T. MORGAN

REVIS PG 33
% ABSORPTION BY MICE
PG 27
MEDIAN SOLUBILITY
IN WATER
3%
27 SAMPLES
SAIC MEDIAN 2.35%
PG 2-10
TURNER RESULTS
PG 3-11
50% PERCENTILE
FROM TURNER
DATA
MEDIAN
EST AT
2%

SUMMARY REPORT

OF REVIEWER COMMENTS

ON THE

TOXICOLOGICAL PROFILE FOR

MERCURY

Submitted to:

The Agency for Toxic Substances and Disease Registry
Executive Park Drive, Building 4
Atlanta, Georgia 30329

Submitted by:

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October 14, 1993

Printed on Recycled Paper

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inhalation.

Revis N, Holdsworth, G.B., King a, and Elmore J. An assessment of Health Risks Associated with Mercury in soil and sediment from East Fork Poplar Creek, Oak Ridge, Tennessee.

1 Adequacy of design, methodology, and reporting

This is an experimental study in mice to determine the toxicity of contaminated soil taken from a creek at Oak Ridge. Large quantities of mercury, of the order of millions of pounds had been lost from an Oak Ridge nuclear facility since the 1950s'. Some of the mercury found its way into a creek at Oak Ridge where local citizens might go fishing and children might play.

This is a large scale toxicity tests involving about 2700 mice. Contaminated soil or sediment was given orally in pellets of Purina chow at a level of 5% of the pellet. for periods of 6 to 12 months to 2400 of these animals. Additional test were carried out on the remaining 300. Positive controls were given soil to which mercuric chloride had been added. Data are also presented on soil and sediment levels of mercury, solubility and bioavailablity of mercury in soils and sediments. The gastrointestinal absorption of mercury from contaminated soil and sediments and the tissue disposition was also measured. The toxic end points tested were: body weight, mortality, kidney function, teratogenicity, neurological function (a swimming performance test) and carcinogenicity. Altogether an enormous amount of data are presented.

In principle , the design of the study looks good. Large numbers of animals are used. An appropriate route of exposure is used. Many measurements are made of mercury in soil, mercury intake, mercury absorption and disposition in mice and several toxic end points. The mercury analytical methods are not described in details and little quality control data are given. Recoveries from spiked samples were measured.

The report is not well written. The introduction is long and in fact constitutes a minor review article. Much of the text in the Results and Discussion section belong in the methods section.

2. Validity of results and author's conclusions

In view of the broad scope of this study, the variety of methods used and the large number of end points tested, it is very difficult to assess the validity of all the data. Indeed, it is difficult to determine the exact experimental protocol. In the main chronic feeding study, different experimental groups are not clearly defined as to mercury intake. Some idea of the mercury dose can be obtained from table 15 where mercury intakes are listed for all 30 groups in the chronic feeding study. Details on the negative controls are missing.

3. Study inadequacies or confounding factors

Some of the references are out of date and no longer valid. For example, the concentration of mercury in seawater quoted in reference 3 is about 1000 times too high.

It is of some concern that this study was completed in 1989 and has not yet appeared in the normal scientific literature.

Confounding factors may have been present as these were soil and sediment samples containing a variety of contaminants. However, the findings were negative despite the potential for additive of synergistic effects from the other contaminants.

Conclusions

This is a tantalizing report in so much that the results may be very useful for risk assessment of mercury in soil if we can only be sure of the

validity of the results. I am concerned that such a major and obviously expensive study has not yet appeared in the scientific literature and apparently not even as a formal report from Oak Ridge., Department of Energy.

The finding that mercury in soil is mainly in the inorganic form,and that it has low bioavailability and low toxicity seems reasonable. Thus despite the concerns expressed above, this study gives some degree of reassurance that gross toxic effects did not result from soils and sediments highly contaminated with mercury.

Lasora BK, Citterman RJ. 1992 Segmental analysis of mercury in hair in 80 women in Nome, Alaska. Govt. Reports Announcements & Index (GRA&I) Issue 10

1 Adequacy of design, methodology, and reporting

A previous study involved the collection of 200 hair samples from women of child bearing age living in Nome, Alaska. Total mercury concentrations were measured. This paper reports a follow up study in which 80 hair samples were collected from 27 participants in the original study plus 53 additional heavy users of subsistence foods. Hair samples were also taken from 7 residents of Sequim, Washington as control subjects. The hair samples were measured for total mercury. Segments of each hair sample, corresponding to one month's growth, were measured sequentially so as to give a recapitulation of past exposure and reveal seasonal changes.

The method of sampling and analysis appear to reliable. They have taken care to conduct quality control checks on standard samples supplied by the National Bureau of Standards and other intra-laboratory checks.

The sample size is small and no indication is given on how representative this samples is of the Nome population.

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mentioning:

1. Only median values are quoted, with NO measure of variation. Thus, although the conclusions drawn, seem reasonable, depending on the variation, the conclusions might be questioned.

2. Thirty days was too short a period of time to reach a constant elimination rate. One wonders why 30 days was chosen as the end point, rather than 90 days with the 20 day group being eliminated in favor of a 90 day group.

TICHENOR, B.A. and GUO, Z. (1991)

1. Only one duplicate test was run, giving rise to a near 50% difference in values. This casts doubt on ALL the conclusions drawn.
2. It is stated that tests for organic mercury were run on half the samples. One wonders how these samples were chosen and why tests were not run on the other samples.
3. No explanation is given for the difference in results for light and dark testing in the CMPA sample and this result is ignored in the summary.

REVIS, N. et al. (1989)

The design for this very costly experiment should have been peer revived, prior to its performance. The design leaves much to be desired, as does the presentation of results. Only major flaws will be listed below:

1. Up front it was decided that mercury was the only major toxic agent. This is bad science.
2. If mercury is the only key toxic agent or one wants to prove that mercury was the agent posing the health risk, for EVERY site considered there must be a CONTROL containing everything else except mercury. Absolutely no attempt was made to do this. Nor was any attempt made to run suitable controls.
3. The literature review pages 1-5 was outdated even for 1989.
4. Mercury has never been shown to be an agent producing skin cancers. Proper controls of soil containing everything except mercury were not run. Positive results obtained with a couple of batches of soil, lead one to wonder what the actual cause might have been and efforts should have been made to determine this.
5. The "swim tests" and the histopathological testing should have been correlated with mercury concentrations and proper controls (soil containing everything except mercury) should have been included. It should be remembered that lead was present in the soil.
6. Proper controls (soil containing everything except mercury) were not used for the teratogenicity testing.
7. With the possible exception of mercury content in soil versus mercury levels in the organs, the results obtained are meaningless due to the lack of proper controls.

TURNER, R.R. et al. (1992)

This is a straight forward account of measurements made. The methodology is standard and a true review would have had to include a field check. Recommendations 1-4 on pages 12-3 seem to be realistic.

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Revis, N., Holdsworth, G., Bingham, G., King, A., and Elmore, J. 1989. Final Report: An assessment of health risk associated with mercury in soil and sediment from East Fork Poplar Creek, Oak Ridge Research Institute, Oak Ridge, Tennessee.

Scientific and Technical Merit Review.

1. Adequacy of design methodology and reporting.

   The experimental design and methods employed for this study were excellent, as well as the analysis of data and discussion of results. Because the soils were contaminated with metals other than mercury, especially with selenium and cadmium, perhaps the study should have included an unpolluted sample of the same soil type with additions of mercury to establish concentrations at intermediate levels found in contaminated soil. Results from such a study would have been useful in assessing whether or not one or more of the other contaminants limited the action of mercury.

2. Validity of results, and authors conclusions.

   The authors derived correct conclusion from data representing health effects in mice due to an exceptionally wide range of mercury and other metal concentrations in diets. Mercury contained in soil which was incorporated into rations did not affect the health of mice. I believe, as they surmised, that the availability of mercury in soil for absorption by mice was too low to produce adverse health effects.

3. Study inadequacies or confounding factors.

   Although some information was presented regarding the distribution of mercury between organic and inorganic forms in contaminated soils, further information about inorganic mercury species in soils and sediments may have been helpful in interpreting the dose-response relationships. It also would have been helpful to have information about changes in mercury species distributions with time after contaminated soil was mixed with feed stuffs.

4. Your conclusions? Do you agree or disagree with those of the author? If not please explain.

   My conclusions do not differ from those of the authors.

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and the flow characteristics give a reasonable approximation of "real life" release from a building or outside wall is beyond my ability to evaluate. Paint thickness on the test surface was not provided. The authors note several reservations to the study, including the use of a single replicate and a weak confidence in the long-term decay calculation.

CONCLUSION: This study is a very preliminary report with many limitations. Nevertheless, some aspects of the findings might be worth reporting: 1) the mercury is rapidly released; 2) there is a rapid fall-off release with long-term, slow decay; and 3) light appeared to have no effect on release. A place for several of these points might be found in the document, and paint as a potential indoor source of mercury contamination might be stressed.

5) Revis, N.: *Assessment of health risk associated with mercury in soil and sediment from East Fork Poplar Creek, Oak Ridge, Tennessee*

This is a long, complicated, and occasionally obtuse document. The goal is laudatory. The question, raised in the review of the ATSDR document, regarding the bioabsorption of complexed mercurials, is one which is urgently in need of answer. This project spreads a very wide net in an attempt to cover all of the potential adverse health effects of mercury (alone and in combination with other toxicants), and includes pathology, carcinogenicity, toxicity, teratogenicity, chemical analysis of both biological and soil samples, etc. A long and generally reasonable review of mercury toxicity is included.

The ambition of this study is perhaps is greatest asset. The conclusions of the study are fully supported by the data, and stress that most of the mercury will be elemental or inorganic in these settings. I concur with the authors that, accepting the model as a "...predictor of the potential health effects of humans eating contaminated soil and sediment...", that the mercury in the Poplar Creek is of no appreciable risk to humans. I would also tender the suggestion that individuals eating soil and sediment probably have more immediate problems than mercury.

The carcinogenicity data is suspect and difficult to interpret; clearly, more work needs to be done. I do not think that these data would support the conclusion that mercury is a carcinogen, and I doubt that it is even a weak initiator.

CONCLUSION: This is a very interesting study, but does not add much to the existing data base. Its major contribution is to confirm what one would have suspected from the existing literature and from first principles. Of the 7 papers, this is one in which negative data actually tells something; ie., that even relatively high levels of release may not translate into dangerous exposure conditions.

However, I differ from the authors in statements made in Section 2, Introduction. They state (Para 2, ln 12) that environmental pollution may represent the greatest health risk, and that industrial contamination would have a greater impact on the human population than would direct industrial exposure. I do not know what they mean by this, but at the levels discussed in this paper and from data in the occupational medical literature, the opposite would appear to be true. High point sources are more likely to affect health than environmental exposure unless the mercury is biomethylated and accumulated in the food chain, as happened in Minamata Bay, Japan.

6) Wren, C.: *Relationship of mercury levels in sportfish with lake sediment and water quality variables*

CONCLUSION: This paper seems reasonable to me. However, the scope and details are beyond my field of expertise. The details of fish-mercury levels in freshwater fish is of interest but is not new data.

7) Turner, R.R.: *Mercury in ambient air at the Oak Ridge Y-12 plant, July, 1986-December, 1990.*

CONCLUSION: Another data-heavy study detailing atmospheric mercury levels at an isotope separation facility at Oak Ridge. I do not see any data in this report which adds to those already in the ATSDR document. The study highlights some of the difficulties of assessing point concentrations within a building. I would not lose any sleep including this data into the ATSDR document; the comparison of the three mercury trapping techniques and the sampling techniques might be of interest but are outside the scope of the document.

Revised
FINAL REPORT

An Assessment of Health Risk Associated with Mercury in Soil and Sediment from East Fork Poplar Creek, Oak Ridge, Tennessee

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Work performed under contract No. DE-AC05-84OR21443 for
the United States Department of Energy

April 1989

Table of Contents

Page

1 OBJECTIVE....1

2 INTRODUCTION....1

3 BACKGROUND....2
- 3.1 Mercury Metabolism....4
  - 3.1.1 Absorption....4
    - 3.1.1.1 Gastrointestinal....4
    - 3.1.1.2 Pulmonary....4
    - 3.1.1.3 Skin....4
  - 3.1.2 Tissue Distribution and Half Life....4
- 3.2 Mercury Toxicity....5
- 3.3 Objective of the Health Risk Studies....6
  - 3.3.1 Toxicity Assessments....7
  - 3.3.2 Carcinogenicity Assessments....8
  - 3.3.3 Teratogenicity Assessments....10

4 PROCEDURES AND METHODS....10
- 4.1 Procedures....10
  - 4.1.1 Sample Collections....10
  - 4.1.2 Chronic Feeding and Skin Painting Studies....13
    - 4.1.2.1 Chronic Feeding Studies....13
    - 4.1.2.2 Skin Painting Studies....14
- 4.2 Methods....14
  - 4.2.1 Test Diet Preparation....14
  - 4.2.2 Food Intake and Urinary Collection Studies....15
  - 4.2.3 Soil Feeding Studies....15
  - 4.2.4 Mercury Absorption Studies....15
  - 4.2.5 Extracts for Skin Painting Studies....16
  - 4.2.6 Skin Studies....16
  - 4.2.7 Teratogenicity Studies....16
  - 4.2.8 Histological Studies....16
  - 4.2.9 Mercury Solubility Studies....17
  - 4.2.10 EPA EP Toxicity Studies....17
  - 4.2.11 Soil and Tissue Digestion....18
  - 4.2.12 Chemical Analysis of Trace and Bulk Elements....18
  - 4.2.13 Urine Analysis....18

5 RESULTS AND DISCUSSION.....19
5.1 Soil Studies.....19
5.2 Soil Feeding Studies.....26
5.2.1 Body Weights and Mortality.....31
5.2.2 Organ Distribution of Mercury.....36
5.2.3 Kidney Morphology and Function.....39
5.2.4 Dietary Intake of Soil or Sediment Required to Achieve Toxic Levels of Mercury in the Kidney.....40
5.2.5 Carcinogenicity and Teratogenicity Effects.....47

6 SUMMARY, CONCLUSIONS, and RECOMMENDATIONS.....52
6.1 Summary.....52
6.2 Conclusions.....53
6.3 Recommendations.....53

7 REFERENCES.....54

8 FIGURES 3 - 28.....59

APPENDIX (Raw Data)

List of Tables

Table 1 Specimens Collected.....11

Table 2 The Concentration of Selenium in Soil from East Fork Poplar Creek and Bear Creek.....20

Table 3 The Concentration of Zinc in Soil from East Fork Poplar Creek and Bear Creek.....21

Table 4 The Concentration of Lead in Soil from East Fork Poplar Creek and Bear Creek.....22

Table 5 The Concentration of Arsenic in Soil from East Fork Poplar Creek and Bear Creek.....23

Table 6 The Concentration of Cadmium in Soil from East Fork Poplar Creek and Bear Creek.....24

Table 7 The Concentration of Mercury in Soil from East Fork Poplar Creek and Bear Creek.....25

Table 8 The Solubility of Mercury from Soil and Sediment.....27

Table 9 The Solubility of Mercury from Soil and Sediment.....28

Table 10 The Solubility of Mercury from Soil and Sediment.....29

Table 11 EP Toxicity Test for Mercury.....30

Table 12 Recoverable Mercury in Mouse Feed.....32

Table 13 The Gastrointestinal Absorption of Mercury from Soil or Sediment in Mice.....33

Table 14 Mortality of Mice Exposed to Mercury Contamination.....34

Table 15 Mortality of Mice Continuously Exposed to Diets Containing Mercury-Contaminated Soil.....35

Table 16 Animals Killed During the Experimental Period Because of Trauma.....37

Table 17 Percent Mortality in Mice Exposed to Mercury-Contaminated Soil.....38

Table 18 Mercury Data Divided into Histology Classes.....41

Table 19 Partial Correlations (and P-values) for 12-Month Data.....43

Table 20 Estimate of Dietary Intake of Mercury-Contaminated Soil Required by Female Mice to Achieve Kidney Levels of 25 µg Hg/g Wet Weight.....44

Table 21 Estimate of Dietary Intake of Mercury-Contaminated Soil Required by Male Mice to Achieve Kidney Levels of 25 µg Hg/g Wet Weight.....45

Table 22 Tumor Promotion and Initiation Study Using Soil Extracts.....49

Table 23 Mice Breeding Studies.....51

List of Figures

Figure 1 Protocol for Tumorigenesis Assay.....9

Figure 2 Map of Bear Creek and East Fork Poplar Creek.....12

Figures 3-28 Statistical Analysis of the Log of Liver and Kidney Mercury over the Log of Soil Mercury.....59-79

1. OBJECTIVE

Heavy metal contamination of floodplain soil and sediment occurred following the release of water containing mercury and other heavy metals by a DOE facility in Oak Ridge, Tennessee. Because of the concern expressed by several regulatory agencies, experimental studies were performed to determine the potential health risk associated with the resulting contaminated soil and sediment. The objective of these studies, to determine the toxicity of the contaminated soil and sediment in experimental animals, was accomplished by feeding mice contaminated soil and sediment through the diet. Because the soil and sediment contained known carcinogens, skin tumor studies were also performed to determine the potential carcinogenicity of the contaminated soil and sediment. Results from the toxicity and carcinogenicity studies provide a basis for evaluating the health risk associated with human exposure to the contaminated soil and sediment.

2. INTRODUCTION

Mercury (Hg) is neither abundant nor widely distributed in the earth's crust (1). It is estimated that the earth's crust contains 0.8 ppm Hg and that seawater contains 0.3 ppb (2). Mercury exists primarily in highly concentrated ores such as cinnabar, metacinnabar, and living stonite. Elemental mercury, refined from its ores, finds extensive use in the electrical industry, electrolytic manufacture of chlorine and caustic soda, industrial control instruments, paint, pigments, and amalgam metallurgy. Mercury salts are used as catalysts in the manufacture of fungicides, herbicides, insecticides, bactericides, disinfectants, and antiseptics. Some Hg salts have been used as cathartics, diuretics, and antisyphilitics (calomel Hg_2); as a topical antiseptic and disinfectant ($HgCl_2$); and in the treatment of syphilis and gonorrhea (mercury benzoate).

As a result of the therapeutic use of and industrial exposure to Hg, the health profession became quite familiar with its toxic effects. However, as new drugs replaced the mercurial formulations, drug-induced signs of Hg poisoning became virtually unknown. At the same time, industrial demand for Hg was increasing, which resulted in an increase in human exposure to this metal. This exposure occurred not only through industrial activities but also through pollution of the environment (i.e., air, water, and soil). Environmental pollution may represent the greatest human health risk from Hg because a larger percent of the population may be exposed. For example, Hg contamination of drinking water following industrial contamination of surface and ground water would have a greater impact on the human population than would direct industrial exposure. Similarly, direct contamination of the water supply would present a greater health risk than would industrial exposure. Nevertheless, both industrial exposure and environmental pollution represent a risk to the environment and human health.

3. BACKGROUND

Mercury levels in the lithosphere have been reviewed by several expert groups. The National Science Foundation and the World Health Organization (3, 4) report that the concentration of Hg in sediment (i.e., soil) in nonpolluted rivers and lakes in the United States is less than 0.07 mg/kg of sediment. Recently, the Federal Drug Administration (FDA) recommended a guideline for sediment of 3 ppm total Hg (5). Similar levels have been recommended for soil. Mercury levels as great as 1000 ppm have been observed in sediments of polluted rivers and lakes in close proximity to chloralkali plants. Furthermore, freshwater fish and other aquatic life from polluted rivers and lakes usually contain higher-than-normal levels of Hg. The consumption of fish and/or sediment containing high levels of Hg may pose a health risk to humans, because Hg (in particular, methylmercury) has long been recognized as a toxic agent capable of inducing neurological and kidney disorders and death.

Effluents from the Y-12 Plant in Oak Ridge empty into the S-3 Ponds and New Hope Pond. These ponds are used to trap particulates from the effluents and to allow for biodegradation of the organics in the effluents. New Hope Pond drains into East Fork Poplar Creek, which eventually drains into Poplar Creek and the Clinch River. The S-3 Ponds drain into Bear Creek, which drains into East Fork Poplar Creek and Poplar Creek. Higher-than-normal levels of Hg and other pollutants (i.e., cadmium, lead, arsenic, organics, and radionuclides) have been observed in the soil and sediment from these holding ponds and in the creeks which drain from them. The S-3 Ponds also contain higher-than-normal levels of trichloroethylene, perchloroethylene, phenols, and chlorinated biphenyls (PCBs). Furthermore, higher-than-normal levels of radionuclides (i.e., uranium and thorium) have been observed in fish in the Clinch River near these creeks.

Because these pollutants present a potential human risk (6) for persons eating the sediment and/or soil associated with these sites, the studies described below were performed.

The toxicity associated with these pollutants has been established in both experimental and human studies (7-10), using a simple matrix such as water and/or organic solvents. The toxicity of these pollutants in a complex matrix, such as soil or sediment, has not yet been established. Therefore, in the studies reported here, we attempted to determine the toxicity of these pollutants in a complex matrix.

Mice were fed, via the diet, soil and sediment from various locations along East Fork Poplar Creek, and the toxicity and tissue distribution of the pollutants were determined at various time intervals. The tissue level relative to toxicity was used to determine the effect of a complex matrix on the gastrointestinal absorption and tissue distribution of these pollutants.

The level of each pollutant in the soil and sediment was known prior to feeding the mice. For some of these pollutants (on an individual basis), the amount absorbed per dose has been established. For example, cadmium absorption and tissue distribution have been established in mice and humans given daily doses of this element (11-14). The amount absorbed in the mouse per dose is assumed to be 2.8%, and 50% of this dose is distributed between the liver and kidney. Because of slow excretion (i.e., 0.1% of body burden), a

biological half-life of 200 days has been observed for cadmium (15). With this information, one can determine the effect of a complex matrix on cadmium absorption and tissue distribution.

Absorption and tissue distribution for lead, mercury, arsenic, uranium, and PCBs in mice have also been reported (16-20). This information is useful in establishing the effect of a complex matrix on absorption and tissue distribution for some of these pollutants. The results of the effect of this matrix on absorption and tissue distribution were used to assess the potential human health risk associated with humans eating soil or sediment from selected areas along East Fork Poplar Creek.

The toxicity of these pollutants on various organ systems was determined morphologically and physiologically. These determinations were based on previous results describing the toxicity of these pollutants, which is, in part, related to the tissue level. However, toxicity may be observed in an organ where the tissue level is below that predicted to induce toxicity. For example, in the kidney the critical concentration of cadmium required to induce renal pathology is 200 µg/g wet weight (21). If the kidney level of cadmium is below this value and renal pathology is observed, the synergism of other pollutants present may explain this effect.

Mercury, lead, and uranium have also been reported to induce renal pathology. Thus, the tissue level for each pollutant may not relate directly to the observed toxicity. Toxicity, therefore, is the most important index for assessing the health risk associated with soil and sediment contaminated with several environmental pollutants. For example, kidney toxicity may not be observed even though the concentrations of mercury, lead, cadmium, and arsenic in the kidney are elevated following exposure to contaminated soil and sediment. If toxicity is not observed [even though the levels of these metals in soil and sediment samples are above the Environmental Protection Agency (EPA) acceptable limits], we would suggest that these samples do not present a health risk at the dose used in these studies. The experiments described herein attempt to address this question.

The results in this report focus on the absorption and toxicity of Hg in soil and sediment samples, because the level of this element in most samples far exceeded the lead, cadmium, and arsenic levels. It was assumed, after measuring the levels of these metals in soil and sediment, that the predominant effects would be associated with Hg levels. However, as mentioned above, if toxicological changes are observed in the kidney following exposure to soil and sediment, attempts will be made to determine the relative importance of these metals in explaining this effect. The kidney was used as the target organ in these studies because the inorganic forms of mercury, lead, cadmium, and arsenic are associated with kidney toxicity.

The toxic effects of Hg have been reviewed (22). However, only in the last two decades have reports described the specific toxic effects of the mercuric salts (organic, mercurous, and elemental mercury). Furthermore, during this time reports in the literature have shown the metabolic fate of the various forms of Hg. Mercury metabolism and toxicity are described in Sections 3.1 and 3.2.

3.1 MERCURY METABOLISM

3.1.1 Absorption

3.1.1.1 Gastrointestinal

Three forms of Hg are absorbed through the intestinal tract, although there are differences in the rates of absorption between these forms. Organic Hg compounds are almost completely absorbed, whereas inorganic and elemental forms are absorbed at rates of 2 to 20% and 0.01 to 0.5% of the ingested dose, respectively (23-25). Several dietary factors have been shown to affect Hg absorption, including cysteine, selenium, and calcium. In humans, inorganic Hg is absorbed at rates of 7 to 15%, whereas in mice the rate is only 2% of the dose. However, these rates are dependent on the solubility of the mercuric compound. For example, since the solubility of mercuric chloride is greater than mercuric sulfide, we would anticipate differences in the absorption rates between these compounds.

3.1.1.2 Pulmonary

Three forms of Hg may enter the body through the lungs following inhalation. Mercury absorbed to particles and/or as a vapor (i.e., elemental and organic Hg compounds) readily diffuses from the lung into the bloodstream. Vapors of elemental and organic Hg readily diffuse from the inhaled air into the bloodstream and may cross the blood-brain barrier (26, 27). Unlike gastrointestinal absorption of elemental Hg, pulmonary absorption may exceed 45% of the inhaled dose (pulmonary absorption rates for inorganic Hg are similar to rates observed in the gastrointestinal tract). Differences in the absorption rate in the pulmonary tract between humans and mice have not been reported.

3.1.1.3 Skin

Mercury may be absorbed through the skin, but the amount depends on the form and carrier (28). Some investigators have observed an absorption rate of 6% for mercuric chloride (29). We assume that the absorption rate in skin would be significantly higher for organic mercurials than for inorganic Hg.

3.1.2 Tissue Distribution and Half-Life

Once absorbed, Hg enters the bloodstream and is transported to several organs. Elemental Hg, after entering the bloodstream, may be oxidized to the mercuric ion (30). In the blood, the mercuric ion quickly equilibrates with blood cells to give a plasma-to-cell ratio of approximately 1 (31). Elemental Hg, before oxidation, can cross the blood-brain barrier and accumulate in nervous tissue. Inorganic Hg entering the bloodstream equilibrates in blood cells to give a similar ratio, but does not appear to accumulate in nervous tissue. Organic Hg compounds, because of their lipid solubility, accumulate in blood cells and nervous tissue. However, unlike the mercuric ions, these compounds can concentrate in blood cells to give a plasma-to-cell Hg ratio approaching 108 (32). Furthermore, the

organic Hg compounds can cross the blood-brain barrier and accumulate in nervous tissue.

Mercury accumulates in all soft tissues by absorption from the blood. Inorganic Hg accumulates primarily in the kidney, followed, in descending order, by liver, spleen, and other tissues. Elemental Hg shows a similar distribution to that of inorganic Hg. However, following the inhalation of elemental Hg vapors, significant amounts are observed in brain tissue. Organic mercury compounds, in accordance with their particular chemical structure, differ considerably in patterns of tissue distribution. Virtually all organs accumulate significant amounts of the organic mercurials. Reports have indicated that the liver-to-kidney ratio may approach 1 after exposure to organic Hg, whereas 0.09 is observed for inorganic Hg. However, the kidney, liver, and brain contain the highest levels of Hg after exposure to methylmercury. Some of the short-chain alkyl Hg derivatives have been shown to accumulate in the fetus.

Mercury can enter cells of various organs by passive diffusion and/or active transport. In the cell, Hg binds to various macromolecules, primarily sulfhydryl-containing compounds. Turnover of cellular Hg is relatively slow; however, once released from the cell it is cleared from the body primarily via the bile, fecal, and urinary routes.

Inorganic and elemental Hg are excreted predominantly via the urine, while the organic mercurials are excreted primarily via the bile and feces (33, 34). Significant amounts of the organic mercurials are excreted via the bile into the intestinal tract and may be reabsorbed and/or excreted in the feces. Estimates for humans indicate a relatively long biological half-life for Hg of 42 to 70 days (35, 36), depending on the form of Hg in the body. The half-life for inorganic Hg is estimated to be 48 and 57 days for males and females, respectively (37). The half-life for organic mercurials in humans is estimated as 70 to 74 days (38). Shorter half-lives for inorganic and organic Hg are observed in experimental animals. For example, the half-lives for inorganic Hg and methylmercury in mice were 2 and 21 days, respectively (39, 40). The half-lives described above were determined for the whole body; it has been demonstrated that the half-life for different organs may be considerably different.

3.2 MERCURY TOXICITY

Toxic effects have been observed in humans and experimental animals following exposure to the three forms of Hg. Ingestion of inorganic Hg produced local necrotic changes in the gastrointestinal tract, circulatory collapse, and acute renal failure with aliguria or anuria. The chronic effects include renal tubular necrosis with proteinuria.

Chronic exposure to elemental Hg vapors occurs primarily in the workplace. Workers are frequently exposed to inorganic Hg through inhalation of dust particles. In clinical studies, attempts to separate the chronic effects of elemental Hg vapor from the effects of inorganic Hg have rarely been made. However, it is known that the respiratory absorption of elemental Hg exceeds that of inorganic Hg. Furthermore, it is known that elemental Hg, unlike the inorganic form, accumulates in brain tissue. This may explain the associated high incidence of central nervous system disorders frequently observed at the workplace (i.e., workers mining cinnabar). The toxic effects

associated with elemental Hg vapors include anorexia, loss of weight, asthenic-vegetative syndrome (i.e., micro-mercurialism), and renal failure. These symptoms are observed in the early stages of toxicity, whereas later stages are characterized by mercurial tremors, psychic disturbances, and changes in personality (erethismus mercurialis).

Animal studies have confirmed some of the effects associated with exposure to vapors of elemental Hg. These experimental studies also show significant levels of Hg in brain tissue following the exposure. Symptoms associated with the ingestion of elemental Hg are few, but include necrotic changes in the intestinal tract and proteinuria. These effects are only observed following acute exposure. Thus, exposure to elemental Hg vapors appears to be more toxic than exposure to equivalent amounts of elemental Hg via the gastrointestinal tract. This difference may be explained by the amount absorbed in the respiratory tract (i.e., 30 to 80% of the dose) versus the gastrointestinal tract (i.e., 0.01 to 0.5% of the dose). Furthermore, as mentioned above, elemental Hg accumulates in brain tissue, and thus an increase in the amount absorbed via the lungs will eventually affect the central nervous system.

Neurological and kidney disorders are observed in humans and mice exposed via pulmonary and/or intestinal routes to organic mercurials. The toxic effects of the organic mercurials are similar to the above descriptions for inorganic and elemental Hg. However, the organic mercurials are more toxic than dose equivalents of inorganic or elemental Hg because (1) the absorption rate (i.e., both pulmonary and intestinal) is greater; (2) they accumulate in nervous tissue; (3) they readily diffuse into cells; and (4) they have a longer half-life.

3.3 OBJECTIVE OF THE HEALTH RISK STUDIES

The overall objective of the studies described here was to determine experimentally the potential health risk associated with humans eating soil or sediment contaminated with Hg and heavy metals from East Fork Poplar Creek. This objective was accomplished by feeding mice a diet containing contaminated soil and sediment for 20 months and assessing the toxicity and organ levels of Hg at various time intervals. Because some of the soil and sediment samples contained known carcinogens, skin tumor studies were also performed to further evaluate potential health risks associated with these samples. Specific objectives were assessments of toxicity, carcinogenicity, and teratogenicity.

The toxicity assessments included (1) body weight changes over the experimental period, (2) liver and kidney weights over the experimental period, (3) a swim test to evaluate neurological changes, (4) levels of Hg in the liver and kidney at various time intervals during the exposure, (5) the ratio of liver-to-kidney Hg, (6) the morphology of the kidney, and (7) the functional physiology of the kidney. Other assessments included determining the levels of heavy metal contaminants in soil and sediment, the relative level of

water-soluble Hg in soil and sediment, and the exposure dose of Hg in the feed after the addition of soil and sediment. The carcinogenicity assessments included tumor promotion and tumor initiation. The teratogenicity assessments included determining litter size in the exposed animal.

3.3.1 Toxicity Assessments

Chronic feeding studies are frequently used in toxicology studies to assess the effect of a single chemical or a specific chemical in a complex matrix. The studies outlined here were designed to determine the amount of Hg absorbed into mice following the feeding of soil and sediments with known concentrations of Hg. These studies were also designed to determine the potential pathological effects of Hg in soil and sediment on the central nervous system and on the various organs described above. If the Hg in soil and sediment was mainly organic Hg (i.e., methylmercury), we would have anticipated changes in the ratio of liver-to-kidney Hg and effects on the central nervous system. However, if the Hg in soil and sediment was mainly in the inorganic form, we would have anticipated morphological and functional changes in the kidney. These pathological changes would, of course, depend on the levels of Hg in the soil and sediment and the amount of Hg absorbed via the intestinal tract. Thus, these chronic studies provide a method for assessing the potential health risk associated with the ingestion of Hg-contaminated soil and sediments. These studies also provide data necessary for determining the effect of soil constituents on Hg absorption and tissue distribution. For example, selenium has been shown to inhibit Hg absorption via the intestinal tract and to alter the tissue distribution and toxic effects of Hg.

The effect of other inorganics on organ systems in the mouse may be assessed by comparing the physiological and biochemical changes with the tissue level of these inorganics. For example, the following associations have been reported: (1) lead with a reduction in heme biosynthesis, (2) methylmercury and lead with neurological disorders, (3) mercury and cadmium with liver cell damage, (4) lead and uranium with bone abnormalities, (5) arsenic with cancer, (6) selenium with white muscle disease, and (7) lead, cadmium, uranium, and mercury with kidney disorders.

The tests outlined below provide a method for determining the toxicity of the various inorganics found in soil and sediments. For example, an increase in the level of lead (Pb) in the brain, kidney, and bone; neurological changes; and a decrease in the activity of aminolevulinic acid synthetase (ALAD) (the rate limiting enzyme in heme biosynthesis) would indicate that the level of Pb found in soil or sediment is toxic. However, if neurological disorders are observed along with increases in brain and kidney Hg, and if Pb in tissue and the activity of ALAD in plasma are both normal, we would assume, *a priori*, that the neurological disorders observed are associated with the level of Hg in the soil or sediments. It is also possible that neurological disorders may be observed when the tissue levels of both Hg and Pb are increased and the activity of ALAD is normal. In this case, we would assume that the neurological changes observed occurred as a result of the level of Pb and Hg in soil or sediments. Previously reported dose-response relationships (i.e., the relationship of tissue level of the above elements to pathological

changes in the mouse) will also be used to assess the potential toxicity of selected elements observed in soil and sediment samples.

The interaction of various elements in the soil sediment on absorption via the gastrointestinal tract was also assessed. By determining the tissue level of these elements relative to the dose, various interactions can be assessed. For example, changes in the calcium and selenium levels in the soil or sediments may affect the amount of cadmium and mercury absorbed, respectively. Interactions between calcium and lead on lead absorption and between zinc and cadmium on cadmium absorption have been previously reported (41). We thus anticipated that absorption and toxicity would be influenced by the various interactions of the elements found in soil or sediments. However, we note that these potential interactions are only assessed if toxicological effects are observed.

3.3.2 Carcinogenicity Assessments

Skin painting tests have frequently been used to determine comparative tumor-initiating activity of some complex mixtures in the Sencar mouse (42, 43). This mouse is used frequently for its increased sensitivity to two-stage carcinogenesis using 7,12-dimethylbenz(a)-anthracene (DMBA) as the initiator and 12-O-tetradecanoylphorbot-13-acetate (TPA) as the promotor. This system is also more sensitive to other polycyclic aromatic hydrocarbons (PAH) such as benzo(a)pyrene (B[a]P). In addition to its well-documented response to PAH, the mouse skin tumorigenesis bioassay system has identified many chemicals other than PAH as potential carcinogens. These chemicals represent a wide variety of structural classes, including aldehyde, carbamate, epoxide, haloalkyether, haloaromatic, haloalkylcarbonyl, hydroxylamine, lactone, nitrosamide, sulfonate, sulfone, and urea. The mouse skin tumorigenesis bioassay can also detect chemicals that cause tumors in the respiratory tract of animals. Of all known animal respiratory carcinogens, the mouse skin tumorigenesis system has, to date, detected PAH, quinolines, and carbamates. For highly suspect occupational respiratory carcinogens, the mouse skin tumorigenesis system has, to date, detected chloromethyl ethers and coke oven emissions. These results indicate that the mouse skin tumorigenesis bioassay can detect both dermal and nondermal carcinogens.

