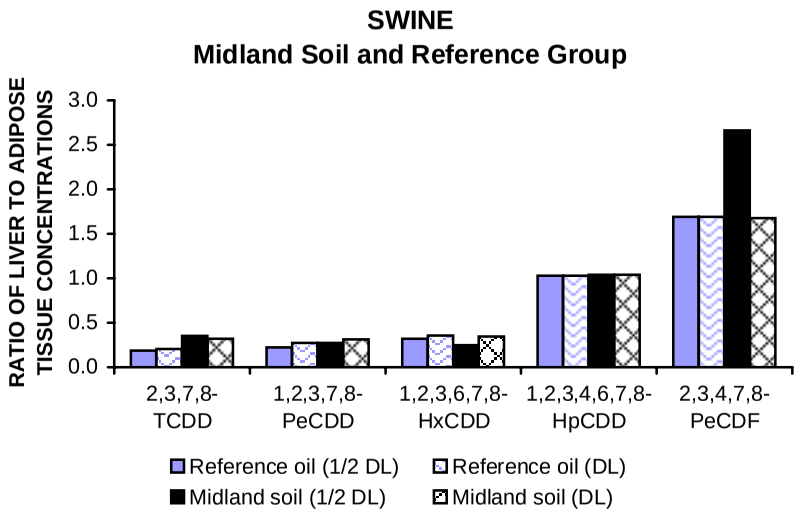


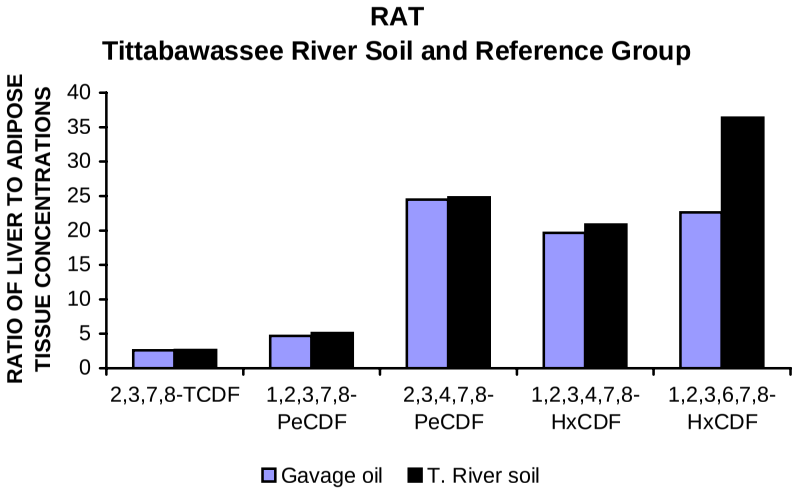


* Liver tissue concentrations were undetected in all soil group animals.

Figure 8. Relative bioavailability estimates for the swine pilot study







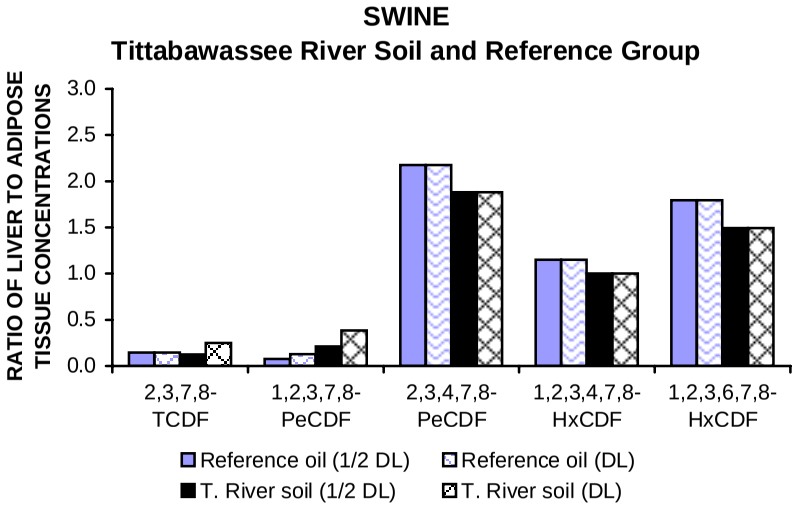


Figure 9. Ratio of liver to adipose tissue concentrations in the rat and swine pilot study