Two basic protocols frequently employed to detect chemical carcinogens in the mouse skin tumorigenesis assay are illustrated in Fig. 1. Multiple application of the test agent for up to 17 weeks will give rise to papillomas and/or malignant carcinomas of the skin. This protocol for complete carcinogens is a test for agents exhibiting both tumor-initiating and tumor-promoting activities. The bioassay protocol for tumor initiators is a double application of test agent followed by multiple applications of a potent tumor promoter 1 week later. Tumor initiation is one step in the multi-step carcinogenic process and involves the conversion of a normal cell into a preneoplastic one. In the case of chemical carcinogens, it involves the interaction of chemicals or their activated forms with cellular DNA. These initiated cells remain dormant for periods of up to 1 year or until they are stimulated to progress into hyperplastic or neoplastic lesions. This stimulation, called tumor promotion, is accomplished by applying croton oil or its most active component, TPA.

PROTOCOL FOR TUMORIGENESIS ASSAY

Tumor Initiation

Soil Extract (two applications) — TPA 2x Weekly

Weeks of treatment

1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17

Tumor Promotion

DBMA (two applications) — Soil Extract 2x Weekly

Weeks of treatment

1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17

Internal Control

DBMA — TPA 2x Weekly

Weeks of treatment

1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17

At weekly intervals, the number of papillomas per mouse and the number of mice with papillomas will be recorded. For solvent controls, mice will be treated with acetone for 17 weeks. The number of mice per treatment group is 5 to 10 of either sex.

Figure 1

An initiated cell is, therefore, an irreversibly formed preneoplastic lesion that can be stimulated to express the transformed phenotype.

Relationships between tumor initiators and complete carcinogens have been previously described (44). Various structurally diverse chemicals are both complete carcinogens and tumor initiators in mouse skin from CD-1 and the genetically related Sencar mouse. Some agents, however, appear to have only tumor-initiating activities in mouse skin. The correlation between potencies as complete carcinogens and as tumor initiators is excellent for the 12 chemicals that show both kinds of activity. The relationship between the production of papillomas and the production of carcinomas in the same animals treated with the skin tumor initiator DMBA or B(a)P has been shown. The results indicate that the number of papillomas per mouse at 15 to 20 weeks correlates well with the number of malignant carcinomas formed at 50 weeks for animals treated with these two strong skin tumor initiators.

3.3.3 Teratogenicity Assessments

Teratology is the study of congenital malformations. Teratogenic effects are defined as alterations in the formation of cells, tissues, and organs resulting from physiologic and biochemical changes (i.e., generative changes). Thus, once the particular cells, tissues, physiological systems, or biochemical systems have been formed, a teratogenic change can no longer occur. The anatomical effect of teratogens thus occur very early in the fetal period. Teratogenic effects are often assessed by anatomical changes such as cleft palate, polydactyly, syndactyly, ectrodactyly, and hydrocephaly. Aborted fetuses and changes in litter size may occur as a result of exposure to teratogens. However, aborted fetuses and/or litter size changes may also occur as a result of chemical toxicity to the fetuses. Thus, if such changes do occur following the exposure to a chemical, it would be necessary to further evaluate the fetuses for congenital malformations. Identification of congenital malformations indicates that the material to which the animal was exposed is teratogenic.

4. PROCEDURES AND METHODS

4.1 PROCEDURES

4.1.1 Sample Collections

Sediment samples were taken with a stainless steel Jenkins sampler at a depth of 30 cm from the areas indicated in Table 1 and Fig. 2. The sampler was thoroughly washed (deionized water) after each sample to avoid contamination between samples. Soil and sediment samples for this study were collected from areas on a transect to include the floodplain and center of the creek bed. In this configuration, five samples were collected from the creek bed and five samples (i.e., five/side) from floodplains. Respective samples represent environmental conditions for aquatic as well as terrestrial systems. Sediment and soil samples within a transect were collected

TABLE 1
Specimens Collected*

Site (see Fig. 1)	Left flood-plain	Creek	Right flood-plain
Origin East Fork Poplar Creek (Y-12 area at guard house)	1	2	3
1.5 miles from New Hope Pond (near NOAA)	4	5	6
K-Mart	7	8	9
Bruners	10	11	12
Sewage Treatment Plant Oak Ridge Turnpike (7-8 miles from New Hope Pond)	13	14	15
Gaging Station Oak Ridge Turnpike (11.5 miles from New Hope Pond)	16	17	18
Fork where East Fork Poplar Creek enters Poplar Creek (14.3 miles from New Hope Pond)	19	20	21
Origin Bear Creek (Y-12 area near S-3 Ponds)	22	23	24
Fork of Bear Creek and East Fork Poplar Creek (8.8 miles from S-3 Ponds)	25	26	27
Origin Poplar Creek (100 ft below origin)	28	29	30

*Five 30-cm cores were taken at each of the above sites.

EAST FORK POPLAR CREEK DRAINAGE

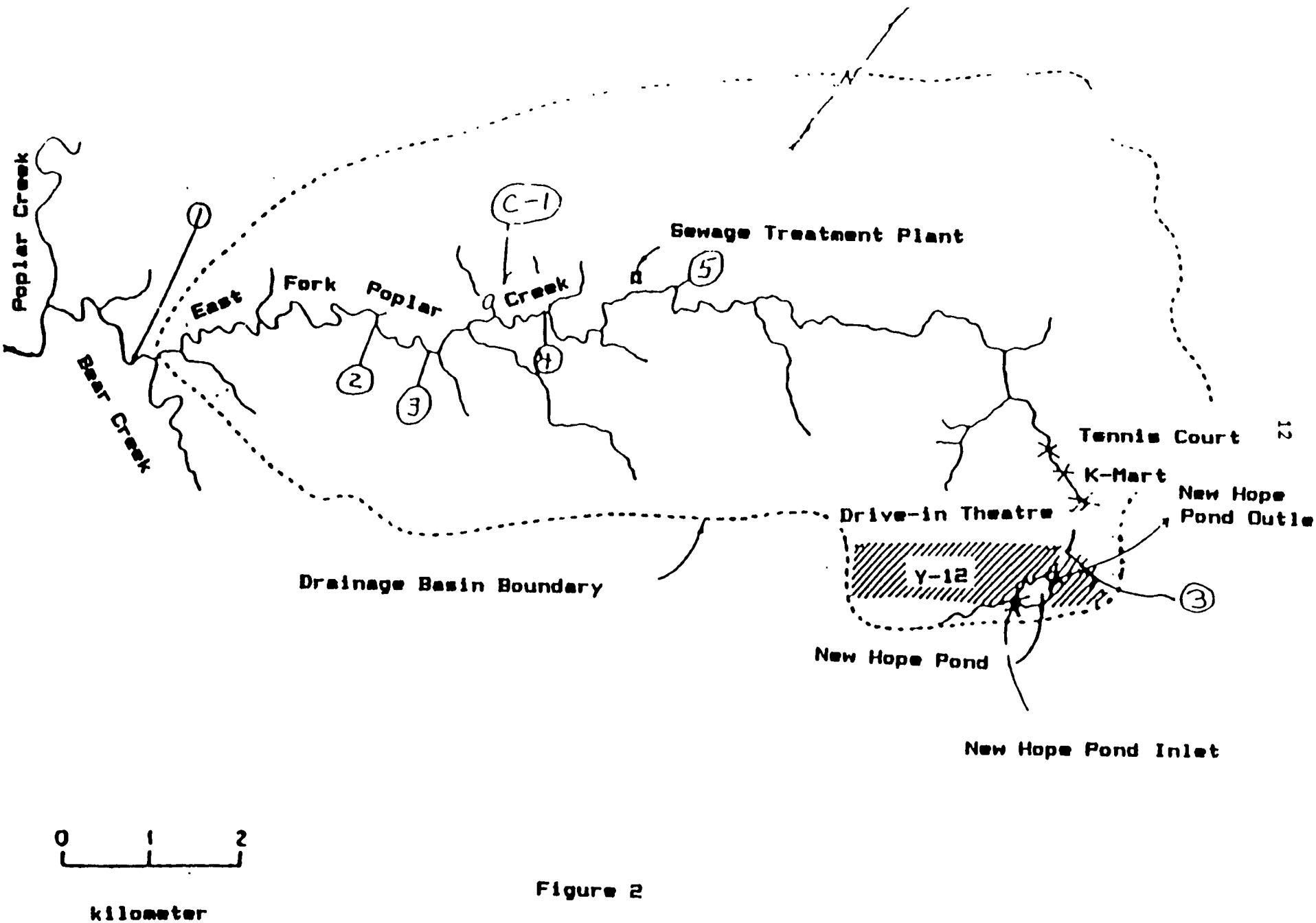

Figure 2

from areas where particle size distribution is appropriate for the feeding experiments.

It is safe to assume that gross changes in the physical and chemical composition of the creek sediments can occur slowly over the period of the experiment, except at times of storm events, when scouring and filling redistribute sediment, and at times of accidental release of pollutants. To address these changes, soil and sediment samples were collected at 0 and 12 months (it is noted that the level of those inorganics measured did change over the experimental period).

Pollutants released into the creek may be partitioned into several fractions, which may be transported from the system at variable rates, with a portion trapped in the sediments and alluvium within the channel and floodplains, respectively. Although pollutants trapped in the sediments and alluvium may be exposed to some physicochemical changes through time, the long-term effects can be assessed through the described feeding experiments. Pollutants most likely to be associated with creek sediments are distributed according to stream morphology and flow dynamics at baseline and storm events.

The collected samples were frozen, cut in half vertically, and analyzed for specified inorganics at 10-cm intervals. The top 10 cm was lyophilized to constant weight and used for feeding and skin painting studies or stored frozen at -60°C. The top 10 cm of the core samples was selected for the animal studies, because it represents the approximate depth at which pollutants may exchange with water in the creek basin. A similar exchange would be expected in the floodplains. The top 10 cm was also selected for these studies because it contained the highest concentration of those inorganics measured.

4.1.2 Chronic Feeding and Skin Painting Studies

4.1.2.1 Chronic Feeding Studies

The chronic feeding studies were performed to assess the potential health risk which may be associated with the various pollutants observed in soil and sediment samples. Soil and sediment samples were obtained as described above. After mixing and lyophilizing the soil and sediments, the dried soil or sediment was added to powdered Purina mouse chow, mixed, and feed pellets were prepared by Ralston Purina. The soil or sediment concentration in the feed was 5%; animals (i.e, 2,700 male and female Swiss mice) were fed these modified diets over a 20-month period. At 6, 12, and 20 months of exposure, 5 to 10 mice from each group were analyzed for neurological changes and muscle coordination by measuring their ability to swim in a tank of water for 100 sec. This simple swimming test has been used frequently to assess central nervous function in rats and mice (45). After this measurement, final body weights were determined, the animals were sacrificed, and the following analyses performed: (1) Hg levels in the liver and kidney, and (2) histological evaluations of the kidney for morphological changes. Some of the mice in this main group were also used in the food intake and urinary collection studies described below.

In some studies, mercuric chloride was added to the dried soil and sediment and mixed with the powdered mouse chow. Animals were fed these diets for 20 months, except for groups 31 and 32. These latter two groups were fed these diets for only 6 months. The groups fed the diets with mercuric chloride added were groups 2, 14, 27, 31, and 32;

the final concentrations of Hg in the each diet were 2,000, 1,000, 500, 4,000, and 8,000 ppm, respectively. Animals in groups 2, 14, and 27 were treated the same as described for the other experimental groups. In groups 31 and 32, body weights, liver and kidney Hg levels, and the morphology of the kidney were determined following 6 months of exposure.

The number of mice used in this study were as follows:

(1) Chronic feeding studies: 2,700 male and female Swiss mice 3 months of age.
(2) Chronic exposure to feed containing mercuric chloride: 80 mice (40 males and 40 females) for groups 2, 14, and 27, and 40 mice (20 males and 20 females) all 3 months of age.
(3) Food consumption and mercury absorption studies: 300 male and female Swiss mice who were previously exposed to the above diets for 6 and 12 months (i.e., groups 1 through 30).
(4) Urinary studies: 150 male and female Swiss mice who were previously exposed to the above diets for 12 months (i.e., groups 1 through 30).
(5) Teratogenicity studies: 30 male and 60 female Swiss mice who were previously exposed for 6 months to the above diets (i.e., groups 1 through 30.
(6) Skin painting studies: 1,060 male and female Sencar mice, 3 months of age.

4.1.2.2 Skin Painting Studies

The procedure used in these studies is shown in Fig. 1. Briefly, the organics in soil or sediment were extracted into chloroform-methanol (2:1 v/v) at 4°C and the samples dried with nitrogen. The dried samples were diluted in acetone and applied to the shaven backs of female and male Sencar mice. The period of application and the volume applied are shown in Fig. 1. At 1-week intervals, the number of papillomas (those greater than 2 mm in diameter) per mouse and the number of mice with papillomas were recorded. Papillomas were removed randomly for histological verification. A total of 1,060 female and male Sencar mice 10 weeks of age were used in this study.

4.2 METHODS

4.2.1 Test Diet Preparation

Thirty test diets were made using soil and sediment samples collected at the beginning of the study and 12 months after the study was in progress. Soil and sediment samples (top 10-cm layer) taken from the sites shown in Table 1 were thoroughly mixed and dried to a constant weight by lyophilization. The powdered soil and sediment samples were maintained at -60°C until required for preparation of the test diets. The soil and sediment samples were pulverized to a fine powder and uniformly mixed with a powder form of Purina mouse chow. This mixture was made into 15-cm pellets by Ralston Purina at 0, 10, and 15 months. Mice were fed this mixture ad libitum for 20 months. The soil and sediment from the 30 sites were added individually to the Purina mouse chow to give a total concentration of 5%. Thus, each

diet contained 5% soil or sediment. A total of 80 Swiss mice (i.e., 40 males and 40 females) were fed these diet for 20 months. A total of 2,700 male and female mice 3 months of age were fed these diets for 20 months.

After preparation of the test diet samples, aliquots were taken for chemical analysis, which included the measurement of mercury, cadmium, arsenic, lead, and selenium. These measurements were made to determine the dose exposure levels.

4.2.2 Food Intake and Urinary Collection Studies

Food intake was measured in male and female mice exposed for 6 and 12 months to diets containing Hg-contaminated soil. The animals (five per experimental group per sex) were placed individually into metabolic cages and acclimatized. After 16 hours, a weighed amount (13 to 16 g) of feed was given to each mouse. After 24 hr, the residual amount of feed was determined. The measurement of food intake was repeated twice, with an overnight interval between measurements, and the results averaged. It is noted that data obtained are subject to a standard deviation of 10 to 18% because, while the animals were eating, some of the feed (small particles) was observed at the bottom of the cage. A total of 300 males and females from the main chronic feeding studies were used to measure food intake and, after these measurements, the animals were returned to the main study. It is noted that food intake was determined in animals fed the respective modified diets. After 12 months, urine samples were collected from mice (five per group per sex) exposed to the modified diet. The animals were placed individually into metabolic cages and acclimatized for approximately 56 hours. Urine samples were then collected over a period of 24 hr. Three collections were made at 8, 10, and 24 hr, and the samples were combined and analyzed for protein, gamma GT, and creatinine. A total of 150 males and females from the main chronic feeding studies were used to collect urine. During the period of urine collection, the animals were fed the respective modified diets.

4.2.3 Soil Feeding Studies

Germ-free 3-month-old Swiss mice were obtained from Jackson Laboratories (Bar Harbor, Maine). For the 30 experimental groups, 80 mice (40 males and 40 females) were used. Animals were fed the test diet *ad libitum*. At 6, 12, and 20 months of exposure, 5 to 10 mice were removed from the study and the various tests and analyses were performed.

4.2.4 Mercury Absorption Studies

The absorption of mercury through the gastrointestinal tract was determined in 3-month-old male and female Swiss mice. Animals (five per experimental group per sex) were placed individually into metabolic cages and fasted overnight. After fasting they were fed the test diet for 24 hr, and their food intake measured. After the 24-hr exposure, they were fed a normal diet and fecal samples were collected over a 96-hr period. Samples were then dried to a constant weight, digested in nitric acid, and Hg determined in the digest. A total of

90 Swiss mice (both male and female) were used in these absorption studies.

4.2.5 Extracts for Skin Painting Studies

Soil and sediment extracts were prepared as described above. Extracts in acetone were used in all studies. A 0.2-mL sample was applied to the backs of Sencar mice for both the initiation and promotion studies.

4.2.6 Skin Studies

Figure 1 outlines the protocol used in the initiation/promotion studies. Briefly, 5 to 15 Sencar mice (male and female) per treatment group were initiated with 0.2 mL of extract (2x) and then promoted with TPA twice weekly (1 µg per application) for 17 weeks. In the promotion studies, animals were initiated with DMBA (two 5-nM applications) and promoted with 0.2 mL of soil extract twice weekly for 17 weeks. The negative controls were treated with 0.2 mL of acetone, DMBA, or TPA for 17 weeks. The positive controls were initiated with DMBA (two applications of 5 nM each) and promoted with TPA twice weekly (1 µg/ application) for 17 weeks. Both TPA and DMBA were dissolved in acetone.

After applying the above mixtures to the skin, the number of animals with tumors and the number of tumors per animal were recorded. Weekly counts of cumulative skin tumors (i.e., tumors that were observed for 3 consecutive weeks) were made for the 17-week exposure period. Results are expressed as the total cumulative number of skin tumors per mouse per treatment group.

4.2.7 Teratogenicity Studies

Litter size has been frequently used to determine the potential teratogenicity of various chemicals (46). To determine the potential teratogenicity of the contaminated soil and sediment samples, male and female Swiss mice were exposed for 6 months to the test diets described above. One male and two females from each group were then time-mated. At 24 and 96 hr postdelivery, the litter size was determined in each group. A total of 30 males and 60 females were used in these teratogenicity studies.

4.2.8 Histological Studies

Mice were exposed to the experimental diets for 20 months. At 6, 12, and 20 months, 5 to 10 animals were sacrificed and a gross necropsy performed. After visible lesions had been recorded, the liver and kidney were removed for further analysis. A section of kidney was removed for microscopic evaluation and the remainder was used for elemental analysis. The kidney section was fixed in a buffered formaldehyde solution. After dehydration and embedding, 10-µ sections were stained with hematoxylin-eosin. The stained sections were examined under a light microscope for necrotic epithelial cells of the proximal convoluted tubules and/or glomerulus, diffuse necrosis, or fibrosis. Epithelial necrosis was defined as karyoklasis and cytoplasmic granulation.

Thirty fields were examined for each kidney section for necrosis and fibrosis. After examination each section was ranked as follows:

0 = normal kidney
1 = 0-2 fields showing one or more necrotic proximal tubules
2 = 2-4 fields showing one or more necrotic proximal tubules
3 = 4-6 fields showing one or more necrotic proximal tubules
4 = 8-12 fields showing one or more necrotic proximal tubules
5 = 12-20 fields showing one or more necrotic proximal tubules and necrotic glomorulus
6 = diffuse necrosis with fibrosis.

4.2.9 Mercury Solubility Studies

The solubility of mercury from soil and sediment samples was determined by adding 10 g of soil to 160 mL of deionized water, benzene, or concentrated nitric acid. This mixture was agitated for 24 hr, and an aliquot of the liquid phase was removed and filtered through a 0.45-μ filter. The mercury level was then determined on the filtrate. In studies using benzene, samples were agitated for 24 hr, then centrifuged at 21,000 rpm for 90 min, and an aliquot of supernatant was removed and the Hg determined. The Hg levels in these samples were determined using the method of addition (i.e., using methylmercury, mercury chloride, and elemental mercury to determine the accuracy of these extraction methods). It is noted that the above species of mercury were all soluble in nitric acid, whereas elemental mercury was sparingly soluble in benzene and deionized water (i.e., 20 μg/L in deionized water and 4 μg/L in benzene), and mercuric chloride was soluble in deionized water and sparingly soluble in benzene (i.e., 6 μg/L).

The factors associated with the different species of mercury in soil samples from East Fork Poplar Creek can be both biological and chemical. For example, chemical and bacterial systems have been identified that convert inorganic mercury Hg^{2+} to elemental mercury Hg^{o}, bacterial systems that convert Hg^{2+} to methylmercury, and systems that convert Hg^{2+} to mercuric sulfide HgS. Methylmercury, elemental Hg, and mercuric sulfide have been identified in soil from East Fork Poplar Creek. The importance of the solubility of the various species of mercury for these feeding studies is discussed in detail in Section 5.1.

4.2.10 EPA EP Toxicity Studies

Soil and sediments obtained from the sites in Table 1 were mixed and weighed. A 100-g sample was placed into a 3-L flask and 1.6 L of deionized water added. This mixture was agitated for 30 min and the pH determined. The pH was adjusted to 5.0 by the addition of 0.5 N acetic acid. During the first 8 hr, the pH was adjusted (if required) at half-hour intervals. Thereafter, pH adjustment occurred every 6 hr. The total incubation period was 24 hr, and the amount of acid added during this period did not exceed 4 mL/g of soil. During the incubation period, samples were agitated and the temperature maintained at 25°C (±5).

After incubation, deionized water was added to the sample using the equation outlined by EPA (i.e., V = (20)W - 14(W) - A (A = mL of acetic acid added). After adjusting the volume, a 100-mL aliquot was

filtered through a 0.45-μ filter and the filtrate analyzed for mercury, lead, cadmium, and arsenic.

4.2.11 Soil and Tissue Digestion

Soil and sediment samples were digested as described by Feldman (47). Briefly, a 1-g sample was added to a 250-mL volumetric flask, and 5 mL of (ultrapure) H_2SO_4 plus 10 mL of (ultrapure) HNO_3 were added. A condenser (wrapped with asbestos tape and further insulated with teflon tape) was placed on top of the flask, and the flask was placed on a hotplate set at 350°C. The sample was heated until all the HNO_3 evaporated (usually 4 hr). A 10-mL aliquot of $HClO_4$ was added to the flask and the temperature increased to 450°C. The sample was digested for 15 hr (or until the $HClO_4$ evaporated) and then allowed to cool before rinsing the condenser with distilled water. After the digestion volume was adjusted to 250 mL with distilled H_2O, the above metals were analyzed.

A 1-g sample of liver, kidney, or brain was lyophilized to a constant weight. An aliquot of the dried sample (100 to 200 mg) was placed in HNO_3-washed vials and 4 mL of HNO_3 added. The sample was heated at 300°C for 8 hr (or until the tissue was completely digested), diluted to 4 mL with deionized water, and the above metals analyzed after further dilution. For both the soil and tissue samples, aliquots of the above metals were added to the sample to determine the percentage of recovery.

4.2.12 Chemical Analysis of Trace and Bulk Elements

The following elements were analyzed using the following methods. Ca^{2+}, AA flame; Zn^{2+}, AA flame; Pb^{2+}, Cd^{2+}, and Se^{2+}, AA graphite furnace; Hg^{2+} and hydride, cold vapor; and As^{2+}, U, ICP.

4.2.13 Urine Analysis

Urine was analyzed for urinary protein, urinary gamma, and creatinine. Protein was measured according to the method of Lowry et al. (48), using bovine serum albumin as standard. Suitable dilutions of albumin and samples were reacted with Folin-Ciocalteau reagent in the presence of alkaline mixtures of sodium carbonate and citrate. The resulting blue color, proportional to the amounts of protein, was read for absorbance at 750 mu, against a similarly treated water blank. Gamma GT was measured in urine by the kinetic procedure described in Sigma Applications Note 418. In this procedure, the enzyme converts L-y-glutamyl-p-nitroanilide to p-nitroaniline in the presence of glycylglycine. The change in optical density at 405 mu, after exactly 5 min of incubation of sample with reagent mixture, is proportional to the enzyme activity. Each assay run was standardized by the inclusion of Acutrol chemistry controls (Sigma) containing accurately known levels of normal or elevated Gamma GT activities.

Creatinine was measured in urine samples using Sigma Diagnostic Kit, No. 555. Samples of urine and of accurately determined standard solutions of creatinine were reacted with picric acid solution to form

a yellow-orange color whose absorbance was measured at 500 mu. Addition of acetic acid solution resulted in a loss of color intensity whose absorbance was measured again at 500 mu after 5 min. The change in optical density was proportional to the creatinine concentration.

5. RESULTS AND DISCUSSION

5.1 SOIL STUDIES

It has been reported that children may eat up to 1 g of soil per day during normal activity (49). Also, in the southeastern United States, there are adults who reportedly eat various quantities of soil per day. Thus, a human health risk may exist in persons ingesting soil with known toxic pollutants. However, human ingestion of soil is not considered an important pathway for human exposure to toxic pollutants. Nevertheless, it is a pathway that requires further examination, as discussed below.

The environmental release of and human exposure to toxic pollutants are regulated by state and federal government agencies because of their potentially harmful effect(s) on environmental and mammalian systems (i.e., humans). These effects have been established under defined experimental conditions which include determining the dose-response relationships for specific chemicals in a simple matrix. However, under normal circumstances humans are exposed to these toxic chemicals in a complex matrix (i.e., foods and/or liquids) which may contain several toxic pollutants. Determining the dose-response relationship for these chemicals in a complex matrix would seem to be a realistic approach in establishing limits for human intake. The objective of the present studies was to determine experimentally the dose-response relationship of toxic inorganic pollutants in soil and sediment (i.e., a complex matrix) on various organ systems. This objective was accomplished by feeding to mice, via the diet, soil or sediment containing a variety of toxic inorganics.

The soil and sediment used in these studies were obtained from an area previously contaminated with arsenic, lead, cadmium, and mercury. These metals were released into a creek, resulting in contamination of the creek basin and the associated floodplains. Samples of soil and sediment were taken from the floodplains and creek basin along this contaminated area. The sites were selected randomly but included areas with relatively high levels of the above-mentioned inorganics. The sites selected are described in Table 1.

Five 30-cm cores were taken from the sites, and sections (10 cm) from the cores were mixed and lyophilized to a constant weight. After acid digestion, aliquots of the dried soil were analyzed for lead, mercury, cadmium, arsenic, selenium, and zinc. The top 10 cm was used in the feeding studies because (1) it represents the approximate depth at which these inorganics may exchange with water, (2) it reasonably represents the depth to which soil-eaters and playing children would be exposed, and (3) the highest level of the above inorganics was observed at this depth. The concentrations of these metals from the various sites are shown in Tables 2 through 7.

Significant site-to-site variations were observed for all the metals studied, but the greatest variation was observed for Hg. Significant variations for this metal were also observed between the

TABLE 2
The Concentration of Selenium in Soil
from East Fork Poplar Creek and Bear Creek

Site	Site number		Selenium mean ± SD (ppm)
Guard-house	1	Left	129.57 ± 39
	2	Middle	190.99 ± 18
	3	Right	197.05 ± 50
NOAA	4	Left	271.51 ± 40
	5	Middle	231.15 ± 38
	6	Right	100.71 ± 27
K-Mart	7	Left	191.47 ± 39
	8	Middle	162.55 ± 53
	9	Right	128.98 ± 16
Bruners	10	Left	190.52 ± 45
	11	Middle	124.68 ± 38
	12	Right	209.11 ± 25
Sewage Plant	13	Left	126.16 ± 18
	14	Middle	59.86 ± 12
	15	Right	69.81 ± 16
East Fork Turnpike Building	16	Left	168.98 ± 13
	17	Middle	90.09 ± 19
	18	Right	142.95 ± 40
Poplar Creek Perimeter Road	19	Left	99.10 ± 16
	20	Middle	73.00 ± 20
	21	Right	93.61 ± 17
Bear Creek	22	Left	111.25 ± 13
	23	Middle	101.29 ± 16
	24	Right	99.27 ± 17
Bear Creek	25	Left	100.69 ± 19
	26	Middle	107.74 ± 13
	27	Right	195.09 ± 14
Bear Creek	28	Left	127.04 ± 16
	29	Middle	101.78 ± 20
	30	Right	122.52 ± 23

TABLE 3
The Concentration of Zinc in Soil
from East Fork Poplar Creek and Bear Creek

Site	Site number		Zinc mean ± SD (ppm)
Guard-house	1	Left	51.40 ± 5
	2	Middle	97.05 ± 8
	3	Right	248.53 ± 23
NOAA	4	Left	294.12 ± 36
	5	Middle	213.78 ± 40
	6	Right	146.48 ± 25
K-Mart	7	Left	91.58 ± 10
	8	Middle	50.93 ± 7
	9	Right	83.83 ± 8
Bruners	10	Left	242.07 ± 20
	11	Middle	78.09 ± 10
	12	Right	190.71 ± 13
Sewage Plant	13	Left	86.97 ± 7
	14	Middle	59.68 ± 8
	15	Right	141.61 ± 24
East Fork Turnpike Building	16	Left	109.83 ± 13
	17	Middle	58.16 ± 4
	18	Right	90.50 ± 8
Poplar Creek Perimeter Road	19	Left	85.66 ± 10
	20	Middle	86.26 ± 5
	21	Right	73.34 ± 11
Bear Creek	22	Left	52.95 ± 6
	23	Middle	59.85 ± 4
	24	Right	86.64 ± 9
Bear Creek	25	Left	68.30 ± 11
	26	Middle	55.44 ± 8
	27	Right	56.46 ± 10
Bear Creek	28	Left	64.20 ± 7
	29	Middle	38.79 ± 5
	30	Right	66.73 ± 5

TABLE 4
The Concentration of Lead in Soil
from East Fork Poplar Creek and Bear Creek

Site	Site number		Lead mean ± SD (ppm)
Guard-house	1	Left	5.65 ± 1.3
	2	Middle	9.85 ± 1.7
	3	Right	10.67 ± 1.3
NOAA	4	Left	17.29 ± 2.1
	5	Middle	12.48 ± 1.3
	6	Right	12.92 ± 2.0
K-Mart	7	Left	5.61 ± 2.1
	8	Middle	7.46 ± 0.9
	9	Right	7.63 ± 1.1
Bruners	10	Left	17.41 ± 3.2
	11	Middle	7.61 ± 1.2
	12	Right	9.57 ± 0.6
Sewage Plant	13	Left	22.17 ± 1.8
	14	Middle	5.84 ± 0.8
	15	Right	11.29 ± 1.0
East Fork Turnpike Building	16	Left	6.55 ± 0.8
	17	Middle	3.86 ± 0.9
	18	Right	5.67 ± 0.7
Poplar Creek Perimeter Road	19	Left	3.95 ± 0.3
	20	Middle	5.58 ± 0.6
	21	Right	5.82 ± 0.9
Bear Creek	22	Left	2.41 ± 0.15
	23	Middle	6.26 ± 0.36
	24	Right	7.04 ± 0.83
Bear Creek	25	Left	5.07 ± 0.22
	26	Middle	3.78 ± 0.74
	27	Right	5.37 ± 0.63
Bear Creek	28	Left	4.43 ± 0.74
	29	Middle	4.23 ± 0.83
	30	Right	4.48 ± 0.63

TABLE 5
The Concentration of Arsenic in Soil
from East Fork Poplar Creek and Bear Creek

Site	Site number		Arsenic mean ± SD (ppm)
Guard-house	1	Left	.59 ± 0.13
	2	Middle	.73 ± 0.06
	3	Right	5.49 ± 0.52
NOAA	4	Left	5.69 ± 0.74
	5	Middle	3.18 ± 0.20
	6	Right	4.32 ± 1.0
K-Mart	7	Left	.58 ± 0.06
	8	Middle	1.32 ± 0.16
	9	Right	2.57 ± 0.38
Bruners	10	Left	6.10 ± 0.27
	11	Middle	3.40 ± 0.68
	12	Right	6.93 ± 0.19
Sewage Plant	13	Left	4.64 ± 0.77
	14	Middle	2.63 ± 0.69
	15	Right	4.17 ± 0.90
East Fork Turnpike Building	16	Left	2.83 ± 0.30
	17	Middle	.57 ± 0.09
	18	Right	2.11 ± 0.11
Poplar Creek Perimeter Road	19	Left	1.79 ± 0.15
	20	Middle	.75 ± 0.06
	21	Right	3.88 ± 0.29
Bear Creek	22	Left	1.32 ± 0.46
	23	Middle	1.90 ± 0.88
	24	Right	2.20 ± 0.71
Bear Creek	25	Left	7.08 ± 0.93
	26	Middle	4.38 ± 0.76
	27	Right	2.37 ± 0.29
Bear Creek	28	Left	4.34 ± 0.85
	29	Middle	2.53 ± 0.46
	30	Right	2.77 ± 0.53

TABLE 6
The Concentration of Cadmium in Soil
from East Fork Poplar Creek and Bear Creek

Site	Site number		Cadmium mean ± SD (ppm)
Guard-house	1	Left	.45 ± 0.03
	2	Middle	12.4 ± 1.2
	3	Right	8.17 ± 1.1
NOAA	4	Left	92.9 ± 18
	5	Middle	2.67 ± 0.63
	6	Right	12.8 ± 1.9
K-Mart	7	Left	26.8 ± 3.7
	8	Middle	6.1 ± 0.63
	9	Right	9.5 ± 1.1
Bruners	10	Left	11.5 ± 2.1
	11	Middle	8.9 ± 0.48
	12	Right	13.2 ± 0.99
Sewage Plant	13	Left	26.1 ± 1.9
	14	Middle	3.3 ± 0.65
	15	Right	35.5 ± 3.9
East Fork Turnpike Building	16	Left	10.7 ± 1.8
	17	Middle	12.2 ± 0.9
	18	Right	9.8 ± 1.6
Poplar Creek Perimeter Road	19	Left	7.3 ± 0.8
	20	Middle	13.6 ± 1.9
	21	Right	7.6 ± 0.8
Bear Creek	22	Left	11.2 ± 2.3
	23	Middle	16.2 ± 1.7
	24	Right	12.3 ± 2.1
Bear Creek	25	Left	1.8 ± 0.7
	26	Middle	5.5 ± 0.9
	27	Right	3.8 ± 0.4
Bear Creek	28	Left	1.13 ± 0.3
	29	Middle	9.5 ± 1.3
	30	Right	1.11 ± 0.2

TABLE 7

The Concentration of Mercury* in Soil from East Fork Poplar Creek and Bear Creek

Site	Site number		Mercury mean ± SD (ppm)	
Guard-house	1	Left	1.97 ± 0.6	
	2	Middle	12.19 ± 1.3	(2000)**
	3	Right	507.55 ± 40.1	
NOAA	4	Left	974.83 ± 63.6	
	5	Middle	49.03 ± 5.9	
	6	Right	97.57 ± 8.6	
K-Mart	7	Left	61.06 ± 7.1	
	8	Middle	16.16 ± 2.3	
	9	Right	48.50 ± 1.7	
Bruners	10	Left	1799.36 ± 88.9	
	11	Middle	17.91 ± 3.2	
	12	Right	987.94 ± 70.6	
Sewage Plant	13	Left	43.52 ± 5.1	
	14	Middle	33.18 ± 5.4	(1000)**
	15	Right	220.85 ± 33.6	
East Fork Turnpike Building	16	Left	78.84 ± 7.9	
	17	Middle	1.33 ± 0.5	
	18	Right	65.43 ± 4.1	
Poplar Creek Perimeter Road	19	Left	22.79 ± 3.6	
	20	Middle	12.51 ± 1.3	
	21	Right	6.13 ± 0.6	
Bear Creek	22	Left	11.23 ± 1.1	
	23	Middle	3.47 ± 0.6	
	24	Right	34.62 ± 2.1	
Bear Creek Mouth	25	Left	6.61 ± 1.3	
	26	Middle	.77 ± 0.06	
	27	Right	2.64 ± 0.93	(500)**
~~Bear~~ Poplar Creek	28	Left	.59 ± 0.09	
	29	Middle	.72 ± 0.10	
	30	Right	13.07 ± 1.7	

* Normal soil levels 3 ppm.

**Mercuric chloride was added to soil samples to levels indicated in parenthesis (ppm).

floodplains and creek basin and from site to site. Contamination of the creek basin for all the metals analyzed was significantly less than that observed for the floodplains. Thus, the major heavy-metal contamination was observed in the floodplains.

The intestinal absorption of significant amounts of the above metals from ingested soil would occur only if these metals are soluble in the digestive fluid. Studies were thus performed with the contaminated soil to determine its solubility in various media and also because the different forms of inorganic Hg show various solubilities in water. Furthermore, the different species of Hg (inorganic, organic, and elemental) vary considerably in solubility in liquid media. For example, in contrast to inorganic forms, organic mercurials are completely soluble in organic media (i.e., benzene), and elemental Hg is completely soluble in nitric acid and sparingly soluble in water (i.e., 20 µg/L). As shown in Tables 8 and 9, the solubility of soil and sediment Hg in water and in benzene was less than 1%. In contrast, the solubility of soil and sediment Hg in nitric acid was much greater (Table 10), ranging from 11 to 100%.

These results suggest that the predominant species of Hg in the selected soil and sediment samples are the elemental and inorganic forms. Significant concentrations of organic mercurials do not appear to be present in the soil and sediment samples. The fact that Hg was not completely soluble in nitric acid from some of the samples may suggest that different inorganic compounds of Hg (such as HgS) exist in these samples. This form is sparingly soluble in nitric acid. Based on these data, we suggest that the intestinal absorption of Hg from the ingested soil and sediment will depend on (1) the binding coefficient of Hg to soil constituents, (2) the relative concentration of the different species of Hg, and (3) the relative concentration of various inorganic forms of Hg in these samples.

In support of the solubility studies are results for the EPA-recommended EP toxicity test. This test is a measure of the solubility and, thus, bioavailability of various pollutants in solid media such as soil. The results of these studies (Table 11) show that the EPA acceptable Hg limit of 200 µg/L was never achieved using this soil. These data suggest, as previously mentioned, that the Hg in soil is not readily bioavailable. Although 23 of the 30 sites had soil Hg levels above normal (3 ppm); only 5 sites had water solubility levels greater than 10 µg/L, which is 20 times below the acceptable limit. It is noted that the soil and sediment Hg solubility studies described above were performed using methods similar to the EP toxicity test, except for the use of three solvents (water, benzene, and concentrated nitric acid). As shown above, the solubility of soil Hg was more apparent in concentrated nitric acid than in the 0.5 N acetic acid, which would be anticipated.

5.2 SOIL FEEDING STUDIES

To determine the long-term toxic effects of contaminated soil and sediment on various organ systems, mice were fed a diet containing 5% soil or sediment for 20 months. At 6, 12, and 20 months of exposure, various tests were performed to determine the toxicity of these inorganic contaminants. A total of 2400 male and female mice were divided into 30 groups (40 males and 40 females) and exposed to the soil and sediment collected from the 30 sites shown in Table 1.

H_2O

TABLE 8
The Solubility of Mercury from Soil and Sediment*

Site	Site number		Mercury ppm in soil	Mercury ppm soluble in water	Percent of total mercury soluble in water
Guard-house	1	Left	1.97 ± 0.6	0 ±	0
	2	Middle	12.19 ± 1.3	0.054 ± 0.016	.44
	3	Right	507.55 ± 40.1	0.973 ± 0.035	.19
NOAA	4	Left	974.83 ± 63.6	0.919 ± 0.026	.09
	5	Middle	49.03 ± 5.9	0.031 ± 0.009	.06
	6	Right	97.57 ± 8.6	0.293 ± 0.035	.30
K-Mart	7	Left	61.06 ± 7.1	0.027 ± 0.009	.04
	8	Middle	16.16 ± 2.3	0.190 ± 0.016	.01
	9	Right	48.50 ± 1.7	0.050 ± 0.009	.10
Bruners	10	Left	1799.36 ± 88.9	0.252 ± 0.025	.01
	11	Middle	17.91 ± 3.2	0.035 ± 0.007	.01
	12	Right	987.94 ± 70.6	0.392 ± 0.014	.03
Sewage Plant	13	Left	43.52 ± 5.1	0.032 ± 0.004	.07
	14	Middle	33.18 ± 5.4	0.146 ± 0.008	.004
	15	Right	220.85 ± 33.6	0.288 ± 0.017	.13
East Fork Turnpike Building	16	Left	78.84 ± 7.9	0.008 ± 0	.01
	17	Middle	13.33 ± 0.5	0 ± 0	0
	18	Right	65.43 ± 4.1	0.012 ± 0	.01
Poplar Creek Perimeter Road	19	Left	22.79 ± 3.6	0.636 ± 0.047	.03
	20	Middle	12.51 ± 1.3	0.392 ± 0.085	.03
	21	Right	6.13 ± 0.6	0.130 ± 0.011	.04
Bear Creek	22	Left	11.23 ± 1.1	0.098 ± 0.006	.009
	23	Middle	13.47 ± 0.6	0.136 ± 0.015	0
	24	Right	34.62 ± 2.1	0.463 ± 0.080	0
Bear Creek	25	Left	6.61 ± 1.3	0.007 ± 0	.005
	26	Middle	.77 ± 0.06	0 ± 0	0
	27	Right	2.64 ± 0.93	0.13 ± 0.003	.05
Bear Creek	28	Left	.59 ± 0.09	0 ± 0	0
	29	Middle	.72 ± 0.10	0 ± 0	0
	30	Right	1.3 ± 0.7	0.003 ± 0.008	.25

MEDIAN POPLAR CREEK .03

BEAR CREEK

*Soil samples obtained from East Fork Poplar Creek and Bear Creek were extracted into distilled water overnight, filtered, and mercury determined by the cold vapor technique. Mean ± SD for triplicate analysis.

Organics = BENZENE

TABLE 9

The Solubility of Mercury from Soil and Sediment*

ite	Site number		Mercury ppm in soil	Mercury ppm soluble in organics	Percent of tot.. mercury soluble in organics
uard-	1	Left	1.97 ± 0.6	0	0
.ouse	2	Middle	12.19 ± 1.3	0.031 ±	.25
	3	Right	507.55 ± 40.1	0.280 ±	.05
DAA	4	Left	974.83 ± 63.6	0.225 ±	.02
	5	Middle	49.03 ± 5.9	0.066 ±	.13
	6	Right	97.57 ± 8.6	0.056 ±	.05
∩-Mart	7	Left	61.06 ± 7.1	0.097 ±	.15
	8	Middle	16.16 ± 2.3	0	0
	9	Right	48.50 ± 1.7	0.042 ±	.08
Bruners	10	Left	1799.36 ± 88.9	0.314 ±	.01
	11	Middle	17.91 ± 3.2	0	0
	12	Right	987.94 ± 70.6	0.479 ±	.04
ewage	13	Left	43.52 ± 5.1	0.031 ±	.07
lant	14	Middle	33.18 ± 5.4		0
	15	Right	220.85 ± 33.6	0.039 ±	.01
ast Fork	16	Left	78.84 ± 7.9	0.027 ±	.03
urnpike	17	Middle	13.33 ± 0.5	0 ±	0
Building	18	Right	65.43 ± 4.1	0.025 ±	.03
oplar					
reek	19	Left	22.79 ± 3.6	0 ±	0
erimeter	20	Middle	12.51 ± 1.3	0.012 ±	.09
oad	21	Right	6.13 ± 0.6	0 ±	0
ear	22	Left	11.23 ± 1.1	0 ±	0
reek	23	Middle	13.47 ± 0.6	0 ±	0
	24	Right	34.62 ± 2.1	0 ±	0
ear	25	Left	6.61 ± 1.3	0 ±	0
reek	26	Middle	.77 ± 0.06	0 ±	0
	27	Right	2.64 ± 0.93	0 ±	0
ear	28	Left	.59 ± 0.09	0 ±	0
reek	29	Middle	.72 ± 0.10	0 ±	0
	30	Right	1.3 ± 0.7	0 ±	0

Soil samples obtained from East Fork Poplar Creek and Bear Creek were extracted overnight with benzene, filtered, and mercury determined by the cold vapor technique. Mean ± SD of triplicate analysis.

CONCENTRATED HNO_3

TABLE 10

The Solubility of Mercury from Soil and Sediment*

te	Site number		Mercury ppm in soil	Mercury ppm soluble in HNO_3	Percent of total mercury soluble in HNO_3
ard-	1	Left	1.97 ± 0.6	1.81 ± 0.19	91.88
use	2	Middle	12.19 ± 1.3	2.71 ± 0.63	22.23
	3	Right	507.55 ± 40.1	326.66 ± 38	64.36
AA	4	Left	974.83 ± 63.6	253.81 ± 45	26.04
	5	Middle	49.03 ± 5.9	36.17 ± 7	73.77
	6	Right	97.57 ± 8.6	33.29 ± 3	34.12
-Mart	7	Left	61.06 ± 7.1	14.25 ± 2	23.34
	8	Middle	16.16 ± 2.3	4.78 ± 0.6	29.58
	9	Right	48.50 ± 1.7	23.93 ± 1.1	49.34
Bruners	10	Left	1799.36 ± 88.9	528.34 ± 69	29.36
	11	Middle	17.91 ± 3.2	11.48 ± 1.3	64.10
	12	Right	987.94 ± 70.6	118.42 ± 12	11.99
wage	13	Left	43.52 ± 5.1	16.47 ± 0.88	37.84
ant	14	Middle	33.18 ± 5.4	8.71 ± 1.21	26.25
	15	Right	220.85 ± 33.6	44.21 ± 3.66	20.02
st Fork	16	Left	78.84 ± 7.9	37.51 ± 4.0	47.58
rnpike	17	Middle	13.33 ± 0.5	4.5 ± 0.13	33.83
uilding	18	Right	65.43 ± 4.1	41.08 ± 4.8	62.78
plar					
reek	19	Left	22.79 ± 3.6	13.05 ± 0.69	57.26
rimeter	20	Middle	12.51 ± 1.3	8.30 ± 1.20	66.35
ad	21	Right	6.13 ± 0.6	2.30 ± 0.11	37.52
ear	22	Left	11.23 ± 1.1	12.12 ± 1.3	100+
eek	23	Middle	13.47 ± 0.6	1.98 ± 0.4	57.06
	24	Right	34.62 ± 2.1	30.59 ± 1.9	88.36
ar	25	Left	6.61 ± 1.3	3.42 ± 0.49	51.74
eek	26	Middle	.77 ± 0.06	.32 ± 0.11	41.56
	27	Right	2.64 ± 0.93	2.12 ± 0.35	80.30
ar	28	Left	.59 ± 0.09	.39 ± 0.14	66.10
eek	29	Middle	.72 ± 0.10	.52 ± 0.35	72.22
	30	Right	1.3 ± 1.7	0.81 ± 1.0	62.28

oil samples obtained from East Fork Poplar Creek and Bear Creek were extracted into concentrated nitric acid, filtered, and mercury determined by the cold vapor technique. Mean ± SD of triplicate analysis of soil.

TABLE 11
EP Toxicity Test for Mercury (RCRA MCL 200 µg/L)

Site	Soil and sediment mg/kg	EP toxicity test µg/L
1	1.97 ± 0.6	2 ± 1.5*
2	12.19 ± 1.3	3 ± 1.8*
3	507.55 ± 40.1	17 ± 2.6
4	974.55 ± 63.6	18 ± 3.4
5	49.03 ± 5.9	10 ± 1.3
6	97.57 ± 8.6	8 ± 1.8
7	61.06 ± 7.1	9 ± 0.9
8	16.16 ± 2.3	4 ± 1.1*
9	48.50 ± 1.7	8 ± 0.6
10	1799.36 ± 88.9	33 ± 5.2
11	17.91 ± 3.2	5 ± 0.6
12	987.94 ± 70.6	23 ± 4
13	43.52 ± 5.1	7 ± 2
14	33.18 ± 5.4	3 ± 1.6*
15	220.85 ± 33.6	13 ± 0.8
16	78.84 ± 7.9	9 ± 1.4
17	13.33 ± 0.5	3 ± 1.4*
18	65.43 ± 4.1	5 ± 1.0
19	22.79 ± 3.6	7 ± 1.9
20	12.51 ± 1.3	7 ± 2.3
21	6.13 ± 0.6	4 ± 1.6
22	11.23 ± 1.1	7 ± 1.3
23	3.47 ± 0.6	6 ± 0.9
24	34.62 ± 2.1	9 ± 2
25	6.61 ± 1.3	0
26	.77 ± 0.06	0
27	2.64 ± 0.93	3 ± 2*
28	.59 ± 0.09	0
29	.72 ± 0.10	0
30	1.3 ± 1.7	0

*Values obtained at the limit of detection.

Soil and sediment from the respective sites were added to the feed, mixed, and made into feed pellets. From each diet, samples of the pellets were digested and Hg concentrations determined. The results in Table 12 outline the Hg recovery following the addition of Hg-contaminated soil and sediment to the diet. The Hg recovery ranged from 73 to 127%. In only two samples was the recovery less than 85%, and in these samples recoveries of 73 and 81% (sites 21 and 25, respectively) were observed. These differences of 15% are within the experimental error in these studies. It is noted that, with some samples, recoveries were greater than 100%. The increased recovery (0 to 27%) is assumed to be associated with experimental error.

To determine the absorption rate for Hg in soil and sediment samples, mice were exposed to the experimental diets for 24 hr. After exposure, fecal samples were collected for 96 hr and Hg determined. The results (Table 13) show that the absorption of Hg from soil and sediment samples varied between 5 and 16%. The factors associated with this variation may be (1) differences in the relative amounts of the various species of Hg, (2) differences in the relative amounts of the various forms of inorganic Hg, and (3) the binding coefficient of Hg for soil particulates. It is difficult to speculate at this time which factor is responsible for the observed variation. However, it is known that the soluble forms of inorganic Hg are absorbed in the intestinal tract of mice at rates of 1.5 to 2.0% (50).

Several tests were used to determine the toxicity of the contaminated soil and sediment. These included the tissue level for the various inorganics, body weights over the experimental period, kidney function tests, morphology of the kidney, and the swim test to determine neurological effects. The raw data and means (± SD) are shown in the appendix. The data from these studies (summarized in the tables and figures) were analyzed using SAS ANOVA on an IBM AT. Results from the data are summarized below.

5.2.1 Body Weights and Mortality

In chronic toxicity studies, body weight changes are frequently used to assess toxicity of ingested compounds. Body weights were measured at 0, 6, 12, and 20 months for both females and males in the 30 experimental groups. In all groups, body weight increased, on average, by 17.5 g in males and 16.3 g in females after a 6-month exposure to the experimental diet. After 6 to 12 month's exposure, the increases were 2.3 and 3.3 g for males and females, respectively. From 12 to 20 months, body weights remained constant for males and increased by 2 g for females (results shown in the appendix). These observed changes in body weight of the Swiss mouse would be anticipated based on previous studies by this investigator and reports in the literature.

Analyses of variance for body weights did not show significant differences between the 30 groups at the various time intervals. This suggests that the experimental diets did not significantly affect growth and, thus, may not be toxic to the exposed animals. Similar conclusions were reached for the organ weights of the liver and kidney. The toxicity of a compound may also be measured by the number of animals that died following ingestion of that compound. Table 14 shows the total mortality data for the study. Table 15 shows mortality in the 30 groups following the exposure to the diets for 6, 12, and 20 months. Apparent differences in mortality were observed

TABLE 12
Recoverable Mercury in Mouse Feed

Site	Site number		ppm in soil	µg Hg added to 95 g of feed	µg Hg recovered in 100 g of feed	Percent difference
Guard-	1	Left	1.97	9.9	10.6 ± 1.3	+ 7
house	2	Middle	2000	10,000	9,999 ± 8	- 1
	3	Right	507.55	2,538	2,502 ± 75	- 1
NOAA	4	Left	974.83	4,874	4,183 ± 279	-15
	5	Middle	49.03	245	283 ± 3	+17
	6	Right	97.57	485	425 ± 7	-12
-Mart	7	Left	61.06	305	315 ± 6	+ 3
	8	Middle	16.16	81	83 ± 4	+ 2
	9	Right	48.50	245	263 ± 5	+ 7
runers	10	Left	1799.36	8,997	9,315 ± 100	+ 4
	11	Middle	17.91	89	74 ± 8	+17
	12	Right	987.94	4,940	5,213 ± 10	- 6
ewage	13	Left	43.52	220	210 ± 3	- 5
Plant	14	Middle	1000	166	166 ± 9	0
_	15	Right	220.85	1,104	1,004 ± 18	- 9
ast Fork	16	Left	78.84	394	349 ± 10	-11
Turnpike	17	Middle	13.33	65	59 ± 0.6	-10
uilding	18	Right	65.43	325	322 ± 4	- 1
Poplar						
reek	19	Left	22.79	114	116 ± 8	+ 2
erimeter	20	Middle	12.51	65	61 ± 3	- 6
Road	21	Right	6.13	31	25 ± 1.3	-19
ear	22	Left	11.23	58	50 ± 3	-14
reek	23	Middle	3.47	17	15 ± 2	+12
	24	Right	34.62	175	175 ± 9	0
lear	25	Left	6.61	30	22 ± 3	-27
Creek	26	Middle	.77	3.9	4.6 ± 1.0	+18
	27	Right	500	2500	2410 ± 0.7	- 4
ear	28	Left	.59	3.3	3.0 ± 0.4	- 9
Creek	29	Middle	.72	3.6	3.9 ± 0.13	+ 8
	30	Right	1.3	6.5	6.4 ± 5	- 1

TABLE 13
The Gastrointestinal Absorption of Mercury
from Soil or Sediment in Mice

Site	Sex	Total intake per 24 hours: Feed, g**	Soil, g	Hg, µg	Soil conc ppm	Total Hg recovered in feces,* µg**	Percent absorption from GI tract
2	F	3.3 ±0.4	0.165	1.9	12 ✓	1.8 ±0.2	5
	M	4.1 ±0.6	0.210	2.5		2.3 ±0.3	8
4	F	2.7 ±0.5	0.135	132	975 ✓	125 ±15	5
	M	3.8 ±0.4	0.190	185		170 ±22	8
8	F	2.9 ±0.1	0.145	2.32	16 ✓	1.95 ±0.4	16
	M	3.4 ±0.8	0.170	2.72		2.39 ±0.8	12
10	F	2.4 ±0.3	0.12	216	1800 ✓	203 ±18	6
	M	4.3 ±0.8	0.220	396		380 ±16	4
15	F	3.1 ±0.5	0.155	34	220 ✓	30 ±1.3	9
	M	3.9 ±0.3	0.196	43		41 ±4.6	5
18	F	2.5 ±0.3	0.125	8.2	65 ✓	2 ±0.8	12
	M	3.9 ±0.9	0.22	13.1		11 ±2.0	16
24	F	2.2 ±0.3	0.110	3.8	34 ✓	3.3 ±0.4	14
	M	4.4 ±0.6	0.220	7.6		7.2 ±0.8	5
30	F	2.8 ±0.6	0.140	1.83	13 ✓	1.60 ±0.22	13
	M	4.0 ±0.9	0.200	2.6		2.2 ±0.3	15
?	F	2.3 ±0.4	0.115	230	2000 ✓	200 ±18	13
	M	3.8 0.1	0.190	380		335 ±40	12

*96 hours post-feeding.
**Mean ± SD for five animals per experimental group.

TABLE 14
Mortality of Mice Exposed to Mercury Contamination

Group	No. mice exposed		No. mice tested		No. mice died		No. mice surviving		No. mice killed because of trauma	
	M	F	M	F	M	F	M	F	M	F
1	40	40	17	22	23	5	0	13	0	0
2	40	40	19	22	12	8	1	10	3	0
3	40	40	22	22	14	8	1	10	3	0
4	40	40	18	22	12	4	0	12	10	2
5	40	40	22	22	16	4	0	11	2	3
6	40	40	22	22	13	5	0	13	5	0
7	40	40	22	22	11	6	0	12	7	0
8	40	40	22	22	15	7	0	11	3	0
9	40	40	19	22	15	3	1	15	5	0
10	40	40	21	22	13	4	1	12	5	2
11	40	40	22	22	13	8	0	10	5	0
12	40	40	22	22	12	3	0	12	7	3
13	40	40	19	22	11	7	0	11	10	0
14	40	40	22	22	12	6	0	12	6	0
15	40	40	20	22	11	4	0	14	9	0
16	40	40	21	22	12	6	1	12	6	0
17	40	40	19	22	12	8	3	10	6	0
18	40	40	18	22	10	5	0	13	12	0
19	40	40	19	22	12	5	0	13	9	0
20	40	40	22	22	6	5	2	13	10	0
21	40	40	19	22	17	5	0	12	4	1
22	40	40	22	22	9	11	3	7	6	0
23	40	40	18	22	15	6	0	12	7	0
24	40	40	16	22	17	3	0	15	7	0
25	40	40	20	22	10	7	0	10	10	1
26	40	40	22	22	7	2	3	16	8	0
27	40	40	18	22	19	6	0	12	3	0
28	40	40	22	22	13	3	0	12	5	3
29	40	40	19	22	15	7	0	11	6	0
30	40	40	22	22	10	8	4	7	4	3
TOTAL	1200	1200	605	660	387	169	19	353	189	18

TABLE 15
Mortality of Mice Continuously Exposed to Diets
Containing Mercury-Contaminated Soil

Group	Intake µg Hg/day		Exposure time							
			6 Mo.		12 Mo.		20 Mo.		Total	
	M	F	M	F	M	F	M	F	M	F
1	0.34	0.27	40/1*	40/0	43/9	35/3	18/13	25/2	40/23	40/5
2	392.0	282.0	40/2	40/0	27/2	35/2	15/8	26/2	40/12	40/8
3	104.0	74.0	40/3	40/0	29/0	35/0	22/11	28/8	40/14	40/8
4	176.0	146.0	40/2	40/0	26/2	33/1	14/8	25/3	40/12	40/4
5	9.3	7.8	40/9	40/1	24/3	32/2	14/4	22/1	40/16	40/4
6	15.0	13.0	40/1	40/0	29/0	35/1	22/12	27/4	40/13	40/5
7	11.9	8.6	40/0	40/0	31/2	35/2	19/9	26/4	40/11	40/6
8	3.1	2.3	40/1	40/1	31/1	34/1	23/13	25/5	40/15	40/7
9	8.7	8.0	40/4	40/0	27/1	35/0	17/10	25/3	40/15	40/3
10	360.0	261.0	40/2	40/0	28/4	33/2	18/7	24/2	40/13	40/4
11	3.5	2.3	40/6	40/2	29/1	33/2	17/6	24/4	40/13	40/8
12	173.0	128.0	40/0	40/0	31/3	35/0	18/9	25/3	40/12	40/3
13	10.4	6.1	40/0	40/1	29/1	34/0	17/10	27/6	40/11	40/7
14	186.0	145.0	40/2	40/0	33/0	35/0	20/10	28/6	40/12	40/6
15	42.0	34.0	40/0	40/0	28/4	35/1	15/7	27/3	40/11	40/4
16	15.0	13.0	40/2	40/3	27/1	33/0	18/9	26/3	40/12	40/6
17	0.24	0.17	40/0	40/1	33/3	34/3	19/9	24/4	40/12	40/8
18	12.0	8.0	40/1	40/1	28/2	34/1	13/7	26/3	40/10	40/5
19	4.2	3.7	40/3	40/0	25/3	35/0	13/6	28/5	40/12	40/5
20	2.6	1.6	40/0	40/0	25/2	35/2	16/4	26/3	40/6	40/5
21	1.2	0.7	40/2	40/0	32/1	34/1	21/14	26/4	40/17	40/5
22	2.3	1.7	40/1	40/1	28/4	34/4	17/4	23/6	40/9	40/11
23	0.68	0.50	40/3	40/0	27/1	32/3	17/11	22/3	40/15	40/6
24	5.2	4.7	40/4	40/1	24/4	34/1	13/9	26/1	40/17	40/3
25	0.99	1.6	40/2	40/1	24/1	33/1	15/7	25/5	40/10	40/7
26	0.14	0.08	40/1	40/0	26/2	35/1	16/4	27/1	40/7	40/2
27	98.0	70.0	40/2	40/0	33/0	35/0	23/17	28/6	40/19	40/6
28	0.11	0.07	40/1	40/0	32/2	33/0	20/10	25/3	40/13	40/3
29	0.13	0.08	40/4	40/1	31/2	34/1	16/9	26/5	40/15	40/7
30	2.6	1.4	40/0	40/1	31/2	32/1	22/8	24/6	40/10	40/8

*No. of exposed animals/No. of animals that died.

between the males and females, but the differences did not appear to be related to the dietary level of toxic metals. High mortality is frequently observed in males housed together because of wounds inflicted by other mice. We therefore suggest that these observed mortality differences were related to wounds inflicted by males on each other. Table 16 shows the number of animals killed during the experimental period because of trauma.

Apparent differences in mortality were observed between the experimental diets for males and females. For example, cumulative mean mortality data (calculated from the data in the appendix) were 5.6 for females and 12.9 for males. One group of females (i.e., 22) and four groups of males (i.e. 1, 21, 24, and 27) exceeded the mean values. Mortality levels in four groups of females (9, 24, 26, and 28) and two groups of males (20 and 26) were lower than the respective means. However, these differences did not appear to be related to Hg or any other toxic metal. Those groups receiving the highest level of Hg showed mortality levels similar to the cumulative mean. Statistical analysis of the data did not show a significant correlation between mortality and Hg intake, which suggests that the ingestion of soil containing Hg at levels approaching 2000 ppm did not affect mortality. Table 17 shows the percent mortality of mice continuously exposed to mercury-contaminated soil.

5.2.2 Organ Distribution of Mercury

Several reports have shown that the critical organs for Hg accumulation are the liver and kidney (51). These studies have also observed that these organs may represent up to 50% of the total body burden following the intestinal absorption of inorganic or elemental mercury (52). The distribution of Hg in the body following exposure to organic mercurials is more evenly distributed throughout these organs (53, 54). Thus, the liver and kidney may not represent 50% of the total body burden in animals exposed to the organic mercurials. We would anticipate in the present studies that, if Hg in the soil and sediment was primarily in the inorganic and/or elemental forms, liver and kidney Hg should represent 50% of the total body burden. However, if Hg was in the organic form in these samples, we would anticipate a somewhat even distribution between the liver and kidney. As shown in the appendix, the Hg level in the liver and kidney appeared to increase as the concentration of soil Hg increased. In general, the concentration of Hg in the kidney was far in excess of the levels observed in the liver. Furthermore, in general, the Hg in the liver and kidney of males exceeded the levels observed in females.

Data in the appendix also show that the ratio of liver to kidney Hg was similar to reports showing this ratio in animals exposed to inorganic Hg. This ratio shows great variation between sex and exposure levels. This ratio was determined because several investigators have observed, in animals exposed to inorganic Hg, a liver-to-kidney ratio of 0.01 to 0.21 (wet weight basis) (32, 55). In contrast, the ratio observed in animals exposed to methylmercury ranged from 0.6 to 0.9 (wet weight basis). As shown in the appendix, this ratio on a wet weight basis following a 6-month exposure ranged from 0.06 to 0.34. However, there were exceptions: group 5 (0.89, F; 0.48, M), group 23 (0.91, F; 0.41, M), and group 24 (0.31, F; 0.41, M). This high ratio in females from groups 5 and 23 may require

TABLE 16
Animals Killed During the Experimental Period Because of Trauma*

Group	Exposure time, months			
	0 to 6		6 to 12	
	M	F	M	F
1	0	0	0	0
2	0	0	3	0
3	3	0	0	0
4	7	2	3	0
5	2	2	0	1
6	5	0	0	0
7	4	0	3	0
8	3	0	0	0
9	5	0	0	0
10	5	2	0	0
11	1	0	4	0
12	4	0	3	3
13	6	0	4	0
14	6	0	0	0
15	7	0	2	0
16	6	0	0	0
17	2	0	4	0
18	6	0	6	0
19	7	0	2	0
20	10	0	0	0
21	1	1	3	0
22	6	0	0	0
23	5	0	2	0
24	7	0	0	0
25	9	1	1	0
26	8	0	0	0
27	0	0	3	0
28	2	2	3	1
29	0	0	6	0
30	4	3	0	0

*Trauma inflicted by other mice within the cage.

TABLE 17
Percent Mortality in Mice Exposed to
Mercury-Contaminated Soil

	Exposure period in months							
	6		12		20		Total	
roup	M	F	M	F	M	F	M	F
1	3	0	26	9	72	8	58	13
2	5	0	7	6	53	23	30	30
3	8	0	0	0	50	29	35	20
4	5	0	8	3	57	12	30	10
5	23	3	13	6	29	5	40	10
6	3	0	0	3	55	15	33	13
7	0	0	7	6	47	15	28	15
8	3	3	3	3	57	20	38	18
9	10	0	4	0	59	12	38	8
10	5	0	14	6	39	8	33	10
11	15	5	4	6	35	17	32	20
12	0	0	10	0	50	12	30	8
13	0	3	4	0	59	22	28	18
14	5	0	0	0	50	21	30	15
15	0	0	14	3	47	11	28	10
16	5	8	4	0	50	12	30	15
17	0	3	9	9	47	17	30	20
18	3	3	7	3	54	12	25	13
19	8	0	12	0	46	18	30	13
20	0	0	8	6	25	12	15	13
21	5	5	3	3	67	15	43	13
22	3	3	14	12	24	26	23	28
23	8	0	4	9	65	14	38	15
24	10	3	17	3	69	4	43	8
25	5	3	4	3	47	20	25	18
26	3	0	8	3	25	4	18	5
27	5	0	0	0	74	21	48	15
28	3	0	6	0	50	12	33	8
29	10	3	6	3	56	19	38	18
30	0	3	6	3	36	25	25	20

additional study to determine the possible factors. Mercury sediment levels in groups 5 and 23 were 49 and 13 ppm, respectively. Extraction studies showed that less than 1% of the total sediment Hg was soluble in water and benzene. Thus, it appears that groups 5 and 23 were exposed to sediment containing primarily inorganic and elemental Hg. Additional studies are in progress to explain this result. It is noted that groups 5 and 23 are the only sediment samples which showed this high ratio.

An important factor in predicting the possible long-term effect of Hg in soil is to determine the dose-response relationship of intake versus liver or kidney Hg. In classical toxicological studies to determine a dose-response relationship, an animal is exposed to a single compound and the level of intake compared to the organ level of that compound. Frequently, these studies provide information necessary for predicting minimal and maximal acceptable doses for specific compounds. Again, these studies are performed using a single compound in a simple matrix (i.e., water). In the present studies, attempts were made to determine the dose-response relationship of soil Hg to liver and kidney Hg. Results showing the dose-response effects (Figs. 3 through 28; p. 59) indicate, in some cases, a significant dose-response relationship. Thus, the exposure dose is correlated with the level of Hg in the liver and kidney. However, it is difficult to determine the minimal and maximal acceptable dose from these data because the dose-response relationships were not linear.

Factors that may be responsible for this observation include (1) the binding coefficient of Hg to soil and sediment, and (2) the various species and inorganic forms of Hg in soil and sediment. For example, the solubility of elemental Hg in water is significantly less than that observed for inorganic Hg. Thus, we would anticipate different absorption rates for the different Hg species. The absorption of inorganic Hg (i.e., $HgCl_2$) in mice has been reported to be 1.5 to 2.0% of the dose, whereas elemental Hg is absorbed at rates of 0.01 to 0.5% (50, 56). Variations in the absorption of mercuric compounds which appear to be associated with the solubilities of the compound have also been reported (57). Mercuric chloride and nitrate are absorbed at significantly higher rates than mercuric sulfide and oxides. To determine the effect of the binding coefficient on Hg absorption, $HgCl_2$ was added to soil at levels ranging from 500 to 8000 ppm. Results of these studies are shown in Figs. 15 through 28. In contrast to the data shown in Figs. 3 through 14, a linear dose-response relationship of intake versus kidney Hg was observed in animals exposed to soil containing the $HgCl_2$. This result suggests that possible binding coefficients of Hg to soil may not be responsible for the lack of linearity observed in Figs. 3 through 14. Thus, the species and/or inorganic form of Hg may be the factors explaining the nonlinear response.

5.2.3 Kidney Morphology and Function

The kidney is the critical organ for Hg toxicity. Renal damage and a decrease in kidney function (i.e., proteinuria) have been observed following chronic exposure to inorganic, organic, and elemental Hg. The observed effects include renal tubular (i.e., proximal tubule) and glomerular damage, proteinuria, and renal failure (58). These changes in kidney morphology and function have been observed when the mercury level in the kidney exceeds 10 to 49 µg/g

wet weight (59-62). To determine the effect of Hg in soil on kidney morphology and function, light microscope studies and urinary analyses were performed. Sections of kidney from all the exposed groups were made, stained with H&E, and evaluated for renal tubular and glomeruli damage and kidney fibrosis. Each section was scored according to the criteria outlined in the methods. Kidney function was determined by measuring the level of protein, creatinine, and gamma glutamyl transferase in the urine.

Table 18 describes the histological results. The histological data were ranked into different classes according to the level of damage observed. Results showed that 87% of females and 85% of males exposed for 6 months to the Hg-contaminated soil did not develop significant morphological changes in the kidney. The groups showing morphological changes were those exposed for only 6 months to soil to which $HgCl_2$ was added to levels of 4000 and 8000 ppm. At 12 months of exposure, the kidneys in 94% of the females and 90% of the males appeared relatively normal. Similar results were observed following 20 months of exposure. Results also showed approximately 15% proximal tubule damage occurred when the mean log kidney Hg exceeded values of 7.1 (i.e., class number 3). The exception was observed in groups exposed to 4000 and 8000 ppm $HgCl_2$. In these groups, four females and two males had mean log Hg levels of 8.4 and 9.2, respectively, and approximately 11% proximal tubule damage.

Although proximal tubule damage was occasionally observed in groups exposed to soil containing relatively high levels of Hg, significant correlations between kidney Hg and changes in three urinary constituents were not observed (Table 19). Partial correlation analysis following 12 months of exposure showed that kidney Hg was not significantly associated with urine protein, gamma glutamyl transferase, and creatinine. Thus, the increase in kidney Hg was not significantly paralleled by increases in urinary protein, glutamyl transferase, or creatinine. This suggests that although slight renal damage was observed, kidney function remained normal. These data imply that soil Hg levels as high as 2000 ppm in East Fork Poplar Creek may not be associated with health effects for those persons eating the contaminated soil.

As discussed above, renal tubule damage with a decrease in kidney function has been observed when the Hg level in this organ exceeds 20 to 40 µg/g wet weight. This relationship has been observed in both human and experimental animal studies. In the present studies, slight proximal renal tubular damage was observed even though the level of Hg in the kidney did not exceed 20 µg/g wet weight. However, this damage was not associated with an impairment to kidney function.

5.2.4 Dietary Intake of Soil or Sediment Required to Achieve Toxic Levels of Mercury in the Kidney

We have estimated the dietary intake of Hg-contaminated soil and sediment from the various sites along East Fork Poplar Creek required to achieve 25 µg/g wet weight of kidney in both female and male mice (Tables 20 and 21). This estimate assumes a daily intake at the levels shown for 6 months. These intake amounts were calculated from the level of kidney Hg observed in female and male mice exposed to soil and sediment from East Fork Poplar Creek for 6 months. It was assumed that at 6 months the intake and excretion of Hg had reached a steady state. If this assumption is correct, the level of Hg in the

TABLE 18
Mercury Data Divided into Histology Classes

Histology No.*	Females			Males		
	No. of mice	Mean log Hg	SE	No. of mice	Mean log Hg	SE
Six months						
Kidney						
0	126	4.4	.10	111	4.9	.08
1	22	6.4	.23	34	6.8	.23
2	4	8.4	.60	2	9.2	.40
3	3	9.4	.26	4	10.2	.18
4	13	9.6	.10	10	10.5	.10
5	1	9.8	-	3	10.7	.14
6	1	10.3	-	1	11.1	-
Liver						
0	126	3.9	.08	111	4.0	.07
1	22	5.8	.18	34	5.8	.15
2	4	7.3	.54	8	7.2	.35
3	3	8.3	.54	4	8.5	.15
4	13	8.6	.13	10	9.1	.15
5	1	8.9	-	3	9.0	.19
6	1	9.1	-	1	9.3	-
Twelve months						
Kidney						
0	162	4.4	.08	149	4.9	.07
1	36	6.1	.15	40	6.6	.20
2	12	6.8	.26	12	6.4	.16
Liver						
0	162	4.0	.05	149	4.1	.06
1	35	5.5	.16	40	5.8	.13
2	12	6.4	.16	21	6.3	.16

Table continued on next page.

TABLE 18 (cont'd)
Mercury Data Divided into Histology Classes

Histology No.*	Females			Males		
	No. of mice	Mean log Hg	SE	No. of mice	Mean log Hg	SE
			Twenty months			
Kidney						
0	129	3.8	.08	82	3.8	.11
1	136	4.4	.11	123	4.8	.13
2	26	6.0	.28	34	6.6	.23
3	4	7.1	.42	7	8.1	.18
Liver						
0	129	4.1	.06	83	3.8	.07
1	136	4.4	.08	121	4.3	.08
2	26	5.5	.25	34	5.0	.16
3	4	6.5	.40	7	6.1	.20

* 0 = normal kidney.
1 = 0 to 2 fields showing one or more necrotic proximal tubules.
2 = 2 to 4 fields showing one or more necrotic proximal tubules.
3 = 4 to 6 fields showing one or more necrotic proximal tubules.
4 = 8 to 12 fields showing one or more necrotic proximal tubules.
5 = 12 to 20 fields showing one or more necrotic proximal tubules and necrotic glomorulus.
6 = diffuse necrosis with fibrosis.