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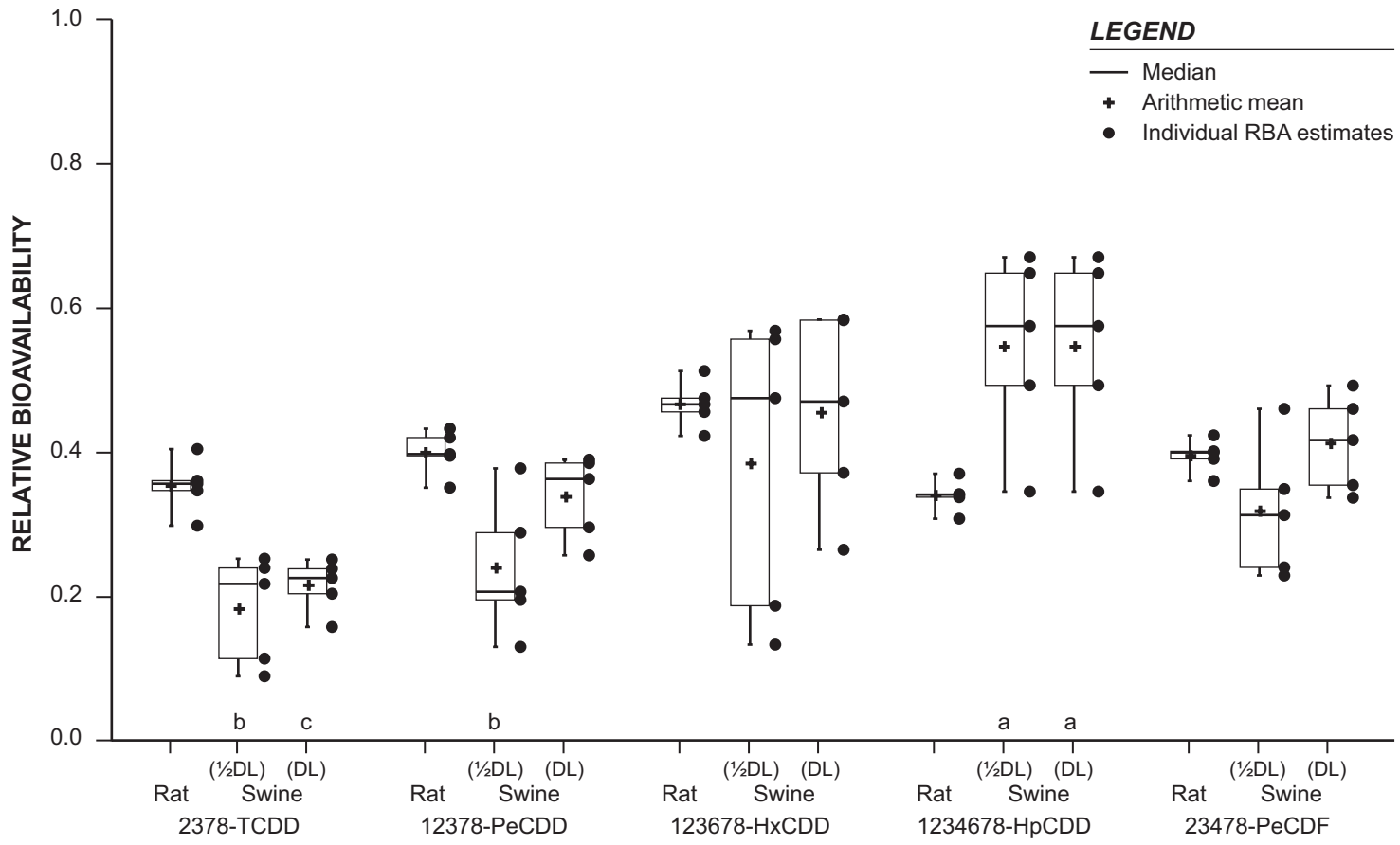
Data\TissueRatio.xls graphs 2/24/2005 (3:09 PM)