TABLE 19
Partial Correlations (and P-values) for 12-Month Data

Correlation of/ With	Kidney Hg	Liver Hg	Urine Protein	Urine -gt	Urine Creatinine
Kidney Hg	1	.08/.27	.03/.70	.00/.99	-.05/.54
Liver Hg		1	-11/14	.08/.26	-166/.03
Urine protein			1	.00/.97	.82/.0001
Urine -gt				1	-/02/.92
Urine creatinine					1

TABLE 20
Estimate of Dietary Intake of Mercury-Contaminated Soil Required by Female Mice to Achieve Kidney Levels of 25 µg Hg/g Wet Weight*

Site conc., ppm)	Soil intake day/kgBW g/kg BW/day	Hg intake day/kgBW mg/kg BW/day	Hg/g wet wt. kidney ng/g	Soil intake** day/kgBW kg	Hg intake** day/kgBW mg
1 (2)	7.8	0.015	77	2.4	4.8
2 (2000)	7.0	14	1822	0.096	192
3 (508)	7.8	4.1	111	1.8	920
4 (975)	8.4	8.2	1144	0.18	179
5 (49)	8.9	0.44	396	0.57	28
6 (98)	6.5	0.65	434	0.38	37
7 (61)	7.8	0.48	230	0.85	52
8 (16)	7.8	0.13	734	0.28	4.4
9 (48)	9.5	0.45	414	0.57	27
10 (1799)	7.0	13	4924	0.037	66
11 (18)	6.5	0.12	334	0.5	8.9
12 (988)	7.0	6.9	5806	0.03	32
13 (44)	7.8	0.34	811	0.24	11
14 (1000)	7.8	8.1	2958	0.068	68
15 (221)	8.4	1.9	489	0.44	97
16 (79)	8.9	0.73	228	0.10	80
17 (13)	6.5	0.085	228	0.72	9
18 (65)	6.7	0.45	119	1.5	95
19 (23)	8.9	0.20	69	3.2	72
20 (13)	6.5	0.08	105	1.5	19
21 (6)	5.7	0.04	67	2.5	15
22 (11)	7.8	0.09	82	2.5	25
23 (13)	7.3	0.03	44	1.3	17
24 (35)	7.8	0.26	204	0.91	32
25 (7)	8.4	0.06	1705	0.13	1.0
26 (.8)	6.2	0.05	75	10	8.0
27 (500)	7.8	3.9	1788	0.11	55
28 (.6)	6.0	0.04	302	5.5	3.3
29 (72)	6.7	0.05	77	2	15
30 (.3)	6.2	0.08	99	36	11

*Results are expressed as mean from mice exposed to mercury-contaminated soil for 6 months.
**Intake required to achieve 25 µg Hg/g wet weight of kidney in the mouse.

TABLE 21
Estimate of Dietary Intake of Mercury-Contaminated Soil Required by Male Mice to Achieve Kidney Levels of 25 µg Hg/g Wet Weight*

Site (conc., ppm)	Soil intake day/kgBW g/kg BW/day	Hg intake day/kgBW mg/kgBW/day	Hg/g wet wt. kidney ng/g	Soil intake** day/kgBW kg	Hg intake** day/kgBW mg
1 (2)	7.6	0.015	137	1.4	2.7
2 (2000)	8.4	17	5003	0.43	85
3 (508)	8.4	4.3	155	1.3	694
4 (975)	8.3	8.1	768	0.27	264
5 (49)	8.7	0.43	468	0.50	23
6 (98)	6.3	0.63	414	0.38	38
7 (61)	8.4	0.51	149	1.4	90
8 (16)	7.9	0.13	548	0.4	6
9 (48)	8.3	0.40	430	0.48	23
10 (1799)	8.4	15	8948	0.039	70
11 (17)	7.8	0.14	263	0.78	13
12 (988)	6.8	6.6	5208	0.032	32
13 (44)	9.3	0.39	598	0.37	16
14 (1000)	7.0	7.3	5181	0.035	35
15 (221)	7.1	1.6	358	0.51	112
16 (79)	7.8	0.59	197	0.95	75
17 (13)	7.0	0.09	316	0.55	7
18 (65)	7.4	0.47	92	2.0	128
19 (23)	7.8	0.17	133	1.4	32
20 (13)	8.0	0.10	264	0.73	9.5
21 (6)	7.2	0.04	67	2.5	14.9
22 (11)	7.2	0.08	189	0.96	11
23 (13)	7.4	0.03	107	0.54	7
24 (35)	5.6	0.19	170	0.79	28
25 (7)	9.1	0.06	1483	0.14	1
26 (.8)	5.9	0.05	153	10	8
27 (500)	8.4	4.1	981	0.21	105
28 (.6)	7.0	0.04	362	4.6	2.8
29 (72)	6.7	0.05	115	0.15	11
30 (.3)	7.2	0.09	207	36	11

*Results are expressed as mean from mice exposed to mercury-contaminated soil for 6 months.
**Intake required to achieve 25 µg Hg/g wet weight of kidney in the mouse.

kidney at 6 months should remain constant if the intake remained the same. The calculations used in Tables 20 and 21 are as follows:

(1) Soil intake in the mouse was determined by measuring the intake of feed containing 5% soil (data shown in the appendix).

Soil intake/body weight x 1000 = soil intake/kg body weight

(2) Mercury intake was determined from the data on soil intake and the analysis of soil for Hg.

Soil intake/kg body weight x soil Hg concentration = Hg intake/kg body weight

(3) Kidney mercury concentrations were determined in the kidneys of mice exposed for 6 months to the diet containing 5% Hg-contaminated soil (data shown in the appendix).

(4) Mercury intake to give a kidney concentration of 25 mg/kg:

25 mg/kg x Hg intake in the mouse (Hg intake/kg body weight)/Hg concentration in the mouse kidney (ng/g) x 1000 = µg of Hg intake/day

(5) Soil intake to give a kidney Hg concentration of 25 mg/kg:

µg of Hg intake/soil Hg concentration = soil intake g/kg body weight/day

The soil and sediment intake would be associated with the level of Hg in this matrix. As shown, the intake of soil/day by mice to give 25 mg Hg/kg body weight varied between 0.03 to 36 kg in females and 0.04 to 36 kg in males. If the mice data were extrapolated to humans, the following intakes of soil would be necessary to give 25 mg Hg/kg body weight: young child weighing 25 kg, 0.75 to 900 kg for females and 1.0 to 900 kg for males; adult weighing 70 kg, 2.25 to 2,520 kg for females and 2.8 to 2,520 kg for males. If one accepts the 1 g/day intake levels reported by CDC for children, the estimated intake levels reported here to achieve toxicity would range from 750 to 900,000 times the CDC estimate. However, this estimates assumes that the absorption rate and the biological half-life in mice and humans are the same. In mice, the absorption rate and biological half-life have been estimated at 2% and 4 days, respectively. In humans, the absorption rate and half-life are estimated at 15% and 40 days, respectively. As compared to the mouse, humans absorb 7.5 times more Hg and have a half-life which is 10 times longer. This suggests that humans are 75 times more sensitive than the mouse and, thus, would require less Hg-contaminated soil to reach the 25 µg limit. The estimated amount required in humans to reach this limit would range from 5.6 to 6,750 g/day for 6 months. These amounts are still higher than the 1 g/day estimate of CDC. A young child would have to eat 5.6 g of soil with Hg levels of 2000 ppm or 6,750 g with soil Hg levels of 0.3 ppm for 6 months in order to reach the 25 µg limit. We thus suggest that the Hg observed in the soil and sediment from East Fork Poplar Creek may not be a health risk to children playing and/or soil eaters. A similar suggestion is made for lead, cadmium, and arsenic

observed in soil and sediment. If the Hg observed in the soil and sediment is significantly converted to the organic mercurials, then further evaluation of these sites may be required.

In addition the neurological evaluation using the swim test following 6, 12, and 20 months of exposure to these diets showed no difference between the 30 experimental groups. Two observations may be made from these results: (1) the swim test will not discern subtle neurological changes and may be only useful in showing acute neurological effects, and (2) neurological effects are not associated with these soil diets. In the absence of any effect, we suggest that this test may not discern those neurological effects that are observed in humans exposed to methylmercury and/or methylmercury may be too low to induce an effect. We note that in our studies, methyl mercury levels were less than 0.01% of total mercury present in the soil and sediment samples.

5.2.5 Carcinogenicity and Teratogenicity Effects

The soil and sediment feeding studies were supplemented by carcinogenicity and teratogenicity experiments. Results for these studies are shown in Tables 22 and 23. Skin tumors were not observed in the tumor promotion studies. However, several sites were associated with tumor initiation. Statistical analysis showed a significant correlation between Hg levels in soil and sediment and the number of tumors. Although these increases in the number of tumors were small, they occurred at sites with the highest levels of Hg. When the number of tumors at these sites is compared to the positive controls (i.e., the DMBA/TPA-treated mice), it is clear that the soil and sediment would be considered only as a weak tumor initiator. For example, in the positive controls tumors were observed for all mice, and the average number of tumors per mice was 7.9; the experimental data showed a high value of one tumor per mouse. We suggest that the samples used in these studies are, at best, weak tumor initiators. However, we recommend additional studies to determine the biological factors that may be associated with observed initiation of tumors in those sites with high levels of Hg.

We also suggest that the observed tumor initiation effect is not directly related to inorganic and elemental Hg because these metals would not be expected to extract into the organic solvent. Thus, an organic metabolite associated with Hg and/or an organic pollutant associated with these sites may be responsible for the observed tumor initiation effect.

To test the potential teratogenicity of pollutants in soil and sediment from the 30 experimental sites, mice breeding studies were performed. As shown in Table 23, litter size was not significantly correlated to the Hg levels in the soil and sediment. Furthermore, changes in the number of digits of the hand and foot, apparent neurological changes, and cleft palate were not observed in the litters. These data suggest that the level of Hg and other heavy metals in these samples may not be sufficient to induce a change in litter size and congenital abnormalities. We thus suggest that the soil and sediment from these sites would not be considered as teratogens.

TABLE 22
Tumor Promotion and Initiation Study Using Soil Extracts

Site	Tumor Promotion*		Tumor Initiation**	
	Female	Male	Female	Male
1	5-0	5-0	5-0	2-3/3-0
2	5-0	5-0	5-0	2-3/3-0
3	5-0	5-0	2-2/3-0	2-3/3-0
4	5-0	5-0	6-12/9-0	6-11/9-0
5	5-0	5-0	6-9/9-0	6-7/8-0
6	5-0	5-0	5-0	5-0
7	5-0	5-0	5-0	5-0
8	5-0	5-0	1-1/4-0	2-2/3-0
9	5-0	5-0	5-0	5-0
10	5-0	5-0	8-10/7-0	1-1/14-0
11	5-0	5-0	7-9/8-0	5-6/8-0
12	5-0	5-0	9-10/6-0	9-15/6-0
13	5-0	5-0	1-1/4-0	2-2/3-0
14	5-0	5-0	5-0	5-0
15	5-0	5-0	5-0	5-0
16	5-0	5-0	5-0	5-0
17	5-0	5-0	5-0	5-0
18	5-0	5-0	15-0	1-1/14-0
19	5-0	5-0	5-0	5-0
20	5-0	5-0	5-5/10-0	4-5/10-0
21	5-0	5-0	5-0	5-0
22	5-0	5-0	5-0	5-0
23	5-0	5-0	5-0	5-0
24	5-0	5-0	5-0	5-0
25	5-0	5-0	5-0	5-0

Table continued on next page.

TABLE 22 (cont'd)
Tumor Promotion and Initiation Study Using Soil Extracts

Site	Tumor Promotion*		Tumor Initiation**	
	Female	Male	Female	Male
26	5-0	5-0	5-0	5-0
27	5-0	5-0	5-0	5-0
28	5-0	5-0	5-0	5-0
29	5-0	5-0	5-5/9-0	2-3/13-0
30	5-0	5-0	5-0	5-0

Controls	Females		Males
		TPA***	
	1-1/9-0		1-1/9-0
		DMBA*	
	3-3/7-0		4-4/6-0
		DMBA + TPA	
	10-73		10-84
		Acetone	
	5-5/10-0		6-6/9-0

* Initiate with DMBA (10 nm) and promote with soil extract (0.2 mL) twice weekly for 17 weeks.
**Initiate with 0.2 mL soil extract (X2) and promote with TPA twice weekly (1 µg) for 17 weeks.
*** TPA = Phorbol 12 Myristate 13 Acetate.
* DMBA = 7,12-dimethylbenz (a,h) anthracene.

TABLE 23
Mice Breeding Studies*

Site	Litter Size**
1	6 ± 1
2	9 ± 3
3	5 ± .6
4	6 ± 1
5	7 ± 1
6	8 ± 2
7	8 ± 1
8	8 ± .5
9	7 ± 1
10	6 ± .6
11	8 ± 1
12	7 ± 1
13	7 ± 2
14	6 ± 1
15	8 ± 2
16	7 ± 1
17	6 ± .5
18	7 ± 1
19	5 ± .5
20	6 ± .6
21	10 ± 3
22	8 ± 2
23	6 ± 4
24	6 ± .5
25	8 ± 1
26	5 ± .5
27	7 ± 2
28	6 ± 1
29	7 ± 1
30	8 ± 3

$\bar{X} = 6.9$
$\sigma = 1.2$
$\mu + 3\sigma = 9.3$

*Mice were exposed to soil and sediment from the sites for 6 months. After exposure, animals were time-mated in pairs of three from each site. The size of each litter was determined within 24 hr and again at 96 hr after delivery

**Mean ± SD for three breeding pairs.

6. SUMMARY, CONCLUSIONS, AND RECOMMENDATIONS

6.1 SUMMARY

Several investigators have established that Hg and other heavy metals are absorbed through the intestinal tract and bioaccumulate in various organs. The organs primarily associated with the bioaccumulation include the liver and kidney. The brain is also associated with heavy metal accumulation. However, reports show that brain accumulation of heavy metals occurs after exposure to metals in the organic and/or elemental form. Furthermore, reports in the literature indicate differences in the organ distribution of metal following the exposure to metals in the organic form versus metals in the inorganic form. For example, after exposure to inorganic Hg, the liver-to-kidney ratio for Hg ranged from 0.01 to 0.31. In contrast, this ratio ranged from 0.6 to 0.9 following the exposure to the organic mercurials. Because metals in the organic form accumulate in brain tissue, toxicity differences are observed between the inorganic and organic metals. Thus, differences in the toxicity and the organ distribution of metals provide a method for determining the relative level of metals in the inorganic and organic forms. These reports provide a means of assessing (1) the toxicity of Hg in a complex mixture, and (2) the interaction of constituents in a complex mixture on the organ distribution of Hg. Because Hg and other heavy metals accumulate in the kidney, this would be used to assess the toxicity of a mixture contaminated with heavy metals. The assessment of interactions may be determined by comparing results showing the organ distribution of Hg following exposure to this metal in a simple matrix versus exposure to this metal in a complex matrix.

The present studies were performed to determine the effect of soil and sediment containing Hg and other heavy metals on (1) distribution of Hg in the liver and kidney, (2) morphology of the kidney, (3) functional physiology of the kidney, (4) animal mortality, and (5) body weight. Other toxicological assessments of soil and sediment included determinations of carcinogenicity and teratogenicity.

The observed results showed:

1. A significant correlation between level of Hg in soil and sediment and Hg in the kidney.
2. A ratio of Hg in the liver and kidney ranging from 0.06 to 0.34 (with the exception of groups 5, 23, and 24).
3. Nonsignificant morphological changes in the kidney in groups exposed for 6, 12, and 20 months.
4. Nonsignificant functional changes in the kidney following 12 months of exposure.
5. A nonsignificant correlation between the level of Hg in soil and sediment and mortality.
6. A nonsignificant correlation between the level of Hg in soil and sediment and teratogenicity effects.
7. A nonsignificant correlation between the level of Hg in soil and sediment and changes in body weight.
8. A significant correlation between Hg in soil and sediment and skin tumor initiation in sites 4, 5, 6, 10, 11, and 12.

6.2 CONCLUSIONS

Based on the above results the following conclusions were reached.

1. Mercury in soil and sediment is absorbed through the intestinal tract and bioaccumulates in the liver and kidney.

2. A significant dose-response relationship was observed, but this relationship was not linear. The factors that may have affected the linear response include the binding coefficient of Hg to soil, the relative level of the various species and inorganic forms of Hg in the different soils, and the effect of various soil constituents on Hg absorption.

3. Mercury was absorbed through the intestinal tract and bioaccumulated in the kidney, but no significant morphological and physiological changes in this organ were observed. If this animal model is accepted as a predictor of the potential health effects of humans eating contaminated soil and sediment, we suggest that no significant health effects associated with heavy metal toxicity should occur in humans exposed to the soil and sediment from East Fork Poplar Creek. This suggestion is supported by the results showing that significant correlations were not observed between the level of Hg in soil and sediment and mortality, body weight, or litter size.

4. The skin tumor initiation and promotion studies showed a significant correlation between Hg in soil and sediment and tumor initiation. Since extracts of soil and sediment were used in these studies, we suggest that an organic pollutant(s) associated with Hg and/or these sites initiates tumors. However, results show that only two areas (of 30 studied) account for the significant increase in tumors. Furthermore, the Hg levels in several sites in these areas which show a significant correlation were below levels observed in sites from other areas in which the tumor incidence was below the calculated mean for all sites. It may be suggested from these data that the areas which show a significant increase in tumors have other organic contaminants that initiate tumors. This suggestion will require additional experiments.

6.3 RECOMMENDATIONS

The three recommendations suggested by the present studies include the following.

1. Determine, in soil and sediment from East Fork Poplar Creek, the levels of elemental, inorganic, and organic mercury as well as the level of mercury sulfide.

2. Perform additional studies to establish the relationship between mercury and tumor initiation.

3. Perform additional statistical analyses of the dose-response data to determine if several levels of response (i.e., kidney mercury) can be discerned. This information will help establish minimal and maximal intake levels for soil and sediment.

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FIGURES 3 - 28

LEGENDS

Figures 3 to 16

Regression analysis plots of log liver or kidney mercury over the log of soil mercury from male and female Swiss mice exposed to diets containing 5% soil with mercury contamination for 6 to 20 months. The plots were made following 6, 12, and 20 months of exposure to these diets.

Figures 16 to 24

Regression analysis plots of log liver or kidney mercury over the log of soil mercury from male and female Swiss mice exposed to diets containing 5% soil with mercury contamination or diets containing 5% soil and mercuric chloride added to levels of 500, 1000, 2000, 4000, and 8000 ppm. The plots were made following 6 months of exposure to these diets.

Figures 25 to 28

Regression analysis plots of log liver over log kidney mercury from male and female Swiss mice exposed to diets containing 5% soil with mercury contamination or diets containing 5% soil and mercuric chloride added to levels of 500, 1000, 2000, 4000, and 8000 ppm. The plots were made following 6 months of exposure to these diets.

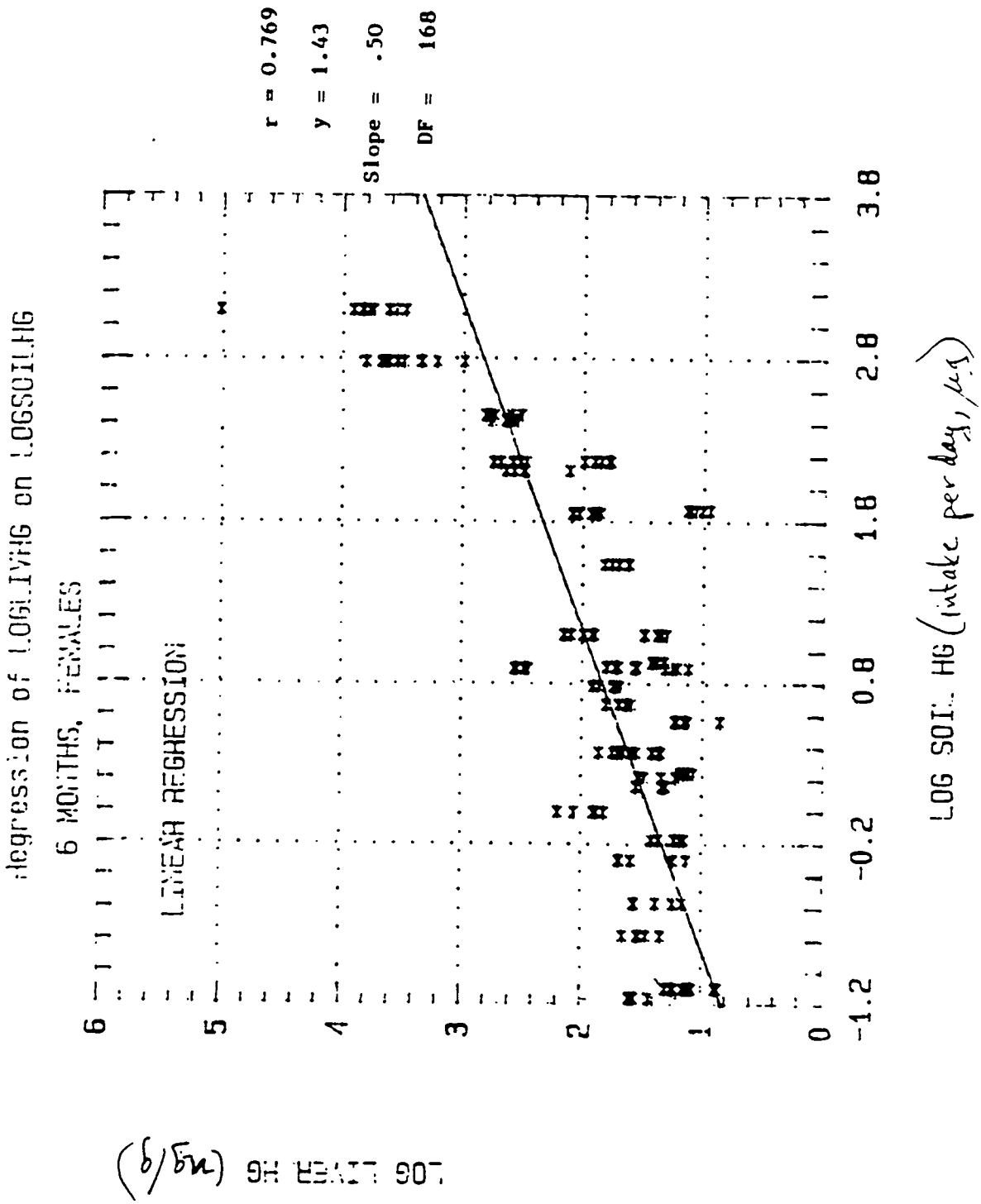

Regression of LOGLIVHG on LOGSOILHG
6 MONTHS, FEMALES
LINEAR REGRESSION
r = 0.769
y = 1.43
Slope = .50
DF = 168
LOG LIVER HG (mg/g)
LOG SOIL HG (intake per day, µg)
6
5
4
3
2
1
0
-1.2
-0.2
0.8
1.8
2.8
3.8

Figure 3

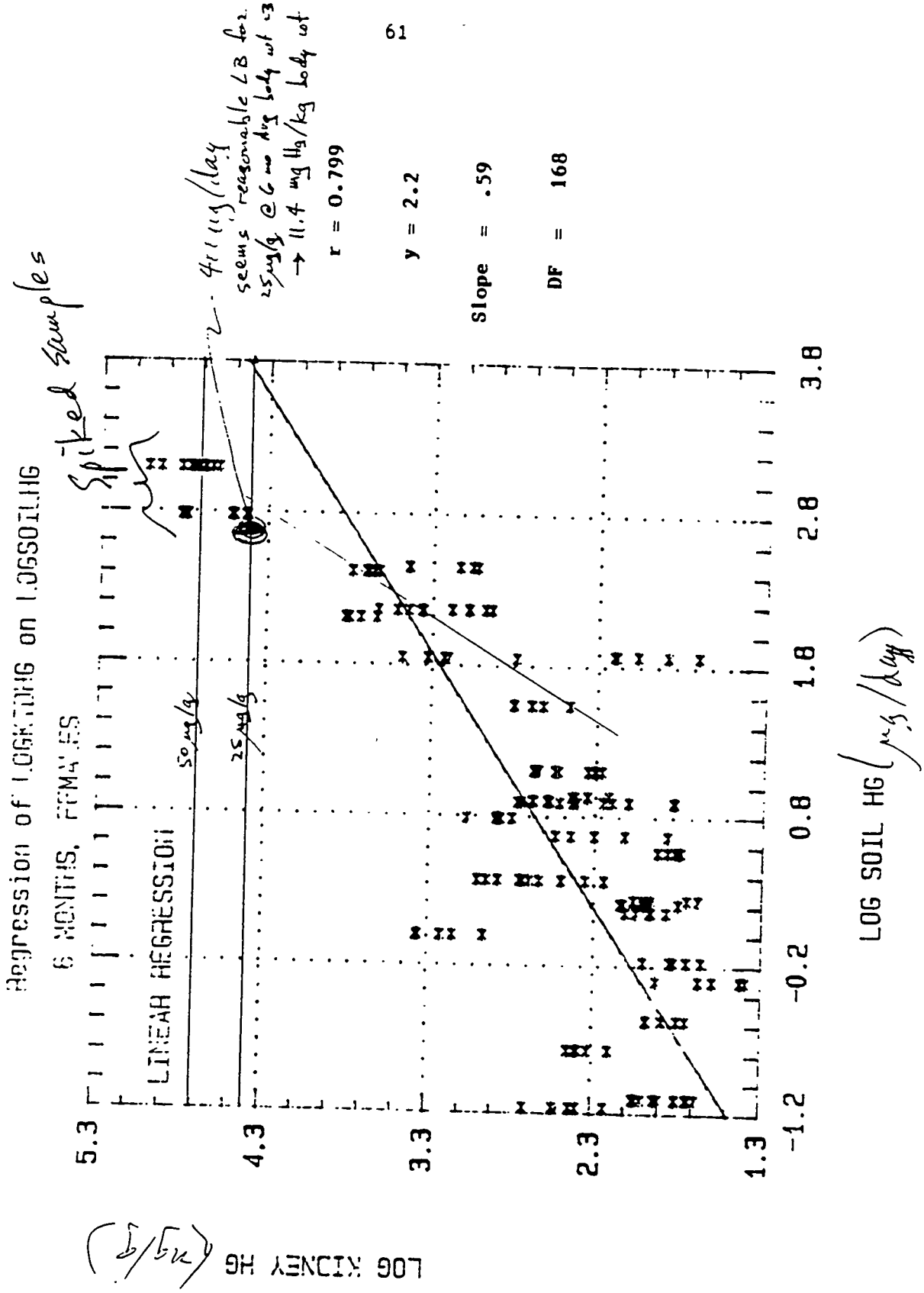
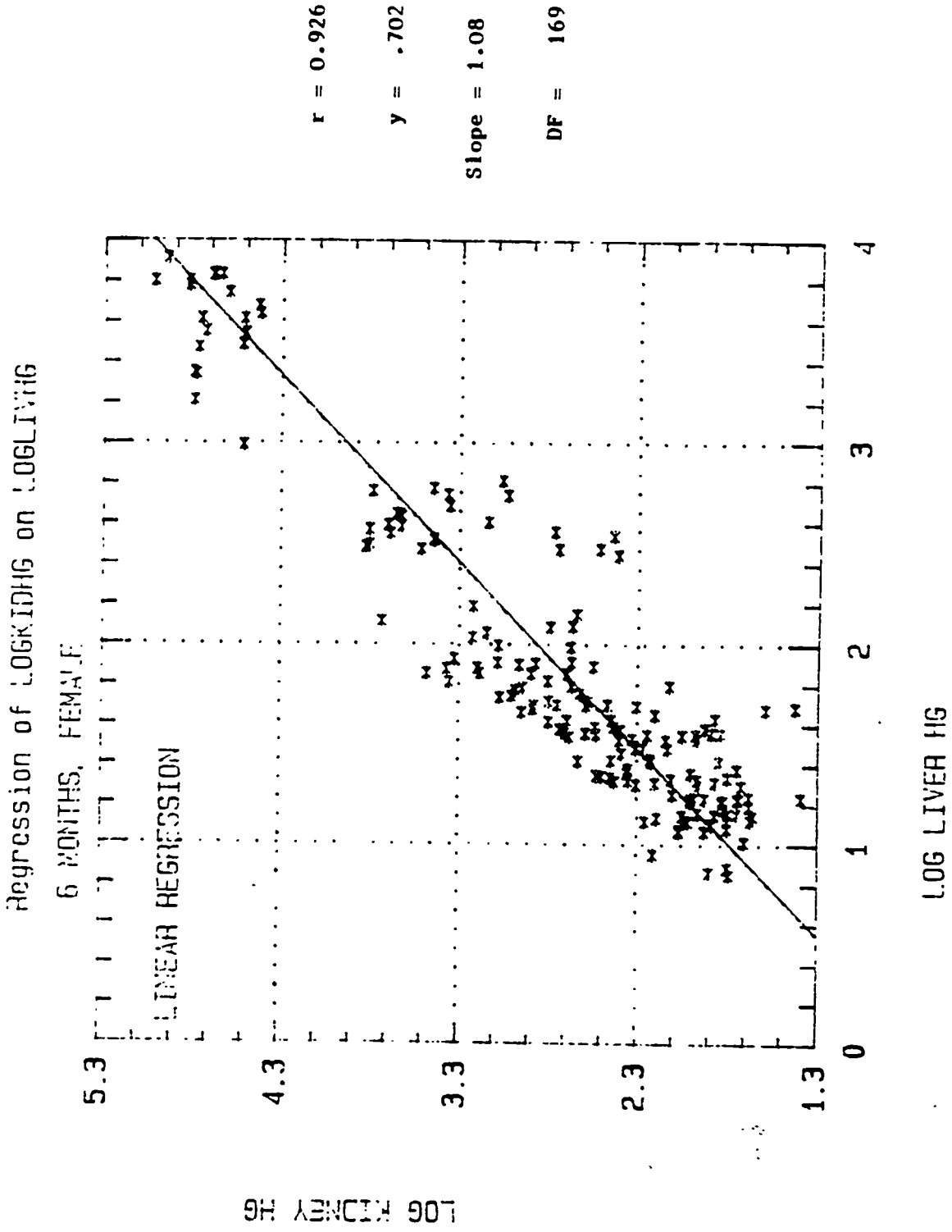

Regression of LOGKIDNG on LOGSOILHG
6 MONTHS, FEMALES
LINEAR REGRESSION
50 mg/g
25 mg/g
r = 0.799
y = 2.2
Slope = .59
DF = 168
LOG KIDNEY HG (mg/g)
LOG SOIL HG (mg/day)
5.3
4.3
3.3
2.3
1.3
-1.2
-0.2
0.8
1.8
2.8
3.8

Figure 4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y = .702
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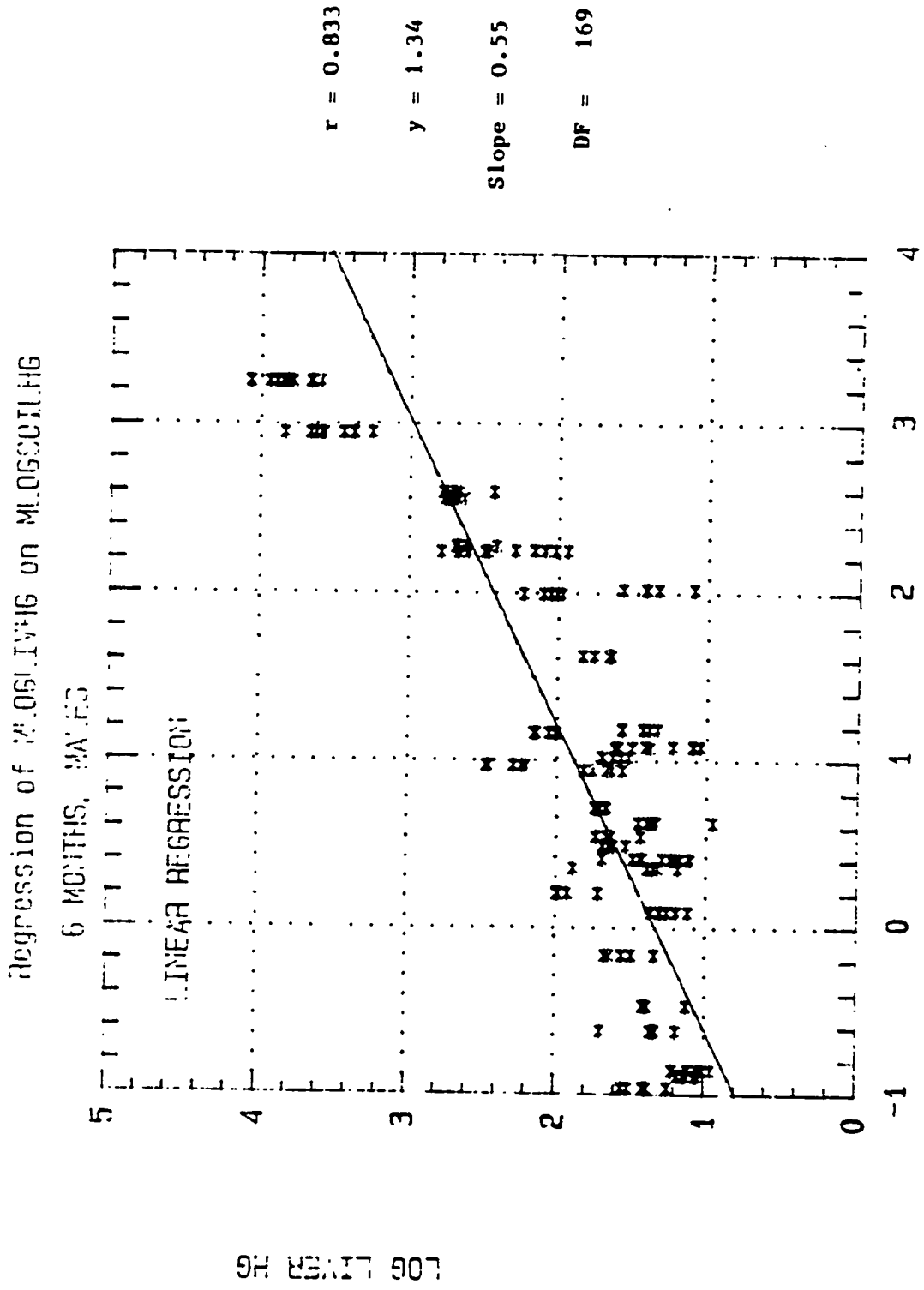
Regression of LOGKIDHG on LOGLIVHG
6 MONTHS, FEMALE
LINEAR REGRESSION
LOG KIDNEY HG
LOG LIVER HG
5.3
4.3
3.3
2.3
1.3
0
1
2
3
4

Figure 5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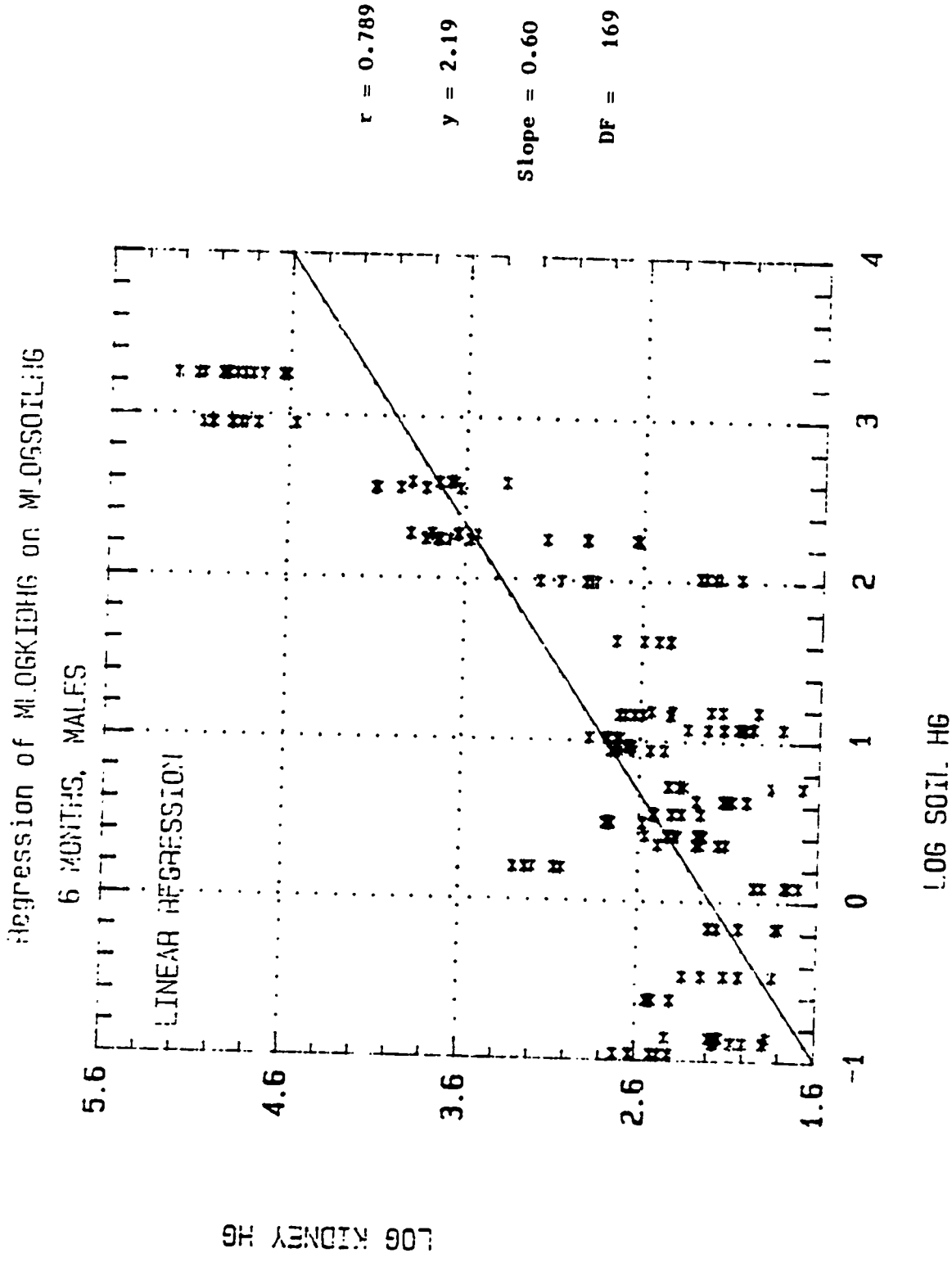
LINEAR REGRESSION
LOG SOIL HG
LOG LIVER HG

Figure 6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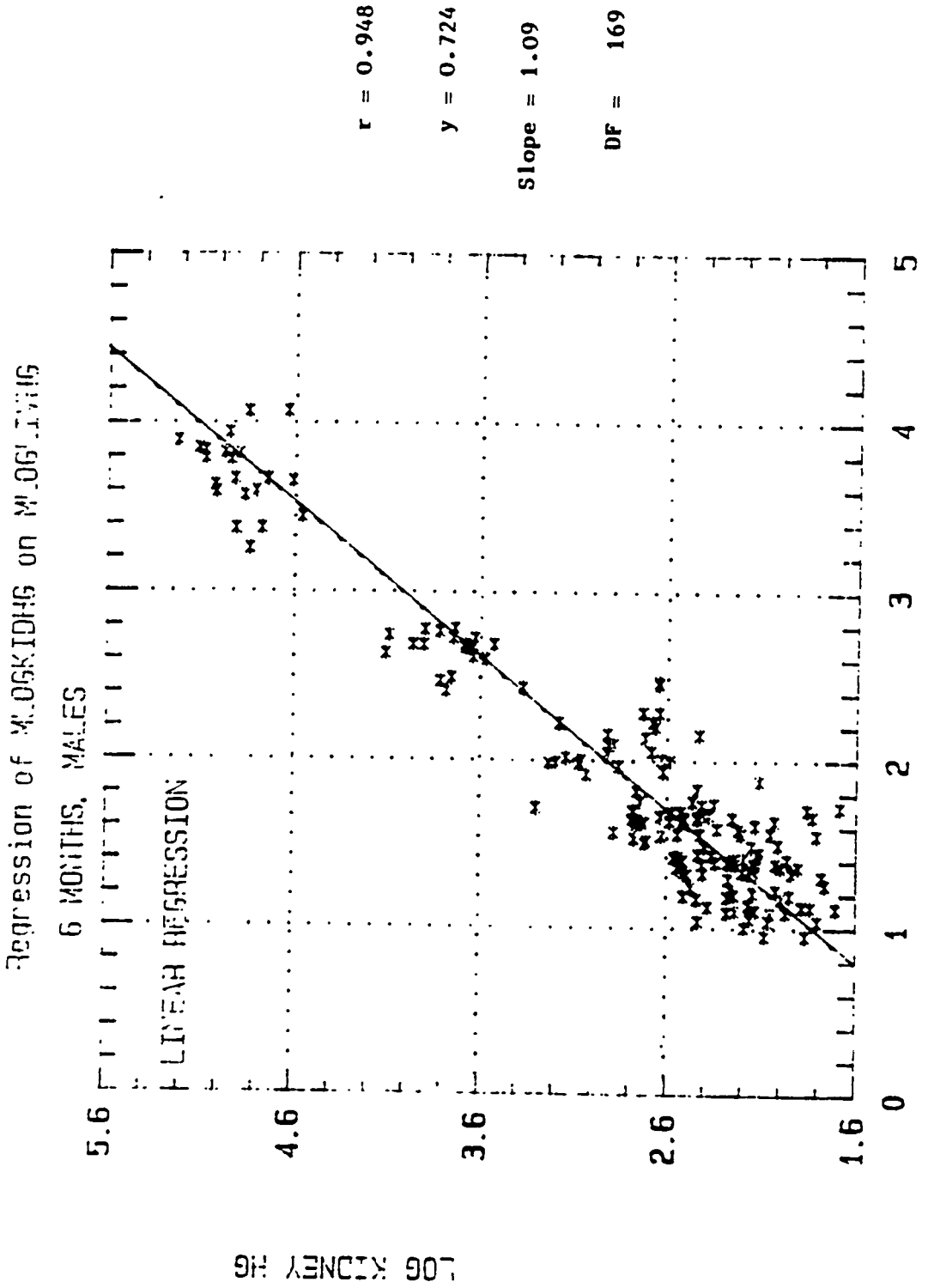
Regression of MLOGKIDHG on MLOGSOILHG
6 MONTHS, MALES
LINEAR REGRESSION
LOG KIDNEY HG
LOG SOIL HG
5.6
4.6
3.6
2.6
1.6
-1
0
1
2
3
4

Figure 7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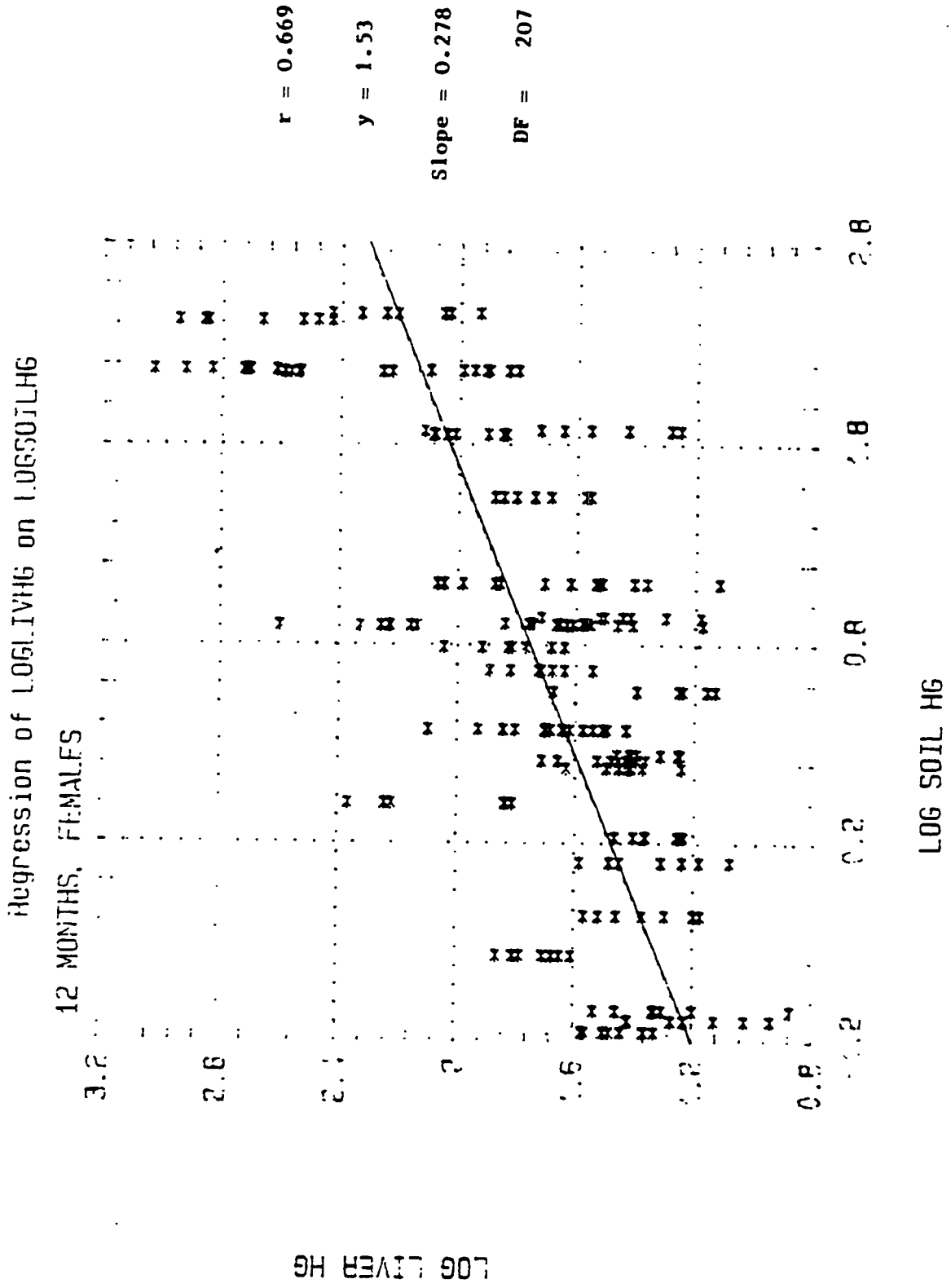
Regression of KIDSKIDHG on KIDSTWHG
6 MONTHS, MALES
LINEAR REGRESSION
LOG KIDNEY HG
LOG LIVER HG
5.6
4.6
3.6
2.6
1.6
0
1
2
3
4
5

Figure 8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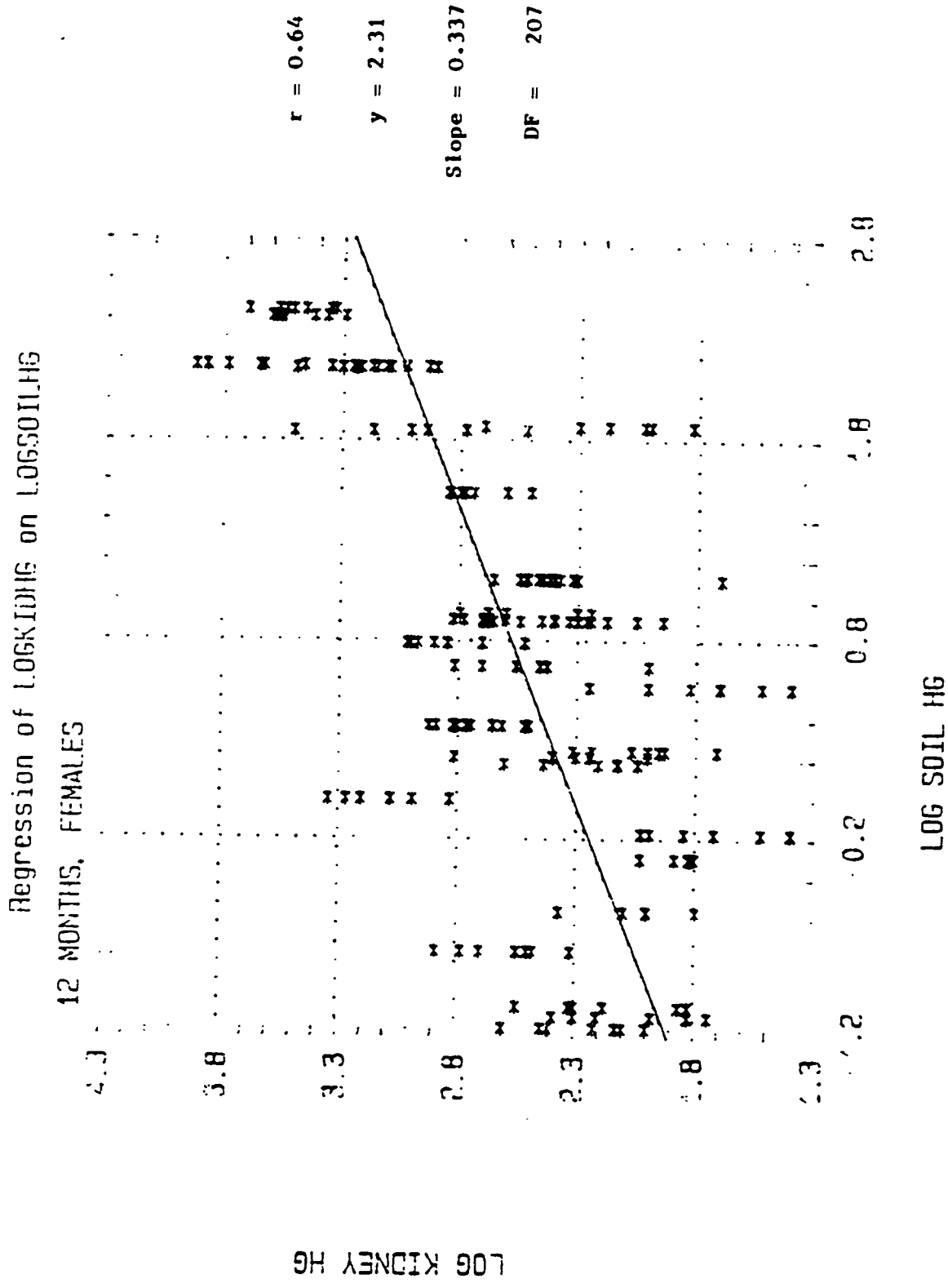
Regression of LOGLIVHG on LOGSOILHG
12 MONTHS, FEMALES
LOG LIVER HG
LOG SOIL HG

Figure 9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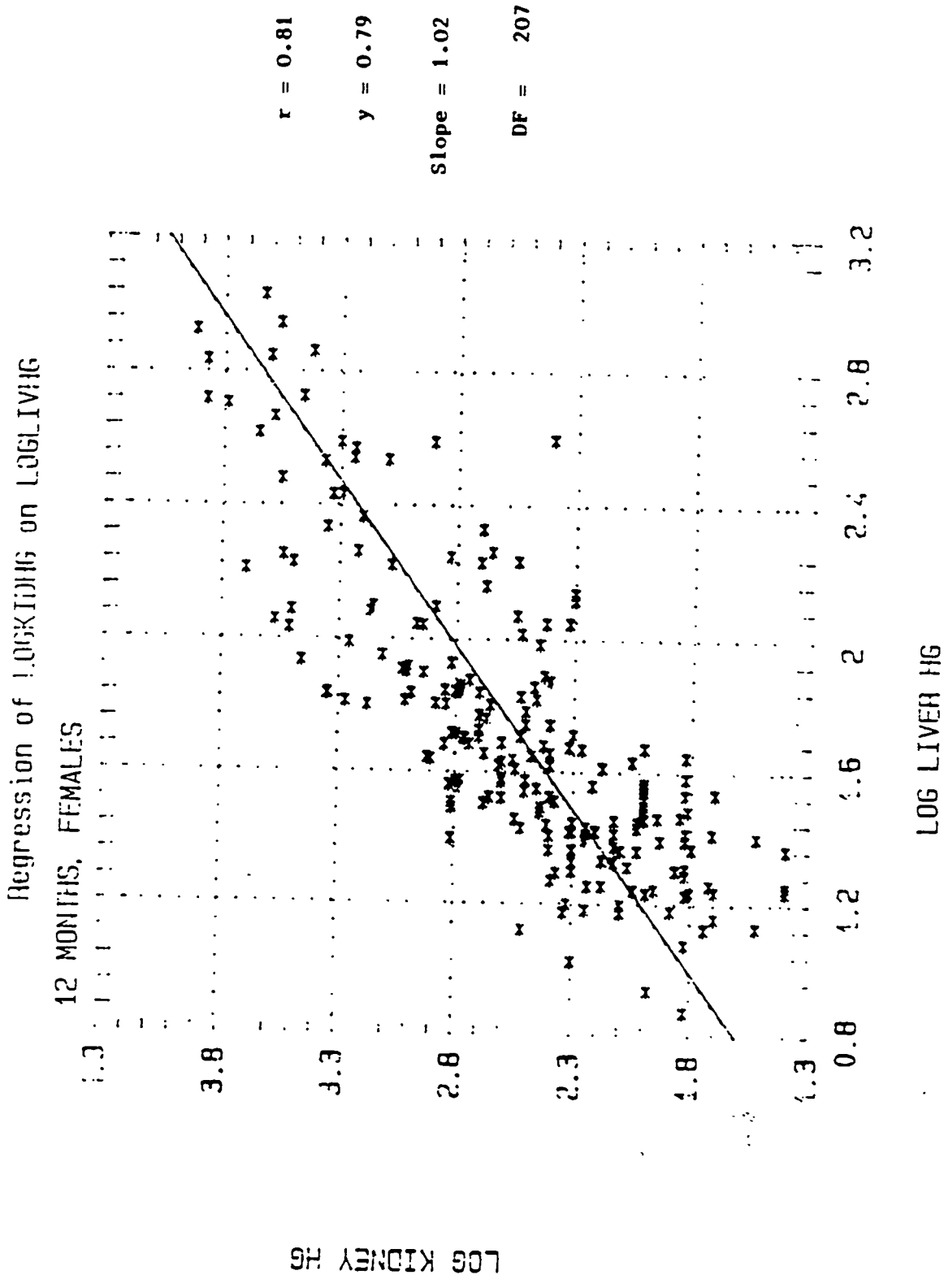
Regression of LOGKIDHG on LOGSOILHG
12 MONTHS, FEMALES
LOG SOIL HG
LOG KIDNEY HG

Figure 10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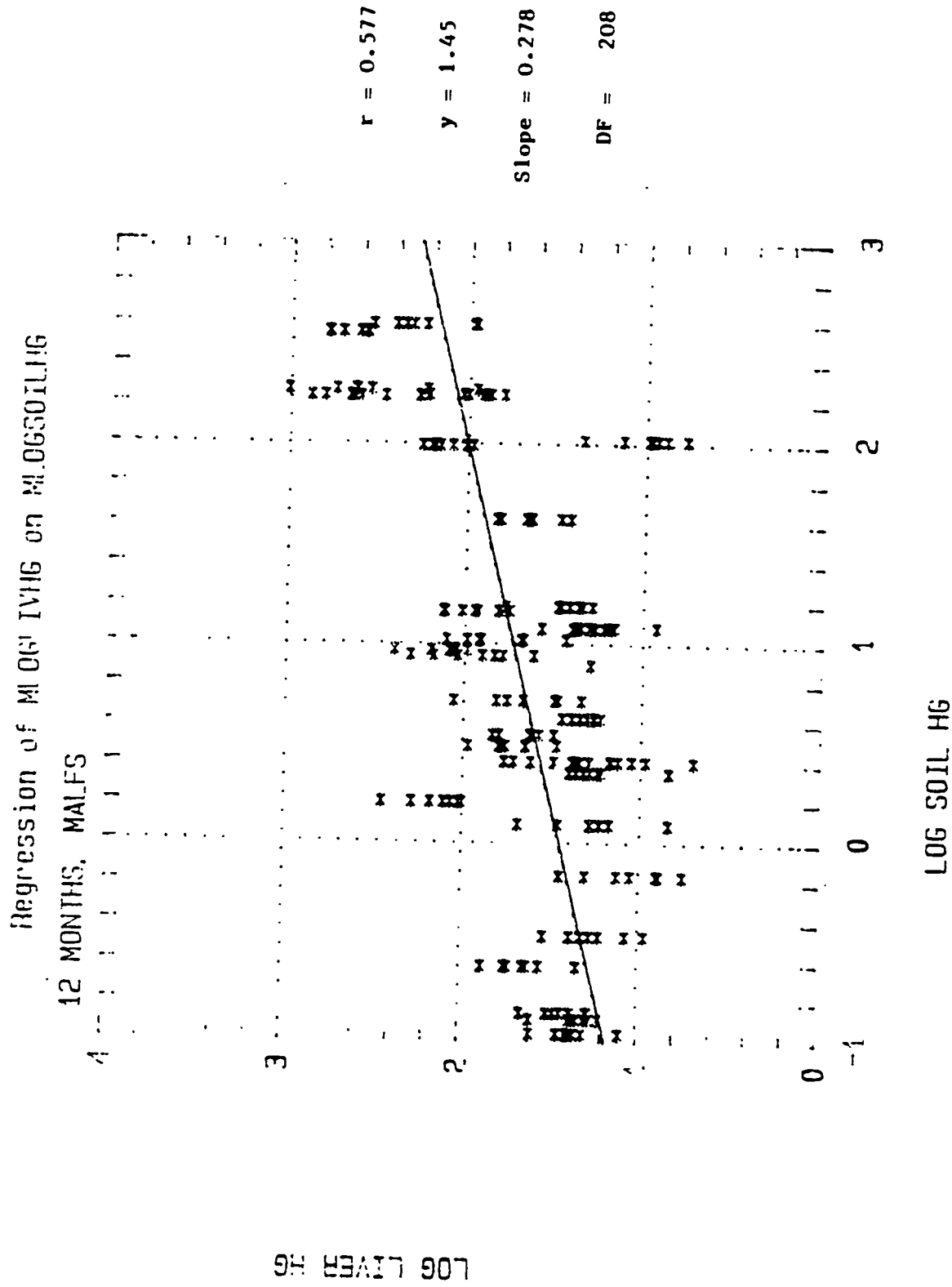
Regression of LOGKIDNG on LOGLIVHG
12 MONTHS, FEMALES
LOG KIDNEY HG
LOG LIVER HG
4.3
3.8
3.3
2.8
2.3
1.8
1.3
0.8
1.2
1.6
2
2.4
2.8
3.2

Figure 11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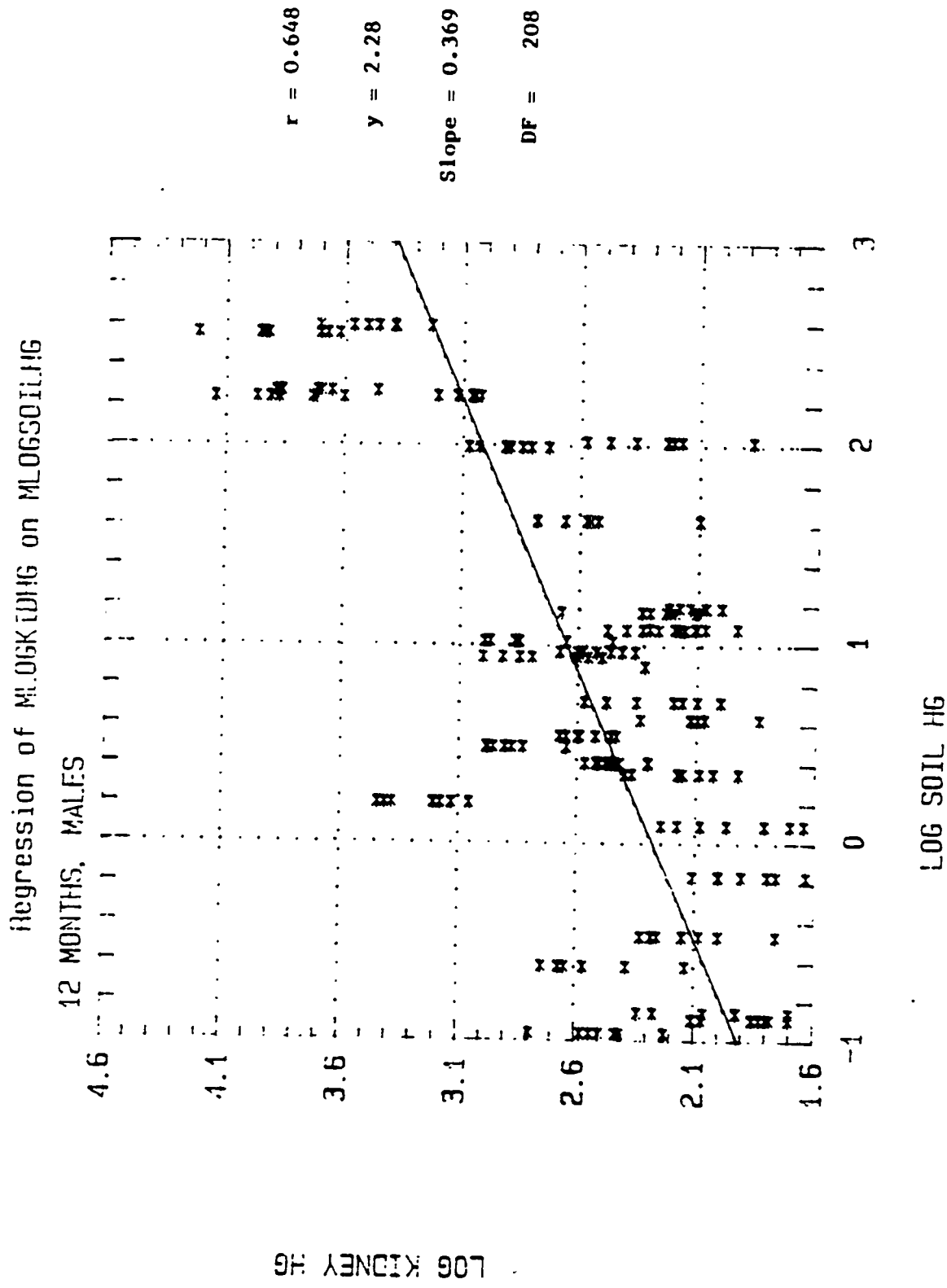
Regression of MLOGLIVHG on MLOGSOILHG
12 MONTHS, MALES
LOG LIVER HG
LOG SOIL HG
4
3
2
1
0
-1
-1
0
1
2
3

Figure 12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y = 2.28
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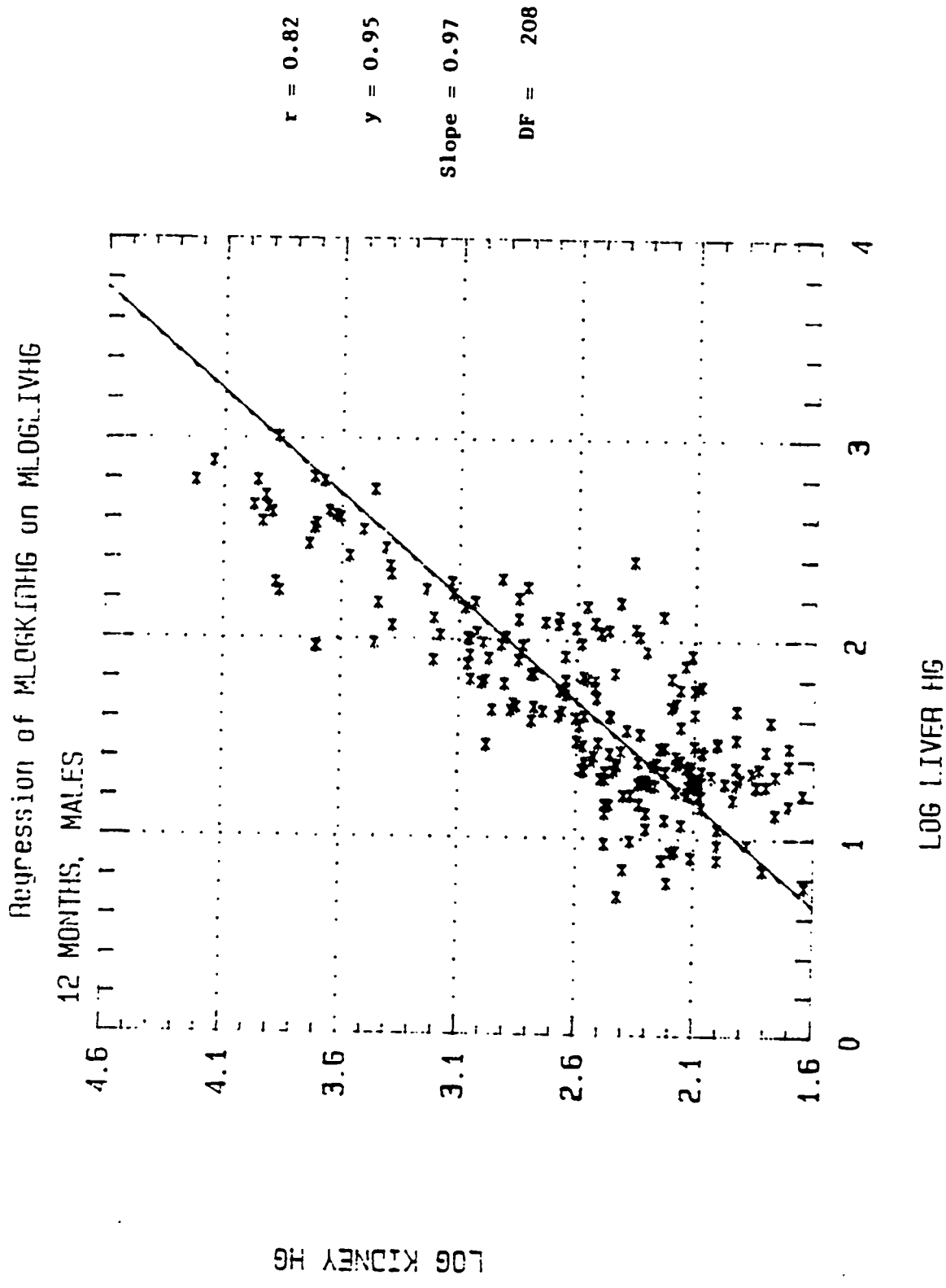
Regression of MLOGKIDNG on MLOGSOILNG
12 MONTHS, MALES
LOG KIDNEY HG
LOG SOIL HG
4.6
4.1
3.6
3.1
2.6
2.1
1.6
-1
0
1
2
3

Figure 13


r = 0.82
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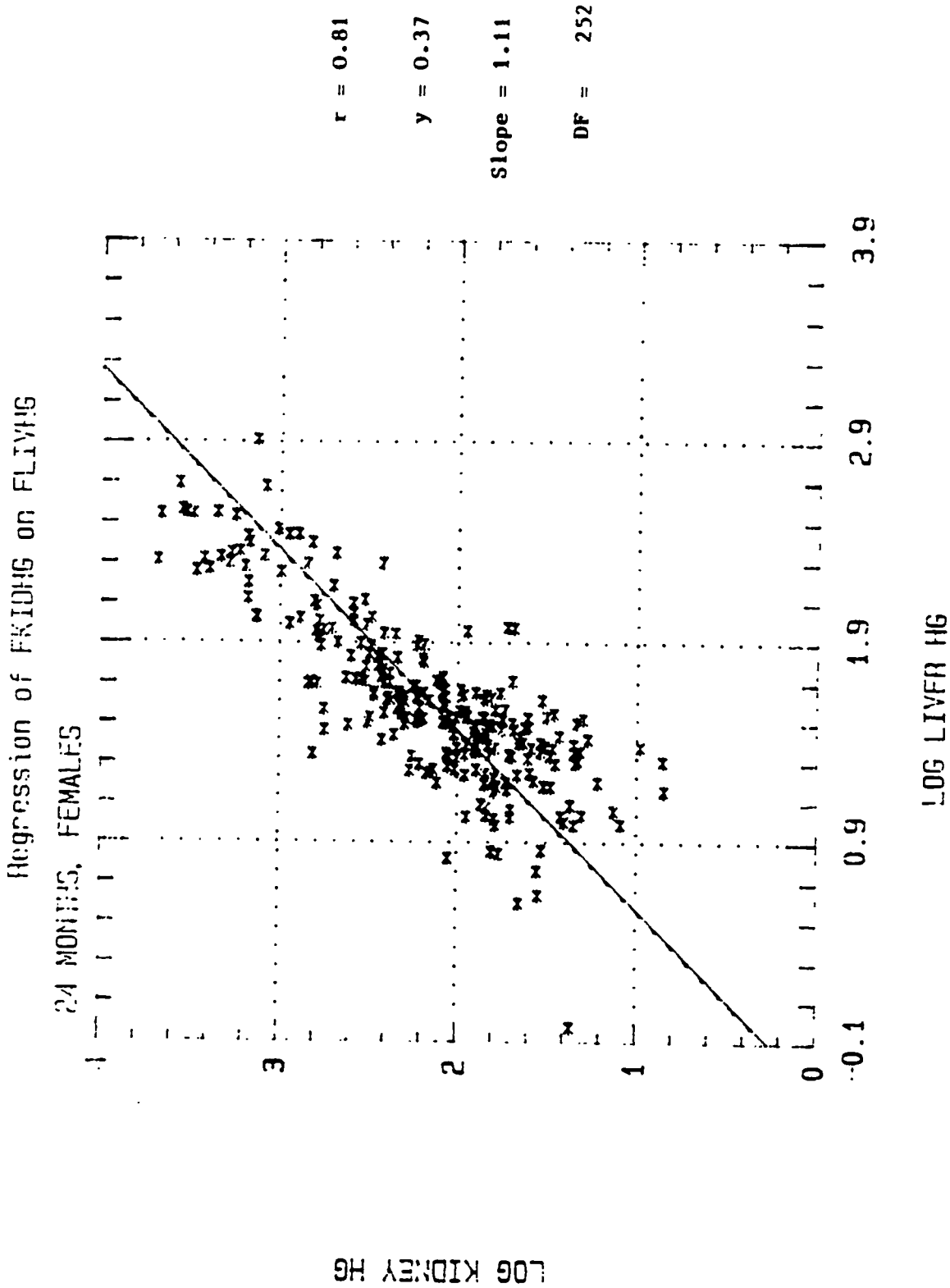
Regression of MLOGKIDHG on MLOGLIVHG
12 MONTHS, MALES
LOG KIDNEY HG
LOG LIVER HG
4.6
4.1
3.6
3.1
2.6
2.1
1.6
0
1
2
3
4

Figure 14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y = 0.37
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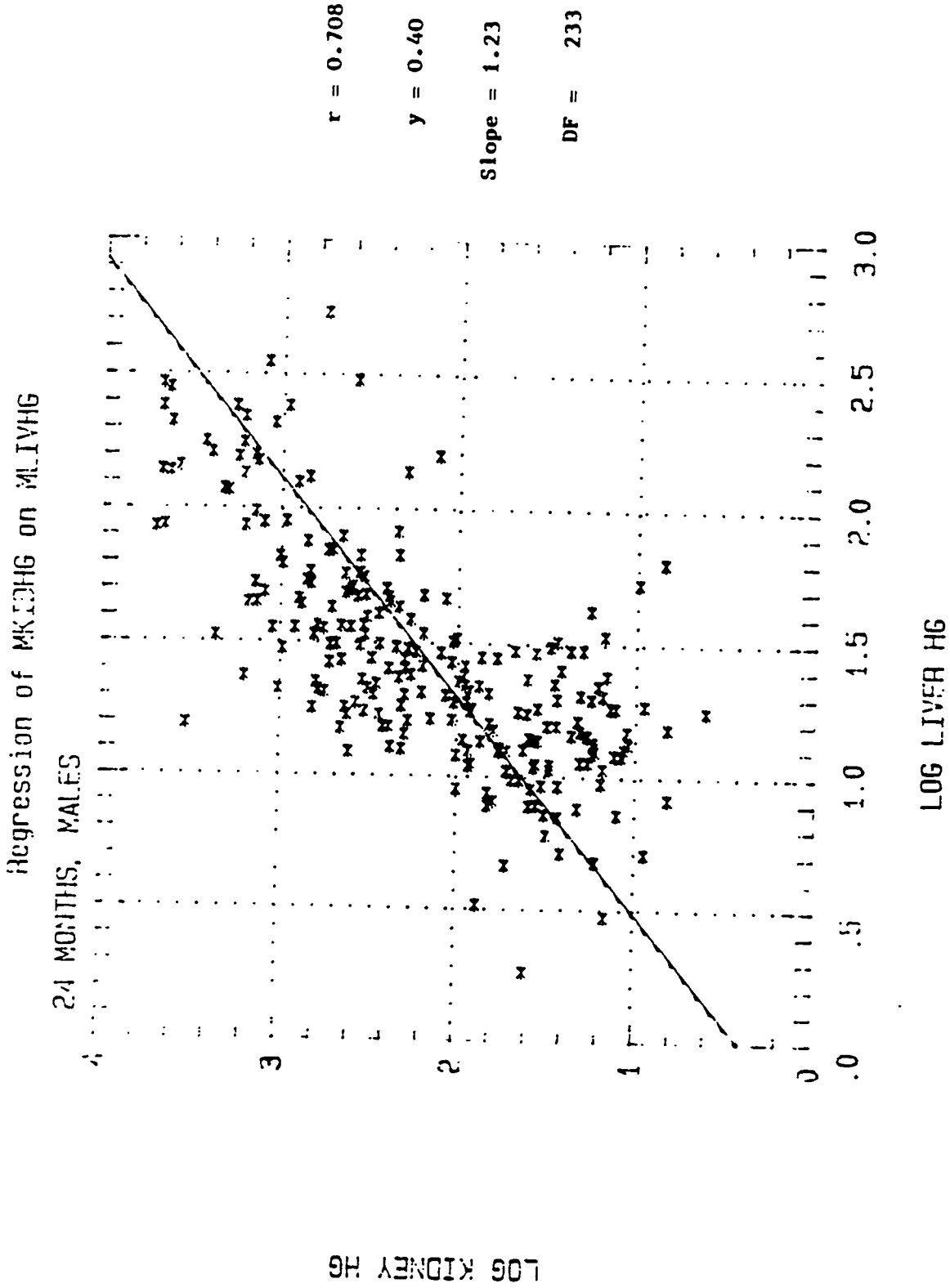
Regression of KIDNEY on FLYING
24 MONTHS, FEMALES
LOG KIDNEY HG
LOG LIVER HG
3
2
1
0
-0.1
0.9
1.9
2.9
3.9