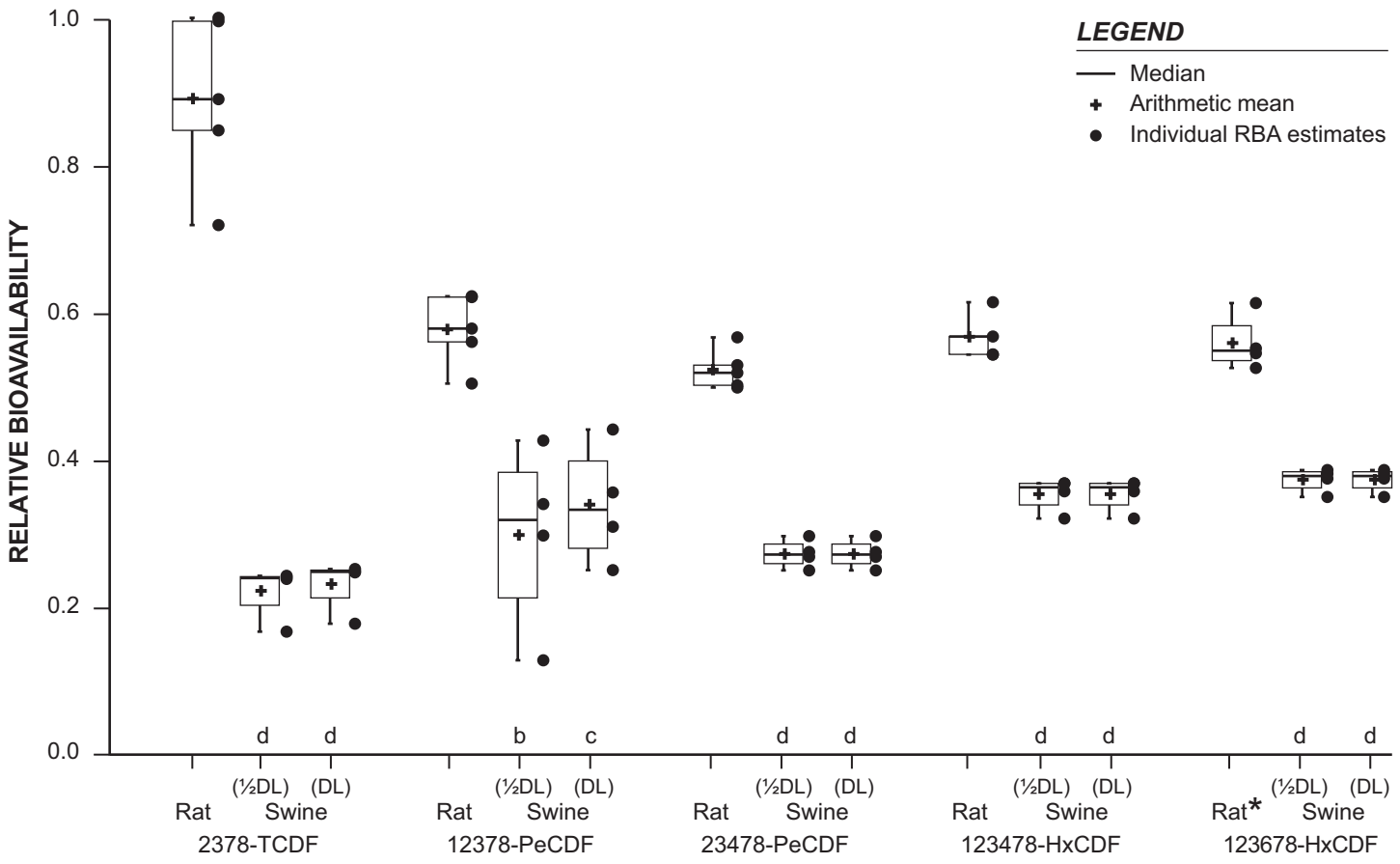


P-values:
Difference between rat and swine RBAs

a = p<0.05
b = p<0.01
c = p<0.001
d = p≤0.0001

Notes:
RBA estimates plotted for each animal (swine) or pair of animals (rats) based on the fraction of administered dose retained in liver plus adipose tissue compared to the average fraction of administered dose retained in liver plus adipose tissue in the respective corn oil reference group
½DL—Calculations performed using one-half the detection limit for non-detects
DL—Calculations performed using the detection limit for non-detects

Figure 10. Relative bioavailability estimates for the Midland soil in rats and swine



P-values:
Difference between rat and swine RBAs

a = p<0.05
b = p<0.01
c = p<0.001
d = p≤0.0001

Notes:

RBA estimates plotted for each animal (swine) or pair of animals (rats) based on the fraction of administered dose retained in liver plus adipose tissue compared to the average fraction of administered dose retained in liver plus adipose tissue in the respective corn oil reference group

½DL—Calculations performed using one-half the detection limit for non-detects

DL—Calculations performed using the detection limit for non-detects

*One outlier excluded from rat RBA estimate

Figure 11. Relative bioavailability estimates for the Tittabawassee River flood plain soil in rats and swine

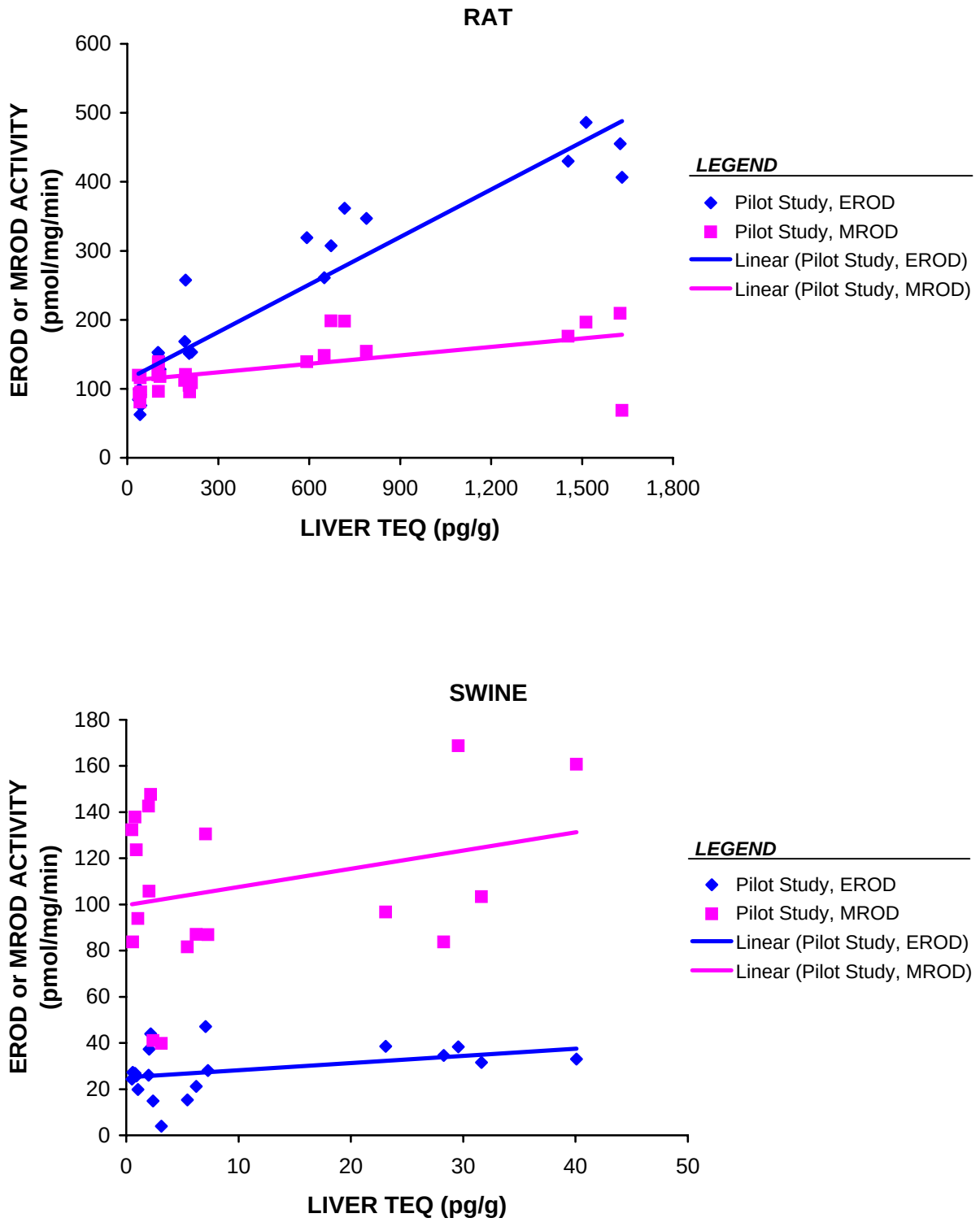


Figure 12. Enzyme activity in rat and swine liver microsomes for the pilot study

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