Figure 15


r = 0.708
y = 0.40
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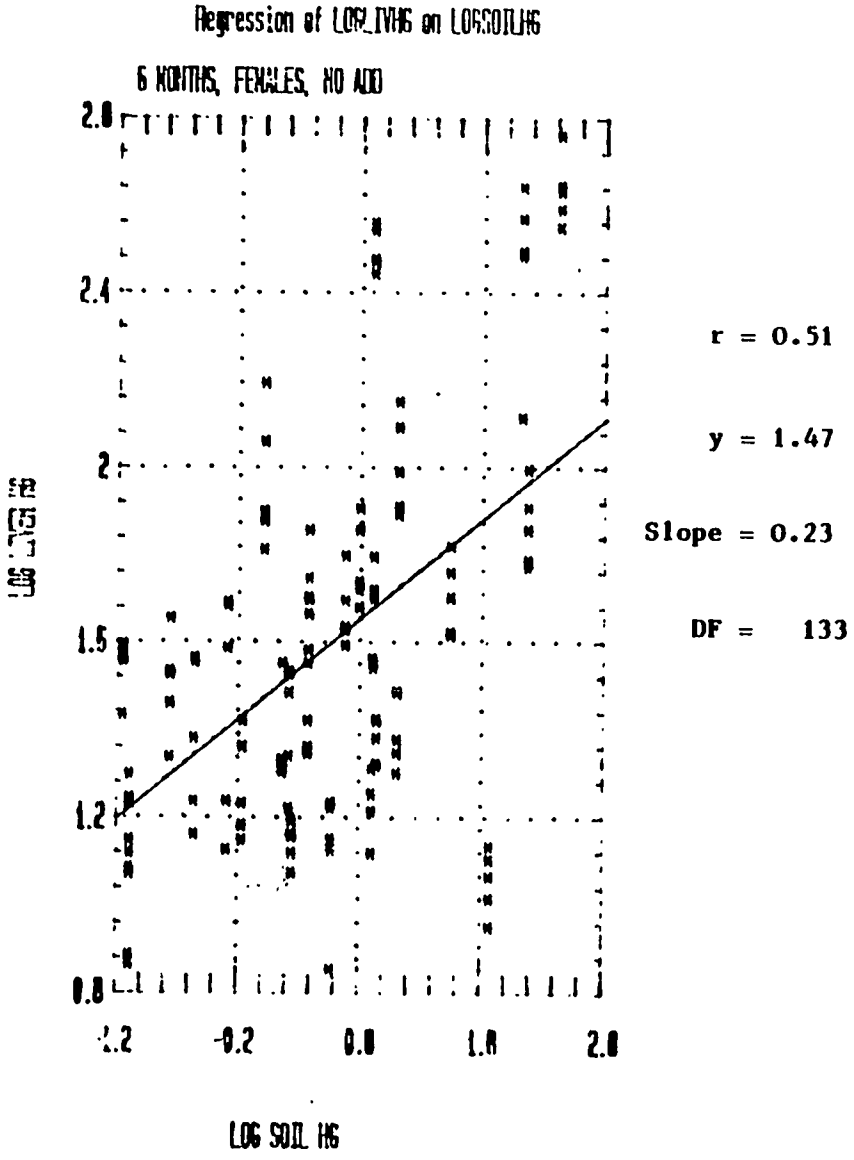
Regression of MKIDHG on MLIVHG
24 MONTHS, MALES
LOG KIDNEY HG
LOG LIVER HG
3.0
2.5
2.0
1.5
1.0
.5
.0
3
2
1
0

Figure 16



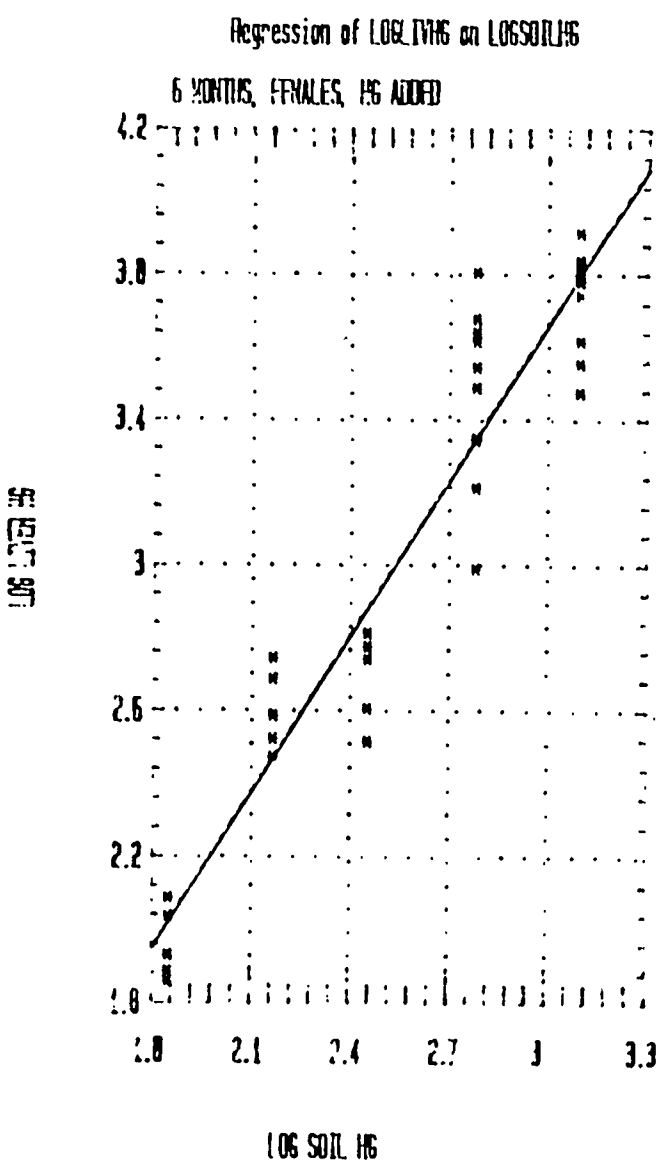
r = 0.51

y = 1.47

Slope = 0.23

DF = 133

Figure 17



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y = -0.61

Slope = 1.43

DF = 33

Figure 18

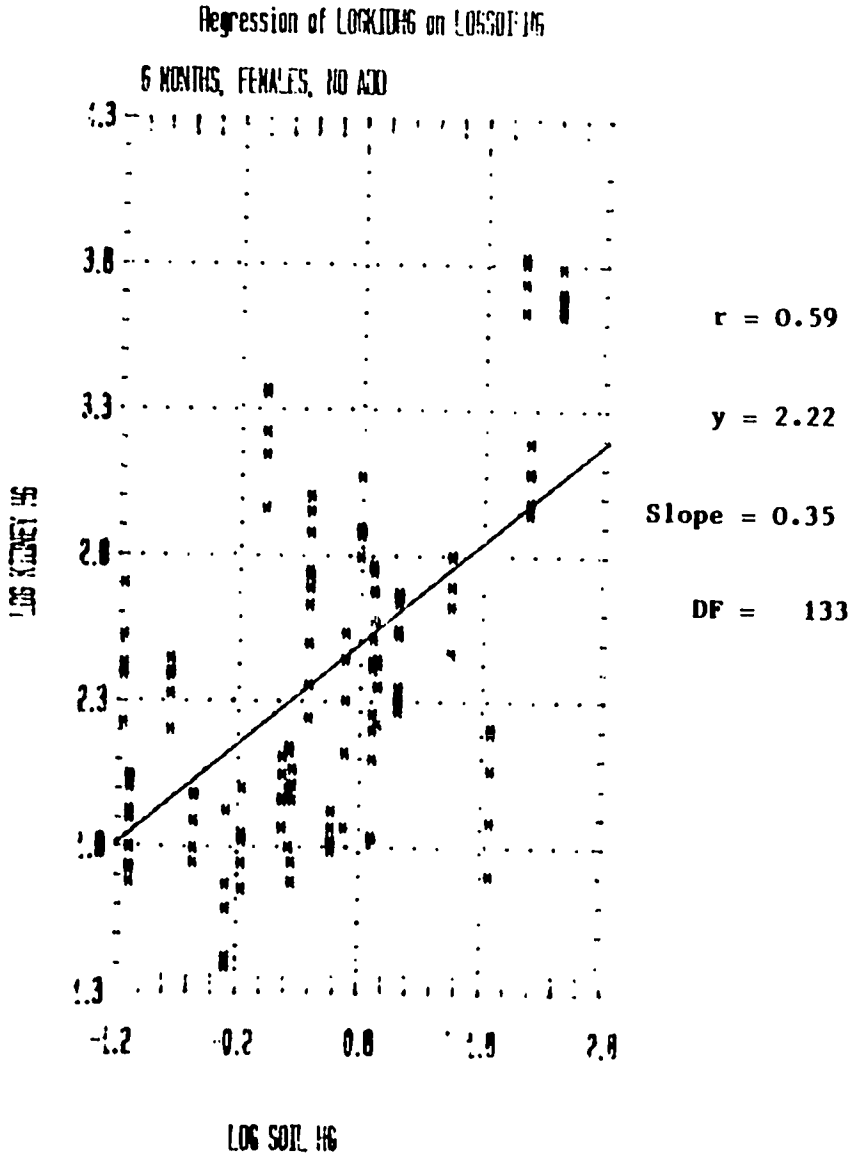


Figure 19

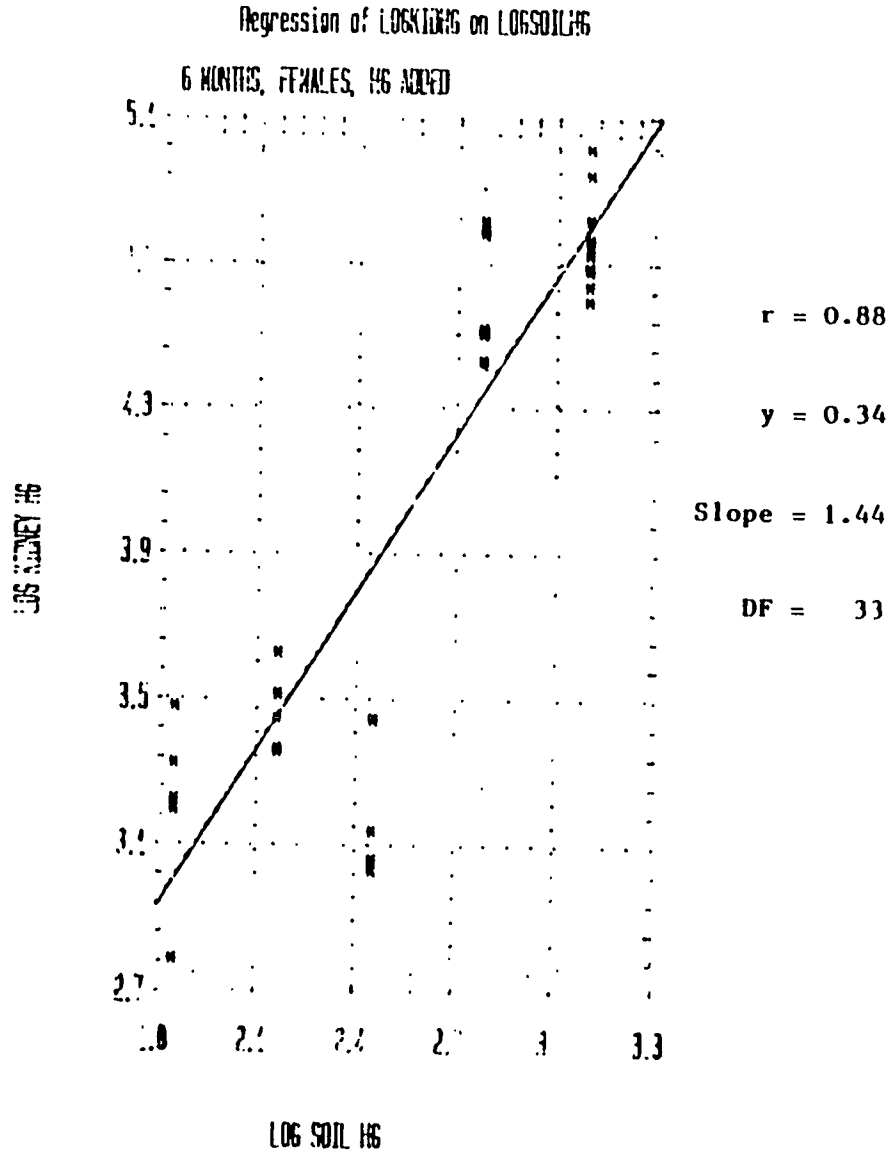
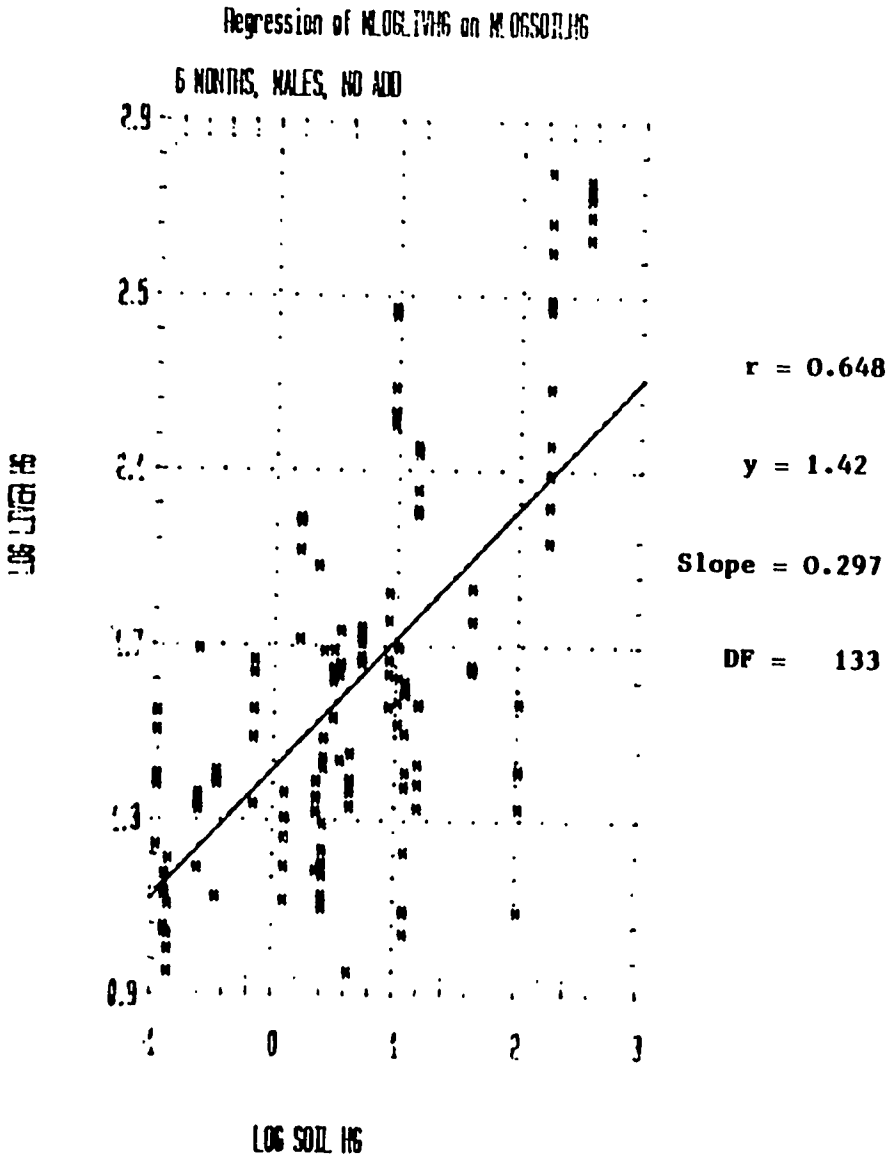


Figure 20



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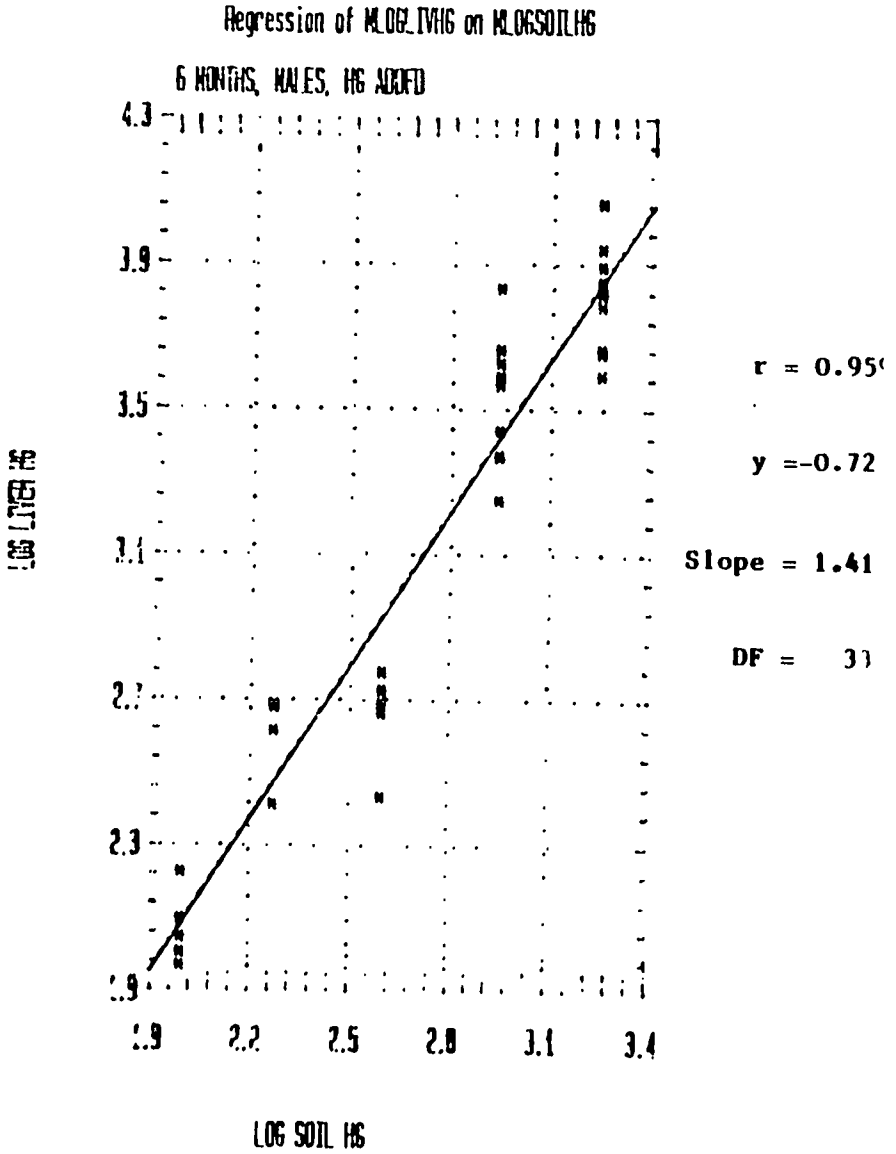

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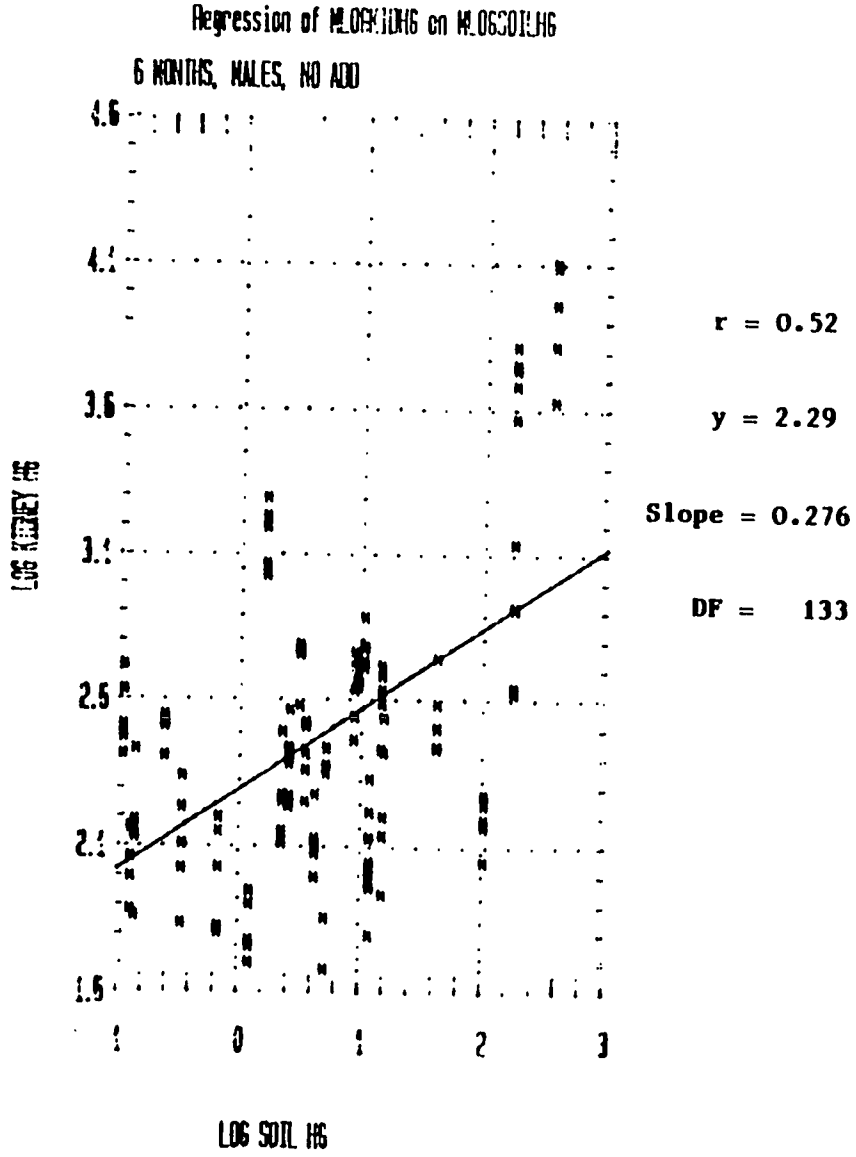

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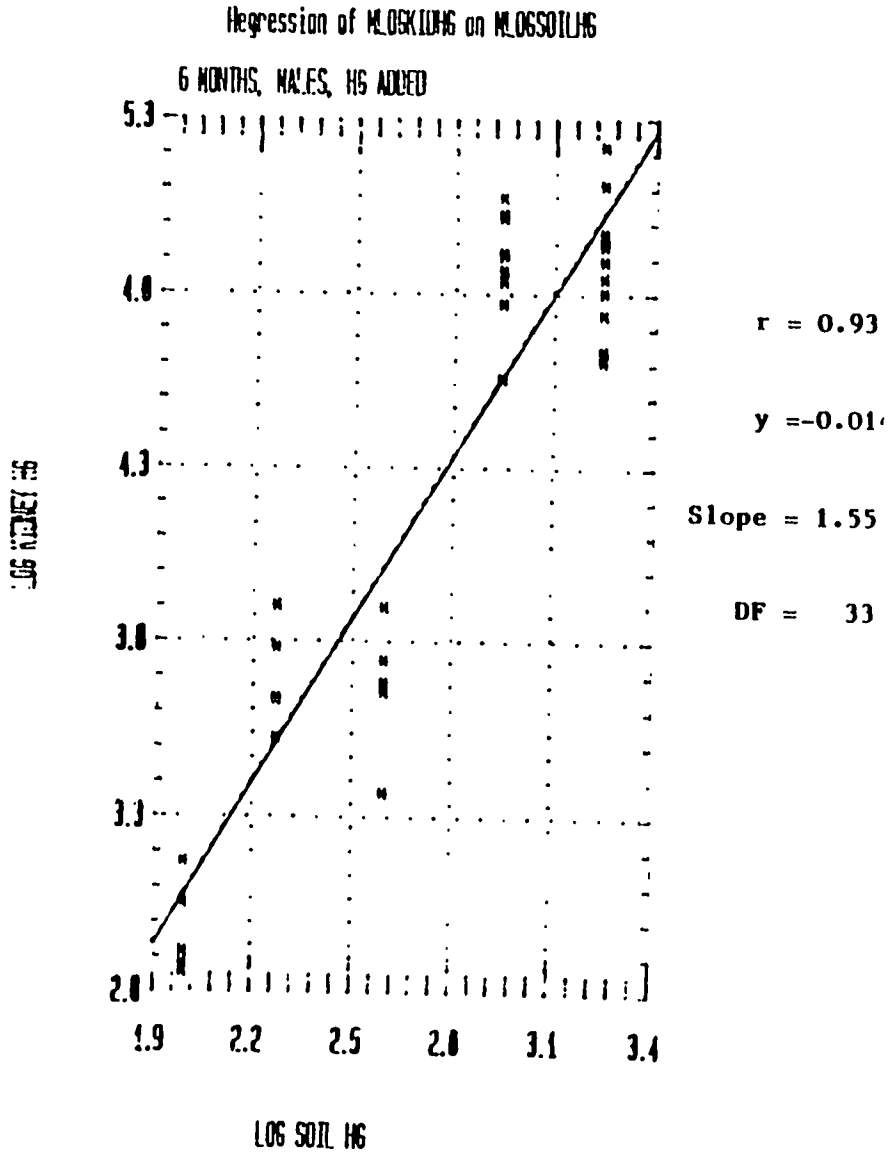

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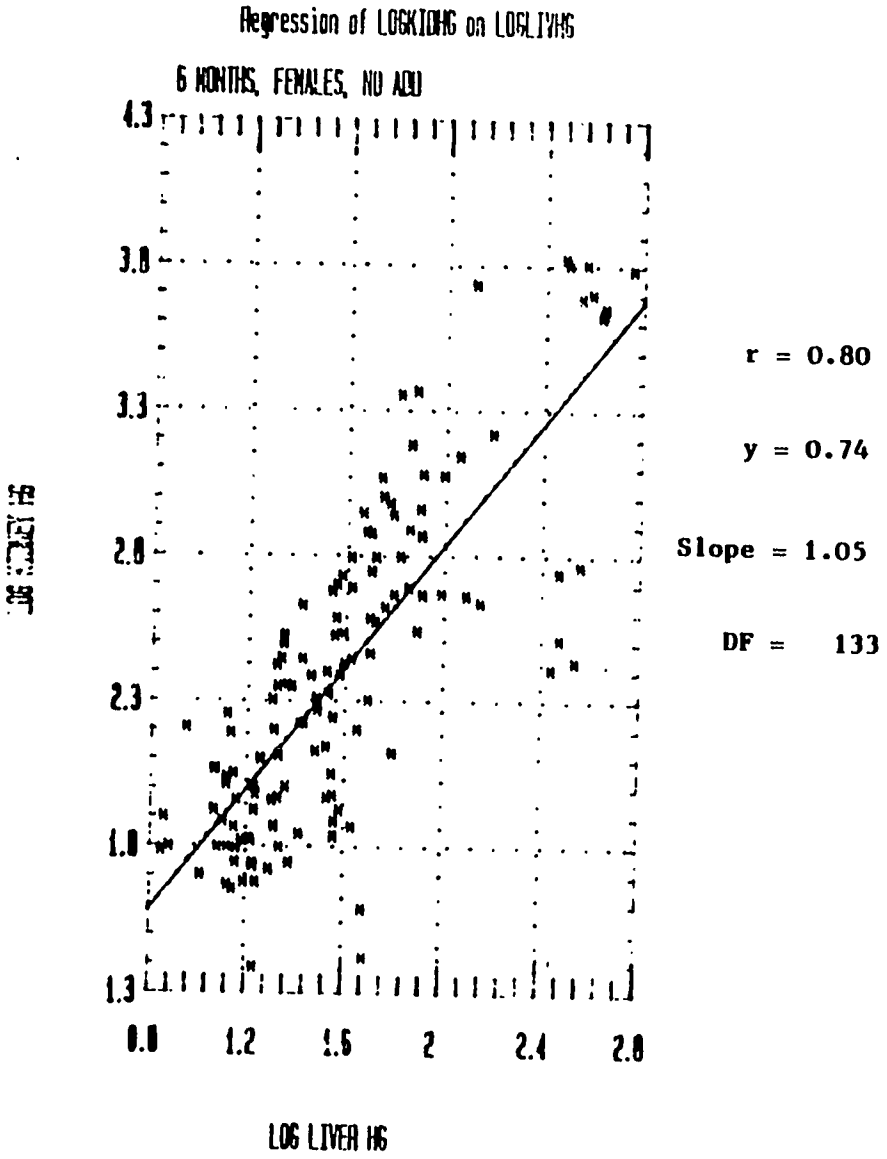

Figure 25

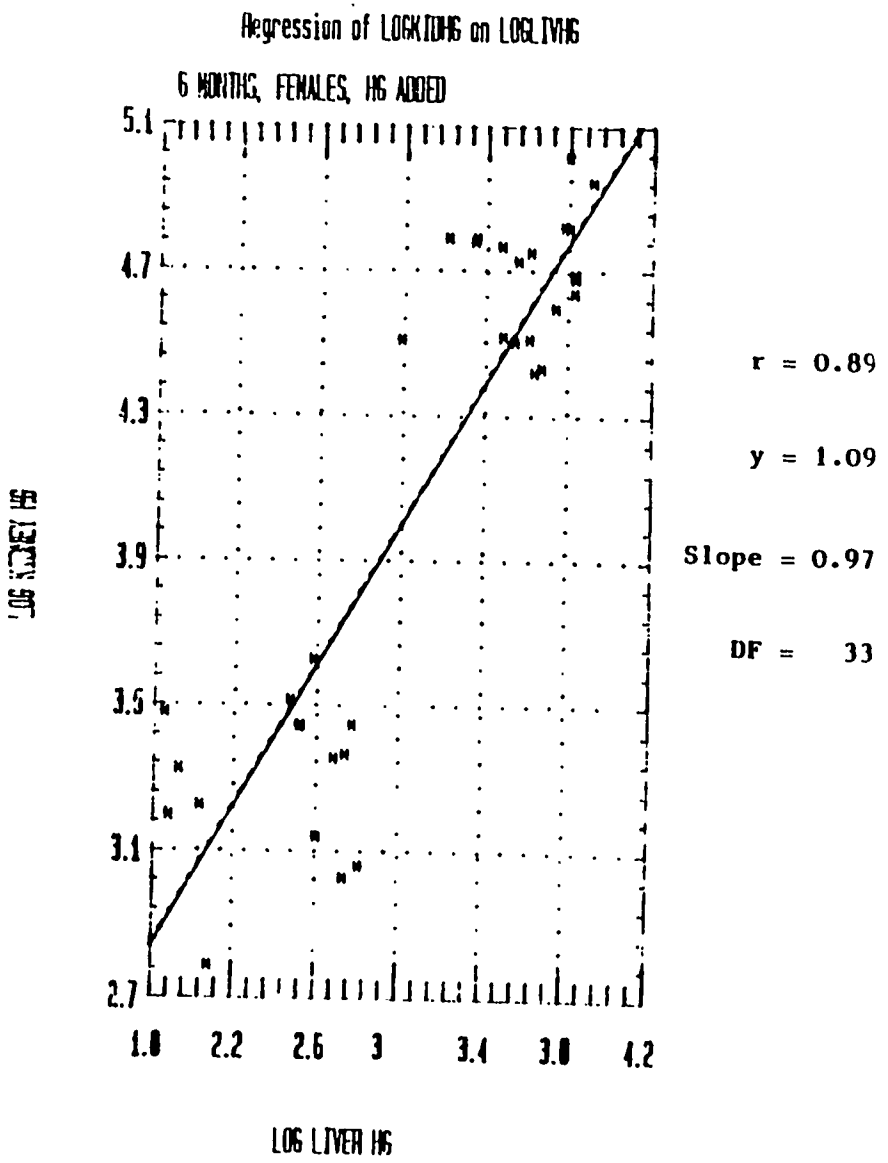

Figure 26

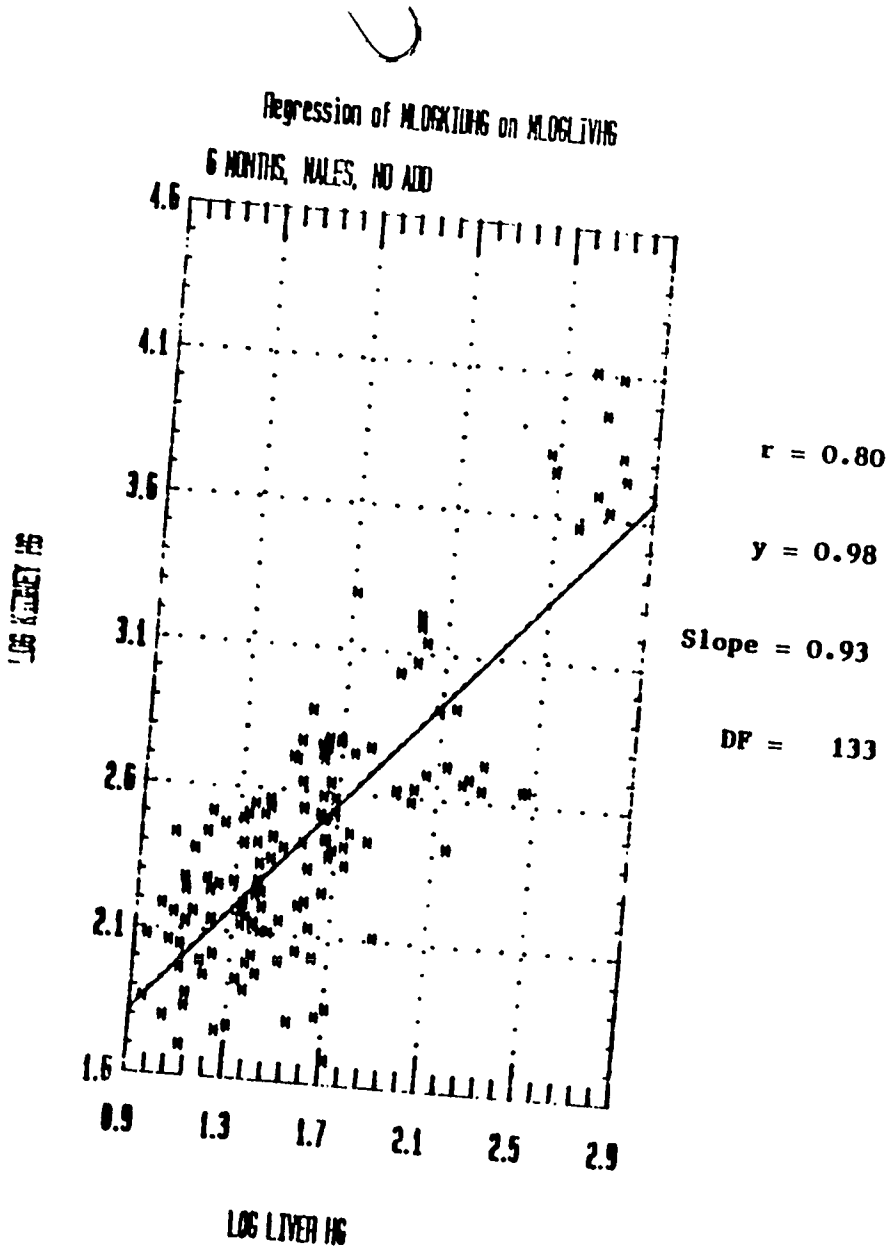

Figure 27

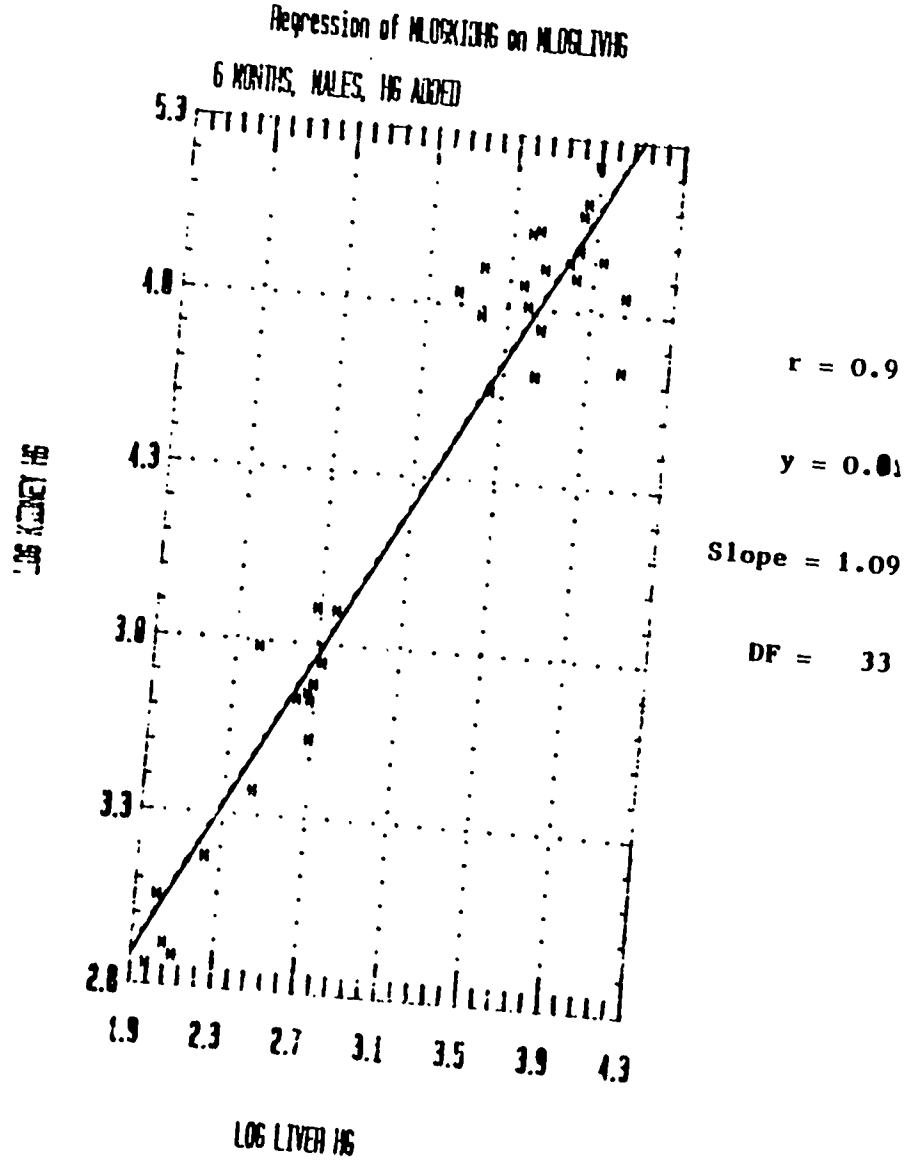

Figure 28

1331.940630.008

Addendum to the East Fork Poplar Creek — Sewer Line Beltway Remedial Investigation Report

This report is an addendum to the *East Fork Poplar Creek - Sewer Line Beltway Remedial Investigation Report*, DOE/OR/02-1119&D2, and does not replace the original document but should be used in concert with DOE/OR/02-1119&D2

Date Published — June 1994

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Submitted to the
U.S. Department of Energy
under contract DE-AC05-91OR21950; ESC

94-070P/062494

SCIENCE APPLICATIONS INTERNATIONAL CORPORATION

contributed to the preparation of this document and should not be considered an eligible contractor for its review.

CONTENTS

TABLES v
FIGURES vii
ACRONYMS ix
EXECUTIVE SUMMARY xi

1. INTRODUCTION 1-1
   1.1 INTRODUCTION 1-1
   1.2 PURPOSE 1-1
   1.3 ORGANIZATION 1-2

2. SPECIAL STUDIES 2-1
   2.1 MERCURY SPECIATION 2-1
      2.1.1 Introduction 2-1
      2.1.2 Mercury Speciation Approaches 2-2
      2.1.3 RI Report Studies 2-4
      2.1.4 Post-RI Report Studies 2-5
      2.1.5 Additional Work 2-23
      2.1.6 Conclusions 2-23
   2.2 WETLANDS 2-24
      2.2.1 Introduction 2-24
      2.2.2 Objectives of the Study 2-25
      2.2.3 Methods 2-25
      2.2.4 Results and Discussion 2-28
      2.2.5 Summary 2-33
   2.3 FOOD WEB 2-36
      2.3.1 Introduction 2-36
      2.3.2 Methods 2-36
      2.3.3 Results 2-38
      2.3.4 Summary 2-41
   2.4 SEDIMENT CHRONIC TOXICITY 2-42
   2.5 CLASSIFIED CHEMICALS REVIEW 2-43

3. DEVELOPMENT OF ALTERNATIVE RGOs 3-1
   3.1 RGOs FOR THE PROTECTION OF HUMAN HEALTH 3-1
      3.1.1 Overview of Absorption, Bioavailability, and Exposure Estimates . . 3-1
      3.1.2 Review of Oral Absorption and Bioavailability of Mercury Species . 3-3
      3.1.3 Selection of Bioavailability Factor for Mercury in EFPC Soils 3-7
      3.1.4 Derivation of an Additional RGO for Mercury 3-7
      3.1.5 Quantitative Uncertainty Analysis of RGO 3-8
      3.1.6 Human Health Remediation Goal Option 3-14
   3.2 DERIVATION OF ALTERNATIVE ECOLOGICALLY BASED RGOs FOR MERCURY 3-14
      3.2.1 Introduction 3-14
      3.2.2 Air, Surface Water, and Groundwater RGOs 3-15
      3.2.3 Sediment RGOs 3-16
      3.2.4 Soil RGOs 3-25
   3.3 RGOs FOR HUMAN HEALTH AND ECOLOGICAL PROTECTION . . . 3-41

4. REFERENCES 4-1

APPENDIX

A DRAFT REPORT: COMPARISON OF MERCURY SPECIATION METHODS A-1

B SUMMARY OF OAK RIDGE Y-12 SITE REMEDIAL INVESTIGATION COMPREHENSIVE ENVIRONMENTAL RESPONSE, COMPENSATION, AND LIABILITY ACT (CERCLA) CLASSIFIED INFORMATION REVIEW (U) B-1

TABLES

2.1 Comparison of selective/sequential extraction schemes for mercury speciation in soil/sediment 2-3
2.2 Leachate results for dry soils 2-10
2.3 Leachate results for field-moist soils; soils at body temperature; and soils with deoxycholic acid, mercuric chloride, and mercuric sulfide 2-11
2.4 Mean, maximum, and minimum values of mercury and methyl mercury in selected soils of EFPC wetlands 2-29
2.5 Mean, maximum, and minimum total mercury values in amphibians from selected EFPC wetlands 2-30
2.6 Mean, maximum, and minimum total mercury values in shrews from selected EFPC wetlands 2-30
2.7 Mean, maximum, and minimum values of total mercury and methyl mercury in crayfish from selected EFPC wetlands 2-31
2.8 Mean, maximum, and minimum mercury values for plant foliage from selected EFPC wetlands 2-33
2.9 Mean, maximum, and minimum values of mercury in trees from selected nonwetland and wetland sites in the EFPC floodplain 2-34
2.10 Mercury in water, periphyton, and fish from Upper EFPC and Hinds Creek . . 2-39
2.11 Mercury in filtered and unfiltered water samples, collected March 7, 1994 . . . 2-40
2.12 Mercury concentrations in periphyton and snails from the laboratory grazing experiment 2-41
3.1 Relative percent absorption of mercury compounds 3-4
3.2 Endpoint, evaluation method, and potential RGO for sediment mercury in EFPC from previously published RI Report 3-17
3.3 EFPC sediment RGOs (mg of total mercury/kg) and assumptions 3-18
3.4 Parameters for sediment RGOs 3-24
3.5 Endpoints, evaluation methods, and potential RGOs for surface soil in EFPC floodplain from the previously published RI Report 3-28
3.6 EFPC soil RGOs (C_{soil}; mg of total mercury/kg) and assumptions 3-32
3.7 Toxicity benchmarks for terrestrial organisms 3-35
3.8 Composition of predators' diets 3-36
3.9 Bioaccumulation factors for prey taxa 3-37
3.10 Evaluation of soil RGO parameters for Scenario 3 3-40

FIGURES

2.1 Sample location for mercury speciation and solubility studies 2-7
2.2 Procedure for extracting and speciating mercury compounds in soil samples . . 2-13
2.3 Mercury speciation by the Environmental Monitoring Systems Laboratory extraction procedure of the surface interval (0 to 3 in.) of EFPC soils 2-14
2.4 Mercury speciation by the Environmental Monitoring Systems Laboratory extraction procedure of the deeper interval (8 to 16 in.) of EFPC soils 2-15
2.5 Ternary plot counts of SK_α and HgM_α, FeK_α, and HgL_α 2-17
2.6a TEM bright field image of an area of mercuric sulfide 2-18
2.6b Dark field image of the mercuric sulfide area in Fig. 2.6a 2-18
2.6c SAED pattern of the grain area in Fig. 2.6a 2-19
2.6d EDS spectra of the mercuric sulfide area in Fig. 2.6a 2-19
2.7 Comparison of the X-ray diffraction patterns for mercury-enriched soil fractions with as-received soil and synthetic metacinnabar 2-21
2.8 Thermal desorption of mercury 2-22
2.9 EFPC 100-year floodplain and wetlands 2-27
3.1 Probability density functions: exposure assumptions 3-9
3.2 Probability density functions: bioavailability of mercury in EFPC soils 3-11
3.3 Probability distribution: RGO for mercury in EFPC soil 3-12
3.4 Sensitivity analysis results 3-13
3.5 Food web relationships of terrestrial biota sampled or modeled for EFPC ERA and RGOs 3-31

1. INTRODUCTION

1.1 INTRODUCTION

Lower East Fork Poplar Creek (EFPC), the associated floodplains, and the Sewer Line Beltway (SLB) have been directly and indirectly contaminated as a result of past releases of mercury from the Oak Ridge Reservation Y-12 Plant. In December 1989, the U.S. Environmental Protection Agency (EPA) placed the Oak Ridge Reservation, including EFPC, on the National Priorities List, thus requiring remediation under the Comprehensive Environmental Response, Compensation, and Liability Act (CERCLA) and the Superfund Amendments and Reauthorization Act (SARA) of 1986. In addition, because the remediation of EFPC may significantly affect the environment, the U. S. Department of Energy (DOE) is considering the potential environmental impacts by integrating the evaluation of National Environmental Policy Act (NEPA) values into the CERCLA process.

DOE's integrated approach results in the preparation of several major documents, including the Remedial Investigation (RI) report, the Feasibility Study (FS), and the Proposed Plan (PP). Following resolution of the comments on the D1 version, the D2 draft of the RI Report (DOE 1994) was issued to EPA and the Tennessee Department of Environment and Conservation (TDEC) in January 1994. This addendum to the RI Report, which supplements the information in that report, was developed to be responsive to suggestions by the regulatory reviewers and input from involved citizens requesting additional information.

1.2 PURPOSE

This addendum to the RI Report presents the results of additional studies that were performed to support and further delineate the recommended remediation goal options (RGOs) to be used for identifying the need for remedial action. These studies also included a review of classified chemicals utilized at the Y-12 facility to ensure that the list of chemicals investigated during the EFPC RI is complete and that there are no contaminants of concern (COCs) other than those previously identified.

The process of deriving an RGO for human health effects consists of establishing an acceptable target risk value for exposure to a contaminant and back-calculating the corresponding concentration in the environmental media under evaluation. Following EPA guidance and methods, an initial RGO of 50 (rounded down from 58) mg/kg (ppm) of mercury in soil was calculated based on the site-specific exposure assumptions that children are the most sensitive receptor group and that ingestion and dermal contact are the exposure pathways of greatest significance. A bioavailability factor of 100% was used to calculate the 50-mg/kg RGO because an EPA-verified or accepted method to justify a reduced factor was not available at the time the D2 draft of the RI Report was issued. The relative bioavailabilities or absorption efficiencies of the various chemical forms of mercury in the floodplain soil were not considered in the RI. The toxicity value for mercury that was used in the baseline risk assessment (BRA) to determine the RGO was based on exposure to mercuric chloride. Mercuric sulfide and metallic mercury, the predominant forms of mercury in the EFPC soils, are considerably less mobile and bioavailable than mercuric chloride.

In parallel with the development of the RI Report, regulatory decisions were made at three mercury-contaminated sites in California that support the use of an efficiency factor to account for the difference in the bioavailability of the various forms of mercury in soils (CDM 1993). Through this addendum, this same approach is now being recommended for calculating a revised RGO for identifying the need for remedial action on EFPC floodplain soil that is considered residential or agricultural land.

To provide better evidence of the predominant chemical forms of mercury in the EFPC floodplain soil and thus further justify the use of the proper absorption factor, several mercury speciation studies were conducted by EPA's Environmental Monitoring Systems Laboratory in Las Vegas, the Environmental Sciences Division of the Oak Ridge National Laboratory (ORNL), the K-25 Materials Science Department, and International Technology (IT) Corporation.

This addendum presents the results of these mercury speciation studies and provides the justification for the use of a bioavailability factor of 30% in the human health risk assessment. This adjustment would result in an alternate RGO of 180 mg/kg of mercury for the scenarios involving ingestion and dermal contact by a child. Using the 180-mg/kg RGO as an action level for residential and agricultural lands would significantly reduce the amount of contaminated floodplain soil that would be above the action level and, thus, require remediation.

In addition to the mercury speciation studies, three studies are being conducted to improve the understanding of the interactions of EFPC contaminants with ecological receptors. One study, "Wetlands Study," focuses on the unique conditions found in the EFPC wetlands and a second study, "Food Web Study," addresses the potential transfer of contaminants in the lower portions of the EFPC food web. Additionally, a "Sediment Chronic Toxicity Study" was performed to directly measure potential toxicological effects from the sediments.

1.3 ORGANIZATION

Section 2 of this addendum describes the technical basis and approach for the special studies undertaken and presents the results and conclusions. In Sect. 3, the conclusions drawn from each of the studies are used to derive new RGOs based on the human health risk assessment parameters for the most sensitive receptor. For the ecological risk assessment (ERA), the speciation information and the results of the wetlands and food web studies are used to develop a range of RGOs that can be used by the decision makers based on the level of protectiveness desired.

Section 3.3 of this addendum briefly discusses the risk management use of the ranges of RGOs derived from the information provided by the special studies.

2. SPECIAL STUDIES

This section describes the various special studies that were conducted in early 1994 to support both the human health and the ecological risk assessments. Section 2.1 describes a series of investigations performed to determine or further strengthen the weight of evidence that mercury in the EFPC floodplain is in a form that is neither readily mobile nor bioavailable. Section 2.2 describes the sampling effort and summarizes the data of a wetlands study, the results of which have been used to refine transfer coefficients for the ERA. Section 2.3 presents a food web study conducted by the Environmental Sciences Division at the K-25 Site. Section 2.4 describes an ongoing sediment chronic toxicity study. Each of these studies is presented in a summary manner, focusing on integration of the data and conclusions into both the human health and the ecological risk assessments. Each study will be published as an independent report that will more fully present the data and data analysis.

2.1 MERCURY SPECIATION

2.1.1 Introduction

The purpose of the EFPC RI Report is to assess the nature and extent of contamination within the boundaries of the 100-year floodplain of the creek and to determine the risk to human health and the environment. Though the majority of the effort in the site characterization phase of the RI was spent in determining the extent and distribution of mercury and other contaminants, specific studies were also performed to assess the form, or species, of the mercury in the floodplain soils. These studies indicate that the mercury within the soil column may have been naturally altered to a mixture of compounds that differ in properties (such as solubility, bioavailability, mobility, toxicity, etc.) from mercuric chloride, which was the basis of the default assumptions that were incorporated into the BRA. The study of a particular compound in trace quantities—especially one with unique properties such as mercury—and the determination of the relative proportions of each compound is not a straightforward or simple task. Much of the information about the form of mercury is derived from behavioral observations or empirical associations. Direct investigative techniques for submicron-sized particles are limited.

Determining the form of mercury allows environmental scientists to incorporate compound-specific reference values into the risk assessment process when considering biological uptake and toxicity. As an alternative to investigating the form of mercury, data were compiled on the solubility and bioavailability of mercury compounds in the EFPC soils. This line of investigation incorporates the site-specific properties of the soil medium without absolute determination of the mercury form.

This section of the addendum presents the approach to mercury speciation that was used during the RI and results from studies conducted since the RI Report was submitted in January 1994. Conclusions concerning the probable form of mercury are presented. These conclusions are incorporated into the risk assessments of Sect. 3 and then used in the development of site-specific RGOs.

2.1.2 Mercury Speciation Approaches

The methods used to characterize the forms of mercury in the EFPC soils include X-ray diffraction (XRD); optical microscopy and scanning and transmission electron microscopy (SEM/TEM); X-ray fluorescence (XRF); and sequential/selective chemical extraction. The level of confidence attributed to each technique ranges from unequivocal "fingerprinting" of the crystal structure to operational definitions that suggest that the mercury compound in question "behaves like" a pure reference compound. A brief discussion of each investigative method and the type of information that can be obtained from its use follows.

XRD. The most reliable technique for identifying a crystalline substance is XRD. A sample is subjected to a monochromatic X-ray beam, causing electrons in the path of the primary beam to emit X-rays, which are scattered in a characteristic manner by the unique lattice structure of the compound. Several conditions must exist for this method to be successful. The material must be in a crystalline or near-crystalline form. The compound must exist in sufficient quantity and the crystals must be of sufficient size to make the reflections distinguishable from background noise. Positive identification can be inhibited by coincident peaks of other compounds; therefore, secondary peaks must be employed for identification.

Optical microscopy, SEM, TEM. Electron microscopy provides evidence on the petrography, morphology, and chemistry of the soil. Combined with analytical capabilities, it can be used to analyze individual contaminant particles and ascertain elemental associations. Electron microscopy can also be used in conjunction with microdiffraction to identify crystalline materials.

XRF. XRF, used with SEM, provides quantitative and semiquantitative analyses of element composition and corresponding elemental associations.

Sequential/selective chemical extraction methods. Sequential/selective chemical extraction methods provide an operational definition of a compound by comparing the responses of compounds in soils to extractive solutions with the responses of reference materials to extractive solutions (see Table 2.1 for a matrix of solutions versus mercury forms used in EFPC extraction studies). The technique is usually calibrated by dosing uncontaminated soil with a pure compound and then observing the success of an extractive solution. This method can be complicated by compound interferences, nonuniform mixtures, or matrix interactions. Though this method of speciation constitutes the largest data set for the EFPC soils, it is subject to the most uncertainty of the above procedures.

The methods discussed in this section were used to provide as much information about the mercury form, species, and solubility as possible. No one method provides the absolute proof that mercury resides in a particular form throughout the 23 km (14.5 miles) of floodplain; however, in combination, the weight of evidence indicates that mercuric sulfide and metallic mercury are the most dominant species within the soils. It has been conclusively shown that the amounts of organic mercury compounds and soluble forms of mercury, such as mercuric chloride, are negligible and that their contributions to the total mercury toxicity are insignificant.

Table 2.1. Comparison of selective/sequential extraction schemes[a] for mercury speciation in soil/sediment

Form extracted/recovered	Revis et al. 1989	Radian/SAIC 1992	EPA/EMSL 1994	Sakamoto et al. 1992
Metallic mercury	5 d @ 150°C	30 min wet purging	1:3 HNO_3	ND[b]
Mercuric sulfide	Sodium sulfide (Na_2S) after 12 *M* HNO_3	Sodium sulfide (Na_2S) after 12 *M* HNO_3	HCl + HNO_3 (aqua regia)	Acidic CuCl in 3% NaCl
Methyl mercury	Toluene extract, gas chromatography	KOH/methanol extract, ethylation, CVAFS	ND	ND
Mercuric oxide	12 *M* HNO_3 digest	12 *M* HNO_3 digest	0.2 *M* HNO_3	0.05 *M* H_2SO_4
Mercurous oxide		30 min wet purging	ND	ND
Mercuric chloride		Ethanol extract	0.01 *M* K_2SO_4 + 0.01 *M* KCl from toluene	ND
Mercurous chloride			HCl + HNO_3 (aqua regia)	ND
Mercury amalgams		Included with 12 *M* HNO_3 digest	1:3 HNO_3	ND
Organic mercury			Toluene	0.01 *M* $Na_2S_2O_3$ from chloroform

[a] Schemes are simplified here to conserve space. See reference for details.
[b] ND means "Not Determined."

2.1.3 RI Report Studies

The following is a synopsis of the data presented in Sect. 3 of the RI Report:

Toxicity Characteristic Leaching Procedure (TCLP). Several samples were subjected to the TCLP, which simulates leaching in slightly acidic rainwater conditions. Though total mercury concentrations in the soil samples were in the 1000- to 2000-mg/kg range, no concentrations of mercury above the analytical detection limit (0.1 ppm) were extracted from EFPC soil.

Treatability Study. As part of the bench-scale treatability tests (Radian 1993) to select a suitable technique for treating the mercury-contaminated soils, extraction studies were performed using a variety of solutions. The list included distilled water, synthetic acid rain water, nitric acid, hydrochloric acid, hydrobromic acid, acetic acid, ethylenediametetraacetic acid (EDTA), citric acid, ammonium hydroxide, sodium hypochlorite, thiourea, and a proprietary reagent using potassium iodide (KI/I_2). Very little mercury is extracted with the above leaching agents, with the exception of unique compounds such as hydrobromic acid, sodium hypochlorite, or the potassium iodide solution, which are not indigenous to the natural environment.

Revis et al. 1989b. Revis demonstrated the effectiveness of sodium sulfide as a selective extractant for mercuric sulfide (see Table 2.1) after a 12-*M* nitric acid extraction of all other inorganic mercury forms and used a thermal treatment [150°C (302°F) for 5 d] to measure the elemental mercury content in separate portions of the soils. These measurements demonstrated that 3 to 8% of the mercury is in elemental form, 84 to 98% is in mercuric sulfide form, and 0.003 to 0.010% is methyl mercury (determined by direct extraction and gas chromatography).

Original Report Attached #1

Methyl mercury analyses by Brooks Rand Laboratory. Three samples were taken in the areas of highest mercury content in the EFPC floodplain and submitted to the Brooks Rand Laboratory for methyl mercury analysis by tetraethylborate derivitization and atomic fluorescence spectroscopy. Results ranged from 0.002 to 0.004% of total mercury and are commensurate with the Revis work. The overriding conclusion of the RI is that methyl mercury constitutes a minor fraction of total mercury and, although it may be significant for ecological exposures, it is not of concern to human health in the floodplain environment.

Original Report Attached #2

Optical microscopy and SEM. Twenty samples were obtained along the length of the floodplain and submitted for both optical microscopy and SEM. Soil cores were inspected under the optical mount for soil morphology, structure, and content. Fly ash and coal fragments were found to be more abundant in the mercury-rich samples and to be less abundant in those samples with less mercury. It is postulated that fly ash, coal fragments, and mercury are transported together and represent releases to the creek during the same time frame; however, no physical or chemical relationship could be established between the mercury and the ash material. Moreover, no genetic or characteristic differences were observed for the various samples over the length of the creek or in relation to the amount of mercury present (with the exception of the aforementioned correlation with fly ash).

Original Report Attached #3

SEM was employed to detect, image, and microanalyze the mercury-bearing matter in the soil samples. Backscatter electron images (dot maps indicating locations of elemental concentrations) displayed a strong association (co-location) between mercury and sulfur in all of

the samples. Additional microprobe work on a number of individual soil particles indicated a sulfur-to-mercury mole ratio in the 0.90 range, compared to a value of 0.98 for a cinnabar (mercuric sulfide) standard. The consistent similarity of the observed ratio to a mercuric sulfide standard would not be expected if only a physical association of elemental mercury and sulfur existed.

Current risk assessment dose calculations for mercury are based on mercuric chloride, a soluble form of mercury not expected to exist in the EFPC environment. The above data, gathered for the RI Report, indicate that mercury within the EFPC floodplain is in a relatively insoluble form and is probably complexed with sulfur in some form (probably mercuric sulfide).

2.1.4 Post-RI Report Studies

After the January 1994 draft of the RI Report was completed, several studies were performed to define the predominant form of mercury in EFPC floodplain soil. The following studies provide additional insight into the species and biological availability of the mercury in the EFPC floodplain. Inconsistencies between observed results and the assumptions incorporated into the development of RGOs indicated additional work was needed to fill a data gap. Discussions were held with researchers at ORNL and scientists at DOE's K-25 Site Materials Science Department and EPA's Environmental Monitoring Systems Laboratory in Las Vegas to seek more definitive methods to speciate the dominant form of mercury and/or to characterize its biological availability. The following is a summary of the results from those investigations. A separate report containing a complete discussion of the methods, results, and conclusions will be published for each study. These reports will be contained within the EFPC project file as part of the administrative record file. Original Report Attached #4

Bioavailability study. A bioavailability study was conducted by Dr. Ralph Turner of the Environmental Sciences Division at ORNL to determine the fraction of mercury in EFPC soils that is available for absorption in the human digestive system, the most sensitive route of human exposure. The bioavailability study was designed to simulate the human digestive system and was adapted from another study, the Almaden Quicksilver County Park Risk Assessment, Santa Clara County, California (CDM 1993), which has undergone review by the State of California. The following is a condensation of Dr. Turner's report.

Two samples were obtained at each of 10 locations (Fig. 2.1) along the EFPC floodplain to represent the range of possible environments along the length of the creek. Locations were also selected to coincide with significant concentrations of mercury (>50 mg/kg). At each location, samples were collected to represent the surface horizon and a subsurface soil horizon. The first sample was collected from 0 to 7.5 cm (0 to 3 in.) to provide evidence for the form of mercury that would most likely exist in an oxidizing environment and that would relate to a direct soil exposure pathway. The second sample was collected from a deeper stratum (see map for actual depths) that appeared to contain the highest mercury concentration. If the location was situated in an area where the water table was near the surface, the second soil sample was obtained below the water level.

In the laboratory, samples were air dried at room temperature [22 to 24°C (72 to 75°F)], lightly crushed with a mortar and pestle, and sieved to <2 mm (0.08 in.) to remove rocks, roots, etc. The samples were then crushed to <180 μm and subsampled for total mercury (SW-846

Method 7471), total carbon, and total sulfur. Aliquots of each soil sample were added to distilled, deionized water adjusted to pH 2.5 with 16 *N* hydrochloric acid. The samples were shaken and the pH was maintained for 4 h. After 4 h, the samples were allowed to settle and a portion of the supernatant was passed through a 0.20-μm filter. The filtrate was preserved and submitted for analysis of total mercury.

The remaining soil-solution suspension was combined with distilled, deionized water to the original solid/solution ratio and the pH was adjusted to 6.5 with sodium hydroxide. Using the procedure described above, filtrate samples were taken after 4 h and submitted for mercury analysis. As a check of potential variables that might affect the results, the leaching procedure was repeated for samples such as: (1) as-received moist soil, (2) soil maintained at body temperature [37°C (98.6°F)] during leaching, and (3) soil with 10 mg/L deoxycholic acid added (constituent of the human digestive system). Also tested as control standards were mercuric chloride and mercuric sulfide in both cinnabar and metacinnabar forms.

Mercury concentrations in these soil samples ranged from 15 to 2700 mg/kg and sulfur concentrations ranged from 26 to 1700 mg/kg. The predominant form of mercury is indicated from other studies to be mercuric sulfide. In 19 of 20 samples, sufficient sulfur was present to bind all of the mercury as mercuric sulfide, and mercury is significantly correlated with sulfur ($r=0.84$) in the 20 samples. By removing the sample with the maximum value (ZN3210127), the mercury-sulfur correlation becomes very strong ($r=0.92$). With the exception of the one sample, significant mercury was not extracted at either pH. Less than 5% of the mercury solubilized in 15 of the samples. Total soluble mercury for the 19 samples ranged from 0.3 to 14.2%, with an average of 3.2% (Table 2.2).

The remaining sample, apparently geochemically different from the other samples, leached significantly more mercury. Of the total mercury concentration in this sample (2700 mg/kg) 570 and 300 μg/L were removed at pHs of 2.5 and 6.5, respectively, for a combined percent removal of 45.9%. Furthermore, this sample was unique among the 20 samples in displaying headspace mercury volatilization at room temperature. The sample was taken at the most upstream location immediately below the outfall of the Y-12 Plant. It is inferred, because of its proximity to the Y-12 Plant, that this sample may represent deposition of mercury that is not characteristic of the downstream locations. Two other sample locations 457 m (1500 ft) downstream of this site did not display similar leaching or sample headspace mercury vapor.

The change in percent solubility for samples leached at 37°C (98.6°F) (percent leached at 37°C minus percent leached at room temperature) ranged from -7.7 to 1.8% (Table 2.3). The change in percentage mercury leached for two samples with deoxycholic acid (percent leached with acid minus percent leached without) was 1.1 and -2.1% but the total percent of mercury leached from the two samples was only 2.1 and 1.3%. Leaching of as-received moist soil showed a wide range of percentage change, though the leachate concentrations were still relatively low. For example, the soil with the largest relative increase in leachate concentration (+426%) increased from 1.50 μg/L to only 7.9 μg/L, well within the range of leachate concentrations of the 19 dry soils. For both the cinnabar and metacinnabar control standards, the fraction of the sample dissolved was much less than 1%. The entire mercuric chloride samples dissolved and the leachate concentrations from these samples were almost 1000 times higher than the highest soil leachate concentration (540,000 versus 570 μg/L).

Table 2.2. Leachate results for dry soils

Sample	Mercury (mg/kg)	pH 2.5		pH 6.5		% Soluble total
		Mercury (µg/L)	% Soluble	Mercury (µg/L)	% Soluble	
ZN3210115	260	0.70	0.4	1.00	0.5	0.9
ZN3210127	2,700	570.00	29.3	300.00	16.6	45.9
ZN3340211	270	0.80	0.4	0.80	0.4	0.8
ZN3340223	1900	77.00	5.5	20.00	1.4	6.9
ZN3340312	230	0.20	0.1	1.80	1.1	1.2
ZN3340324	2100	30.00	1.9	11.00	0.7	2.6
ZE5470412	85	0.20	0.3	0.40	0.6	0.9
ZE5470424	1300	73.00	7.6	14.00	1.5	9.1
ZE5380512	67	1.60	3.2	1.10	2.2	5.4
ZE5380524	2100	26.00	1.7	8.20	0.5	2.2
ZE5340619	140	0.10	0.1	0.20	0.2	0.3
ZE5340621	1200	10.50	1.2	4.90	0.6	1.8
ZE5150717	230	1.80	1.1	2.60	1.5	2.6
ZE5150729	900	13.00	2.0	3.20	0.5	2.5
ZE3770810	480	2.40	0.7	1.50	0.4	1.1
ZE3770822	15.3	1.10	9.7	0.50	4.5	14.2
Z43740918	55	0.20	0.5	0.20	0.5	1.0
ZE3740920	780	14.00	2.4	5.80	1.0	3.4
ZE3181013	28	0.03	0.1	0.20	1.0	1.1
ZE3181025	390	6.30	2.2	1.90	0.7	2.9

Table 2.3. Leachate results for field-moist soils; soils at body temperature; and soils with deoxycholic acid, mercuric chloride, and mercuric sulfide

Sample	Mercury (mg/kg)	pH 2.5		pH 6.5		% Soluble total
		Mercury (µg/L)	% Soluble	Mercury (µg/L)	% Soluble	
ZN3210127-37[a]	2,630	580.00	29.0	170.00	9.2	38.2
ZN3210115-37[a]	260	0.72	0.4	1.10	0.6	0.9
ZN3340223-37[a]	1,900	97.00	6.9	14.00	1.0	7.9
ZE3740918-37[a]	55	0.06	0.1	0.80	2.0	2.1
ZE5470424-37[a]	1,300	88.00	9.1	16.00	1.7	10.8
ZE5470424-W[b]	1,300	110.00		27.00		
ZE3740918-W[b]	55	0.10		0.87		
ZE3770810-W[b]	480	1.20		7.90		
ZE3740920-W[b]	780	7.80		13.00		
ZE3740918-C[c]	55	0.07	0.2	0.80	2.0	2.1
ZE3740920-C[c]	780	6.50	1.1	1.10	0.2	1.3
Mercuric chloride		540,000.00	98.8	450,000.00	109.3	100.0
Cinnabar		0.34	0.0	42.00	0.0	0.0
Metacinnabar		0.05	0.0	0.50	0.0	0.0

[a] Conducted at 37°C.
[b] Field-moist soils. Percentage leached not calculated because mercury measurements were not made on bulk soils.
[c] With 10 mg/L deoxycholic acid.

Conclusions from this study are based on site-specific data rather than information drawn from reference materials. Although the procedure is a simple representation of a complex system, the human digestive system, the solubility, and hence bioavailability, of mercury in EFPC soils is obviously substantially different than pure mercuric chloride. Consequently, incorporation of a bioavailability factor of 1 (i.e., 100%) in the human health risk assessment may be unduly conservative.

EPA's Environmental Monitoring Systems Laboratory speciation procedure. EPA's Environmental Monitoring Systems Laboratory in Las Vegas agreed to assist DOE in the EFPC speciation effort by applying a new method under development for the speciation of mercury compounds (Miller 1993). This procedure takes advantage of sequential/selective extraction of mercury, as does the Revis method, but uses different extractive solutions in its procedure (see Table 2.1). A major difference involves the determination of metallic mercury using acid extraction. Revis first extracted all of the inorganic mercury, except mercuric sulfide, with 12-*M* nitric acid and then employed sodium sulfide to extract mercuric sulfide. Metallic mercury was estimated by measuring the difference between unheated and heated [150°C (302°F), 5 d] total mercury content of separate soil aliquots. The Environmental Monitoring Systems Laboratory procedure (Fig. 2.2) groups its compounds into water soluble, acid soluble, metallic/amalgamated mercury, and mercuric sulfide. Water-soluble mercury represents the mercuric chloride fraction, and the acid-soluble mercury represents the mercuric oxide portion of the soil. This method uses only aqua regia (hydrochloric acid + nitric acid), instead of sodium sulfide, to extract the mercuric sulfide fraction.

Original Reports Attached (Miller 1993) # 5 (EPA 1994b) # 6

The following paragraphs summarize a report by Dobb, Miller, and Cardenas (EPA 1994b). Splits of the 20 soil samples taken for the bioavailability study were supplied to the Environmental Monitoring Systems Laboratory for speciation work. The samples were stored on ice and shipped overnight to the Environmental Monitoring Systems Laboratory's contract laboratory, Lockheed Environmental Systems & Technologies Company, for analysis. Upon receipt, the samples were dried at 45 to 50°C (113 to 122°F) and weighed to determine moisture content. The samples were pulverized to pass a 60-mesh sieve (<250 μm) in a tungsten carbide-lined ball mill. A 2-g (0.07-oz.) aliquot of dried and pulverized EFPC soil was processed using the Environmental Monitoring Systems Laboratory extraction procedure. After performing the extraction procedure, XRF analysis showed a 98 to 99% extraction of mercury from samples that originally contained 2000 to 3000 mg/kg of mercury. Each extract in the process was analyzed for mercury by inductively coupled plasma - mass spectrometry (ICP-MS). Mercury concentrations for each step in the process determined by ICP-MS were summed and compared with total mercury determined by cold vapor atomic absorption spectroscopy. Percent relative standard deviations ranged from 1 to 27%, and 8 out of 20 samples had percent relative standard deviations >15%. Replicate analyses performed on two samples showed relatively close correlation. Matrix spikes indicated recoveries ranging from 102 to 115%.

Results of this sequential extraction procedure are documented in Figs. 2.3 and 2.4. No organic mercury forms were detected by the Environmental Monitoring Systems Laboratory process; however, sample preparation (e.g., drying, grinding) may have affected the test. Analyses performed by the Brooks Rand laboratory (discussed in Sect. 3 of the RI Report) confirm that organic mercury constitutes <0.01%. Water-soluble forms (mercuric chloride) were <1%, and most were <0.1 to 0.3%. The 0- to 7.5-cm (0- to 3-in.) interval samples had metallic/amalgamated mercury as the principal compound, and mercuric oxide increasing in the

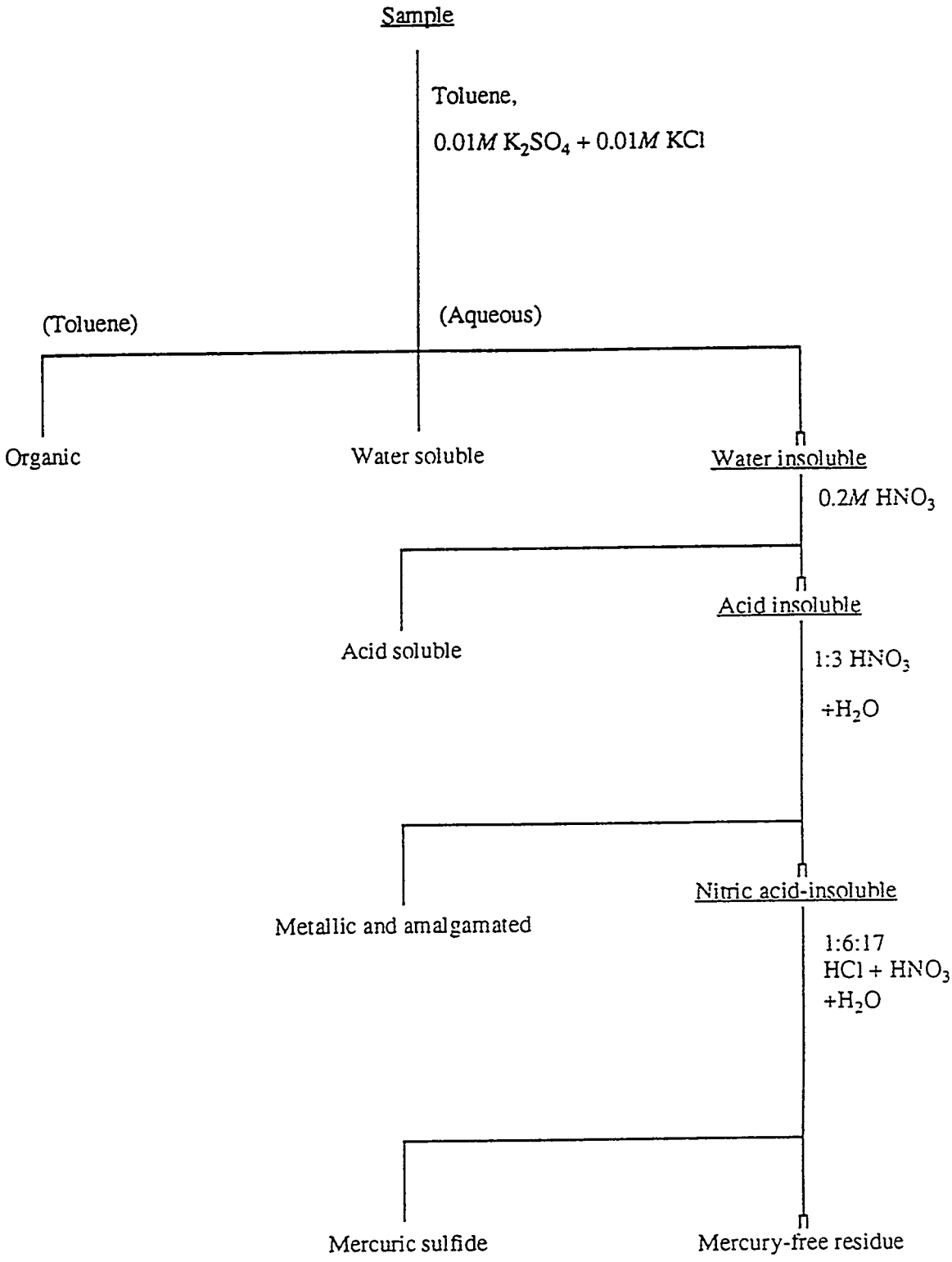
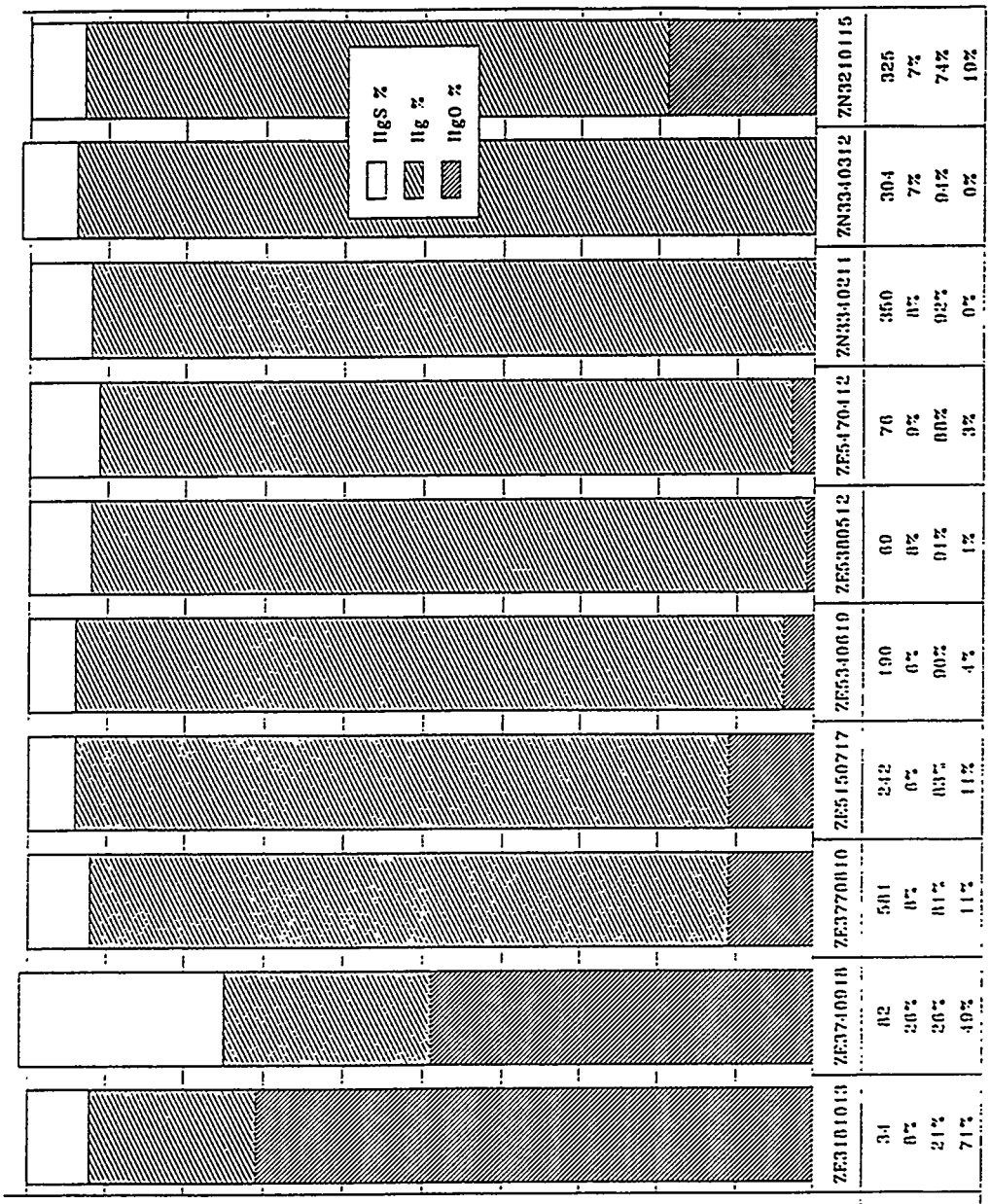
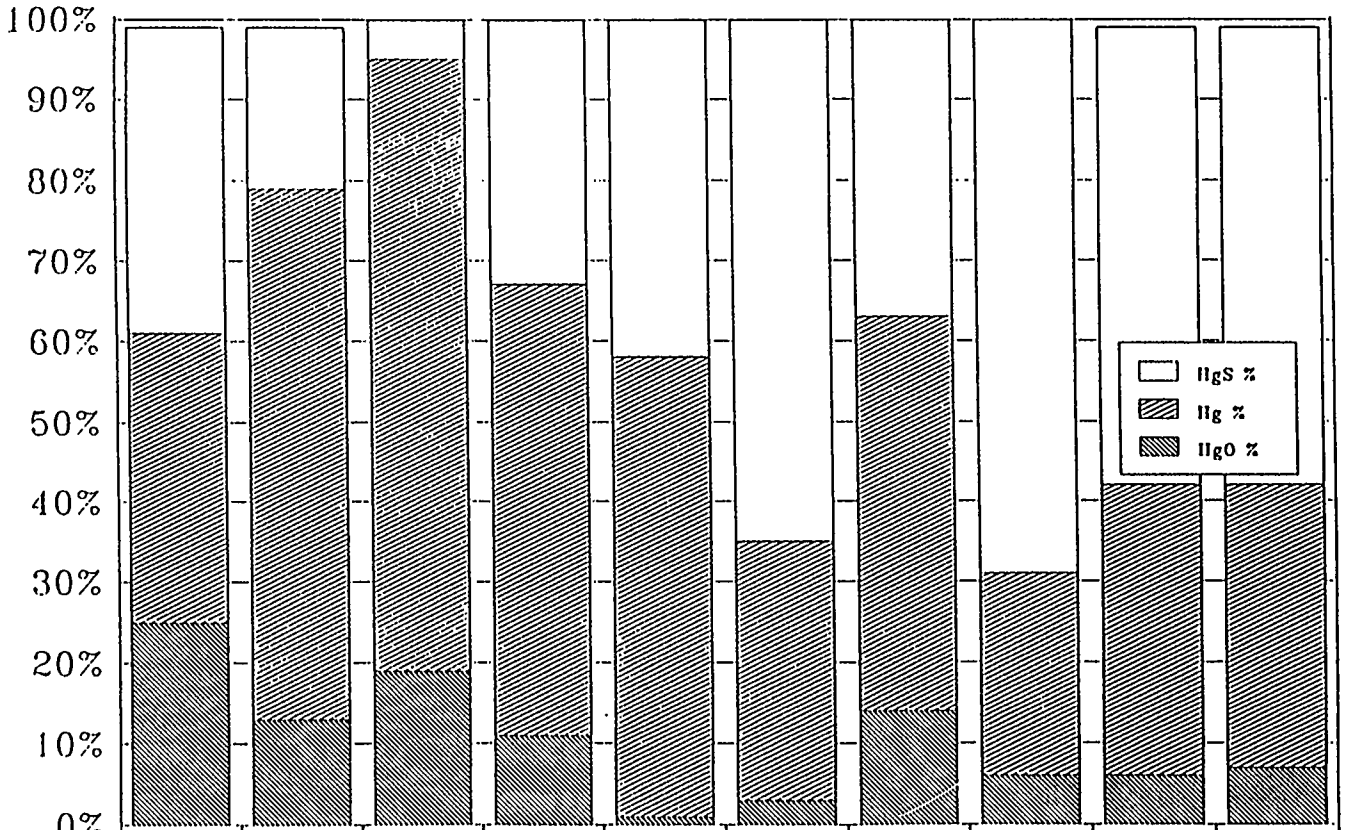


Fig. 2.2. Procedure for extracting and speciating mercury compounds in soil samples.
Note: a single vertical bar above an analyte, |, indicates a solution; a double vertical bar, ||, indicates a solid.)



	ZE3101013	ZE3740910	ZE3770910	ZE5150717	ZE5340610	ZE5300512	ZE5470412	ZN3340211	ZN3340312	ZN3210115
Total Hg mg/kg	34	82	501	242	190	60	76	350	304	325
HgS %	0%	20%	0%	0%	0%	0%	0%	0%	7%	7%
Hg %	21%	20%	11%	83%	90%	91%	88%	92%	91%	74%
HgO %	71%	40%	11%	11%	4%	1%	3%	0%	0%	19%

Fig. 2.3. Mercury speciation by the Environmental Monitoring Systems Laboratory extraction procedure of the surface interval (0 to 3 in.) of EFPC soils. The most upstream location is to the right.



	JE3181025	ZE3740920	ZE3770822	ZE5150729	ZE5340621	ZE5380524	ZE5470424	ZN3340223	ZN3340224	ZN3210127
Total Hg mg/kg	477	813	271	1002	1667	1962	1226	2045	2420	3036
HgS %	38%	20%	5%	33%	42%	65%	37%	69%	57%	57%
Hg %	36%	66%	76%	56%	57%	32%	49%	25%	36%	35%
HgO %	25%	13%	19%	11%	1%	3%	14%	6%	6%	7%

Fig. 2.4. Mercury speciation by the Environmental Monitoring Systems Laboratory extraction procedure of the deeper interval (8 to 16 in.) of EFPC soils. The most upstream location is to the right.

downstream samples. Mercuric sulfide was indicated to be <10% in all but one of the samples from this interval. Samples from the deeper interval [~20 to 40 cm (8 to 16 in.)] showed higher levels of mercuric sulfide, but metallic/ amalgamated mercury was still the dominant form. These results are discordant with other results. Additional laboratory work (Appendix A) and technical discussions between the Environmental Monitoring Systems Laboratory team and the EFPC team are ongoing to discern the reason.

Electron diffraction and XRD. The following is a summary of a study by the K-25 Site's Materials Science Department, which employed TEM and XRD to provide positive identification of mercuric sulfide in EFPC soils. XRD is a bulk analysis tool that requires sufficient concentrations of the compound in question; TEM can utilize low concentrations of the material. By adding selected area electron diffraction (SAED) to TEM, particles <0.1 μm can be investigated. Mercury-enriched EFPC soil samples were characterized with a JEOL 2000FX transmission electron microscope. The microscope was equipped with an energy dispersive spectrometer (a type of XRF detector) for elemental analysis. This arrangement allowed for characterization of material by morphology, chemistry, and electron diffraction on small particles and groups of particles. An additional technique, dark field imaging, was used to identify the source of observed diffraction rings/spots. With dark field imaging, only the diffracted beams of electrons are used to observe the grain, and a correlation can be made between a diffraction pattern and an individual grain.

Original Report Attached #7 (Barnett, 1994)

Mercury-enriched samples from a small number of highly contaminated sites consisting of soil particles <2 μm in size were processed, placed on grids, and scanned for the presence of mercury and sulfur with a JEOL 840 SEM. Grid openings (~8000 μm square) that contained these elements were identified and used for the TEM. Because mercuric sulfide is relatively opaque to electron transmissions, the chemistry of each opaque grain was determined using the energy dispersive spectrometer (EDS). If the characteristic peaks of mercury and sulfur were observed, a photograph of the grain or grains was taken to show its morphology, and the SAED image photographed to document the diffraction pattern. Finally, a 100-s chemical analysis was performed to confirm the chemistry. If a mercuric sulfide grain was located on a clay grain, the grain would not be used because the clay SAED image can interfere with the unequivocal identification of metacinnabar. In many instances, the grains were associated with or near clay material, as would be expected in a natural soil. Most of the SAED images, therefore, have some component of clay diffractograms in them.

Thirty-seven analyses of grain areas are displayed in a ternary plot in Fig. 2.5. A ternary plot shows the percentage contribution of three end members. At its apices are the counts for sulfur (Kα) and mercury (Mα), lower left; iron (Kα), top; and mercury (Lα), lower right. The majority of the grains are located very close to the composition of the mercuric sulfide standard (asterisk symbols) but away from the mercuric oxide standard (triangle symbols). A small number of grain areas contain higher amounts of iron than most of the mercuric sulfide but these grains still display similar mercury-to-sulfur ratio.

Figure 2.6a is a TEM bright field image of a mercuric sulfide particle not associated with clays; Fig. 2.6b is a dark field image of the same area. The bright areas in Fig. 2.6b indicate the source of the diffraction rings and spots used for positive identification of mercuric sulfide in the SAED image (Fig. 2.6c). The grain areas are made up of many small individual mercuric sulfide grains (<50 nanometer) composed of crystalline mercuric sulfide (as indicated by the

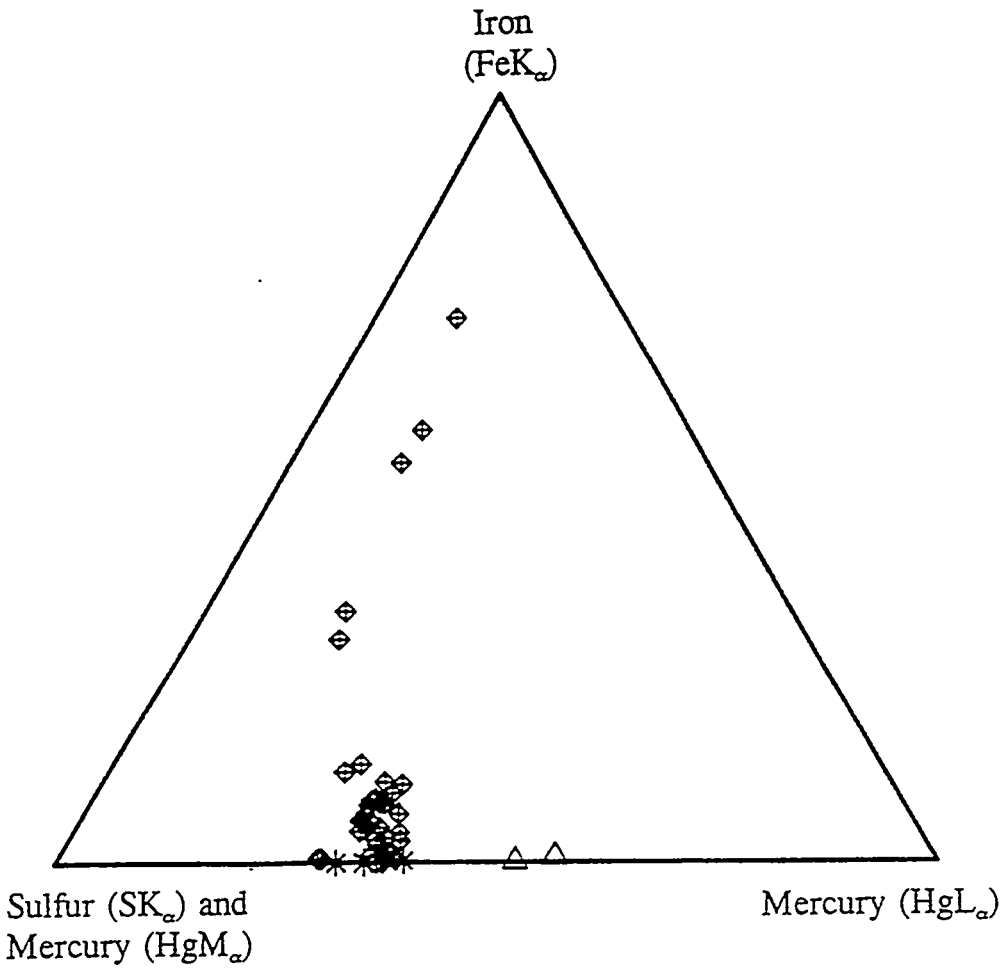


Fig. 2.5. Ternary plot of counts of SK_α and HgM_α, FeK_α, and HgL_α. The bulk of the mercuric sulfide grains (diamonds) plot near the location of the mercuric sulfide standards (asterisks) but they plot away from the mercuric oxide composition (triangles).

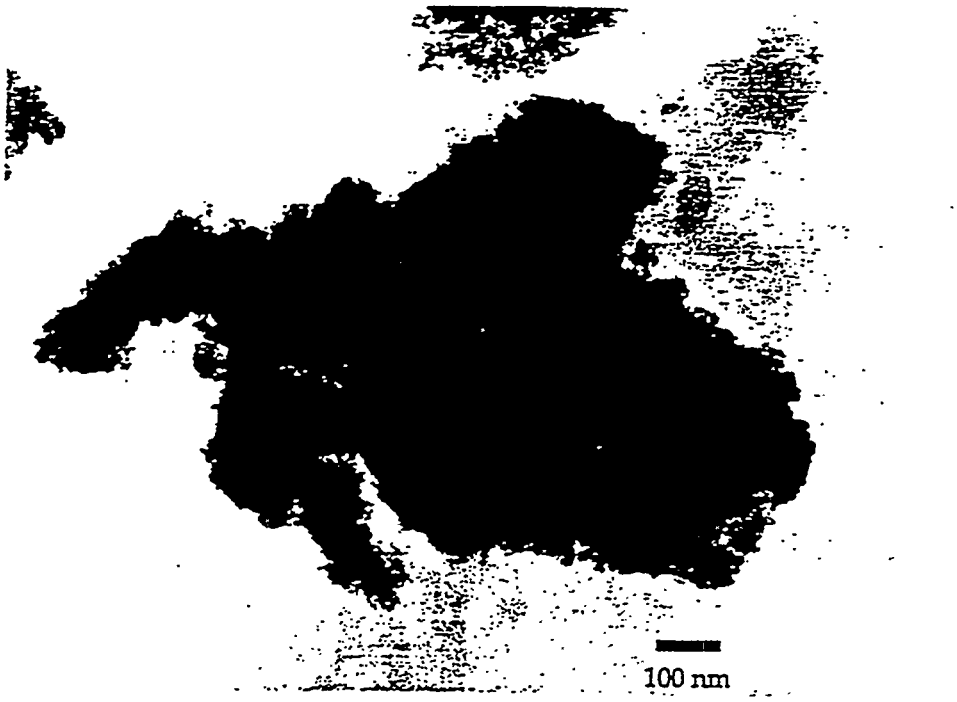


Fig. 2.6a. TEM bright field image of an area of mercuric sulfide. The bar is 100 nanometers long.

Fig. 2.6b. Dark field image of the mercuric sulfide area in Fig. 2.6a. Note that the bright areas indicate the source of the diffraction rings and spots in Fig. 2.6c. The grain area is really made up of many very small (<50 nanometers) mercuric sulfide grains. The bar is 100 nanometers long.

Fig. 2.6c. SAED pattern of the grain area in Fig. 2.6a. There are multiple points on the diffraction rings indicating that the grain area is made up of many individual mercuric sulfide grains and less crystalline mercuric sulfide. The circle indicates where the diffracted beams have come from to form the dark field image of Fig. 2.6b.

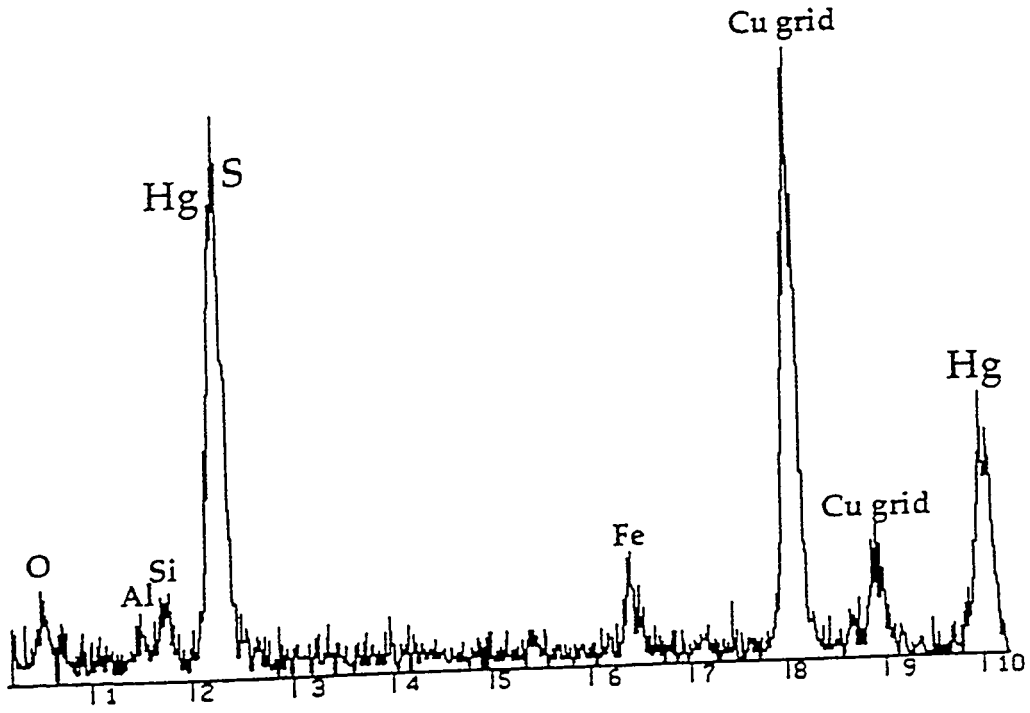


Fig. 2.6d. EDS spectra of the mercuric sulfide area in Fig. 2.6a. The spectra is dominated by the mercury (Hg) and sulfur (S) peaks with a minor amount of iron (Fe).

presence of diffuse rings). Figure 2.6d shows the EDS spectra of the mercuric sulfide area, which is dominated by mercury and sulfur with minor amounts of iron.

Prior to the TEM investigation, standard XRD was employed using a Phillips high-angle diffractometer. XRD analysis of as-received EFPC soils containing up to 3000 mg/kg of mercury failed to reveal any mercury compounds. Laboratory enrichment of EFPC soils resulted in two samples submitted for XRD analysis: (1) one consisting of particles under 2 μm containing 0.96 weight percent mercury and (2) one consisting of particles 2 to 5 μm in size containing 0.89 weight percent mercury. Figure 2.7 compares the XRD patterns for the above samples with the diffraction pattern for as-received soil and synthetic metacinnabar. Lines drawn through the maxima of the metacinnabar peaks at B and C highlight weak, broad peaks in the diffraction pattern of the mercury-enriched samples. The breadth of the peaks in the enriched samples reflects the very small crystal size seen in the TEM/SAED study. In contrast, these lines cut across flat regions of the as-received diffraction pattern. Though the less concentrated samples do not respond to bulk analysis by XRD, the presence of metacinnabar is confirmed by XRD in the enriched samples.

Though the above techniques cannot be applied to large numbers or volumes of samples within a reasonable time frame, they do supply unequivocal identification of mercuric sulfide in EFPC soils, which substantiates that geochemical conditions favorable to the formation of crystalline mercuric sulfide exist within the EFPC floodplain. This evidence, in combination with the similarity of petrographic, chemical, and morphological characteristics of the other samples, yields strong proof that analogous conditions exist throughout the floodplain environment.

Thermal desorption test. A final test was initiated, which might supply additional information about the dominant form of mercury in EFPC. The IT Corporation Process Development Laboratory in Knoxville was tasked to perform a thermal desorption test on EFPC soils and soils spiked with metallic and mercuric sulfide standards. The basis of the test was that metallic mercury volatilizes at a lower temperature than mercuric sulfide. The test was only recently completed, and the results reported here should be considered preliminary and subject to change. IT will issue a final report in the near future.

As-received EFPC background and mercury-spiked soils were placed in a rotary thermal apparatus (RTA), heated, and maintained at various temperatures for 30 min. An off-gas sampling system measured mercury content in the RTA's impinging air stream, and residual mercury concentrations were measured in the soil. Results for the EFPC and spiked soils are displayed in Fig. 2.8. Metallic mercury appears to show significant volatilization between 100 and 200°C (212 and 392°F); EFPC soil and soil spiked with mercuric sulfide only begin to show mercury releases at 200°C (the spiked metallic mercury soils were not heated above 200°C). Between 200 and 300°C (392 and 572°F), EFPC soils show higher mercury releases than the mercuric sulfide standard, but the results are well within the range of experimental and measurement error. The flattening of the metallic mercury curve is not understood and may be a consequence of the test method. This may result from the occlusion of metallic mercury in soils as a result of inadequate dispersion throughout the sample prior to the test. Overall, it can be said that the thermal release of mercury from EFPC soils better reflects the mercuric sulfide standard than the metallic mercury standard.

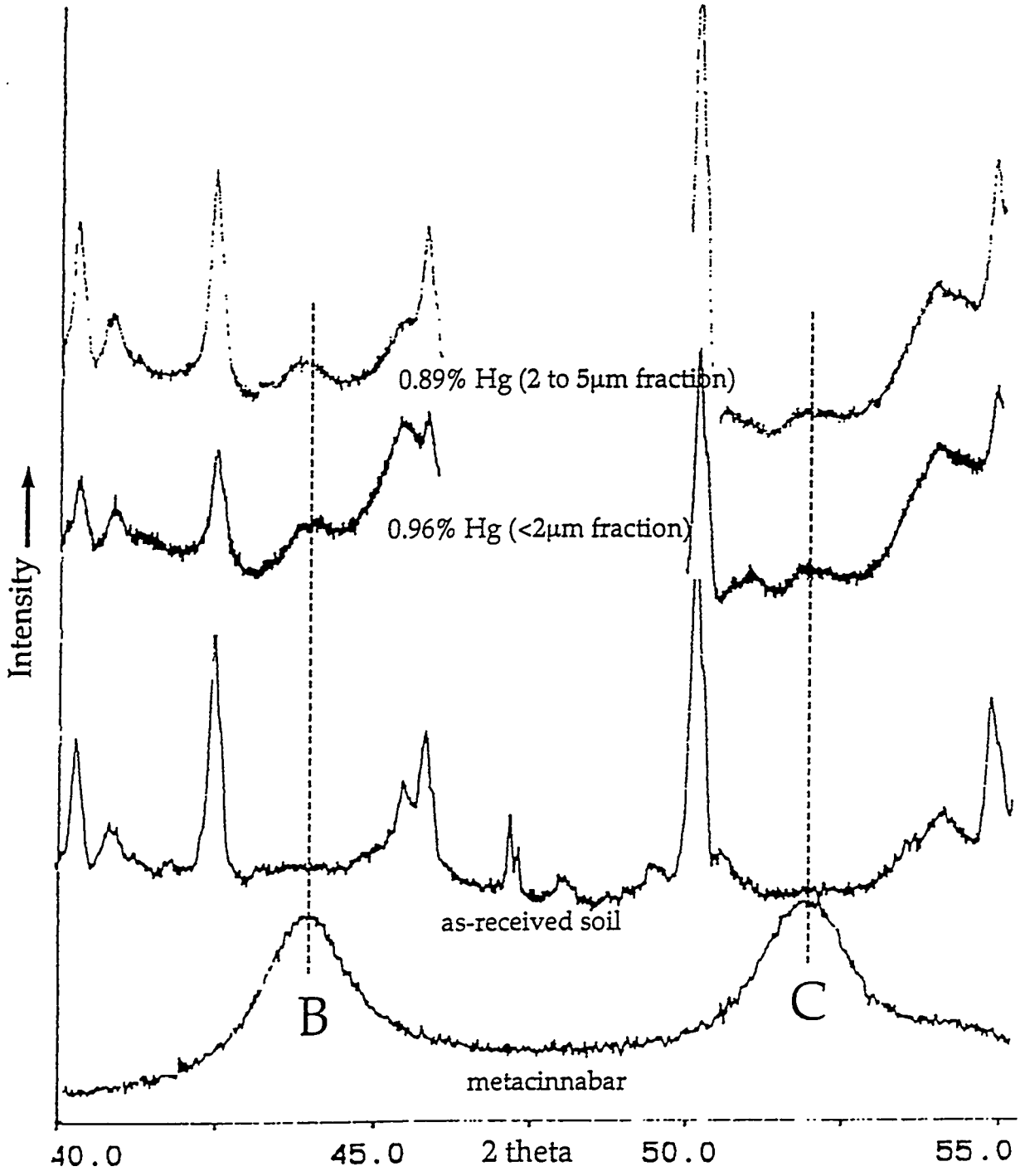


Fig. 2.7. Comparison of the X-ray diffraction patterns for mercury-enriched soil fractions with as-received soil and synthetic metacinnabar.

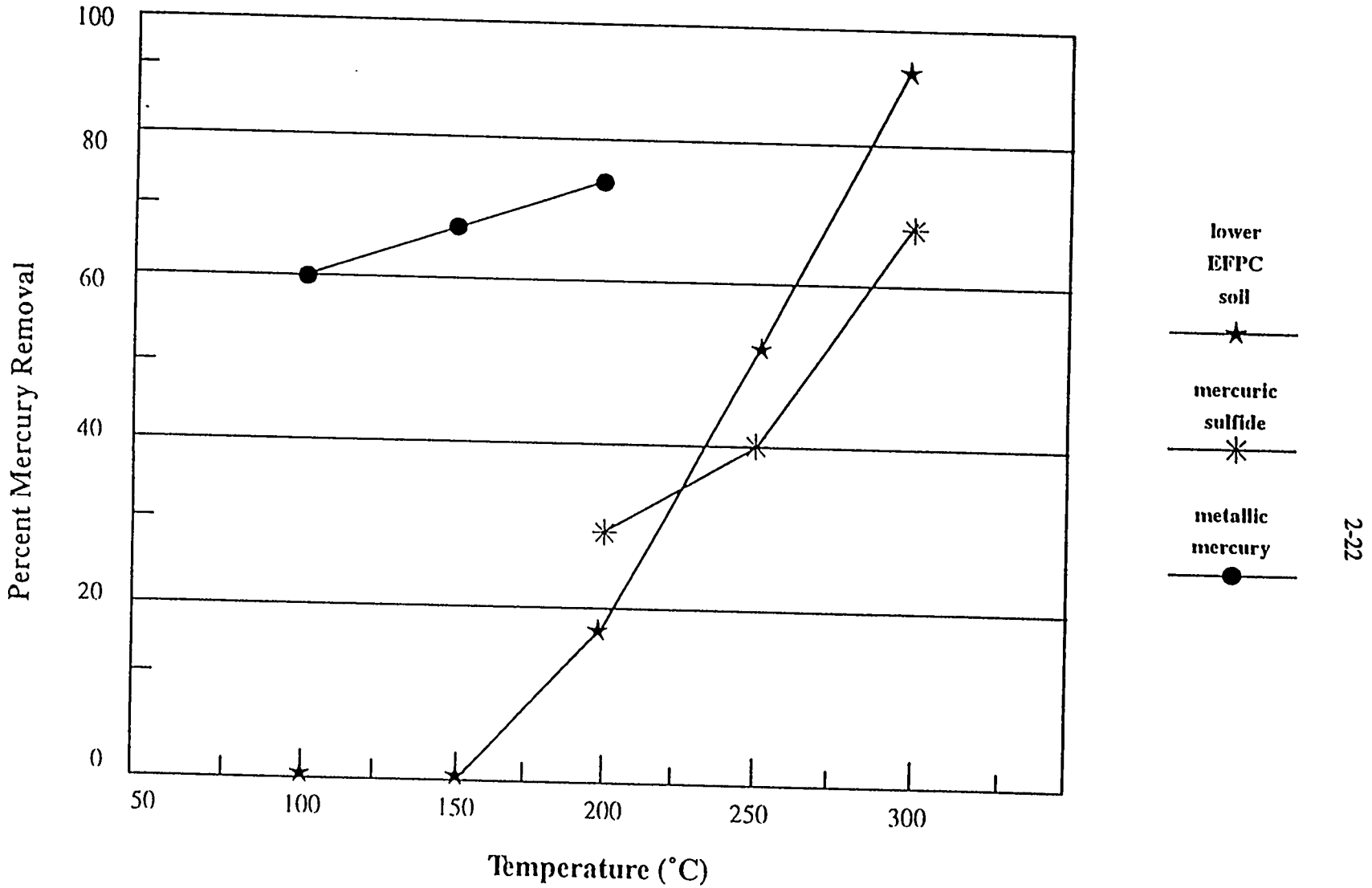


Fig. 2.8. Thermal desorption of mercury
(Lower EFPC-mercury sulfide-metallic mercury).

Original Report #7
Appendix A #8
Attached

2.1.5 Additional Work

Additional tests were conducted to resolve the disagreement between the Environmental Monitoring Systems Laboratory conclusions and the other body of speciation work. Three selective/sequential extraction schemes were applied to five soil samples from the EFPC floodplain. Included in this series were the Revis method, the Environmental Monitoring Systems Laboratory method, and a novel method described by Sakamoto et al. but not yet tested on previous EFPC soils (see Table 2.1 for a comparison of the methods). Four of the five soils used in this comparison were taken from the set of 20 used by Environmental Monitoring Systems Laboratory for their speciation work and by ORNL for the bioavailability study. The four soils were selected on the basis of: (1) the range of total and speciated mercury concentrations and (2) location and depth contrasts. The fifth soil was collected fresh from one of the locations previously sampled for the Environmental Monitoring Systems Laboratory and bioavailability work. The latter soil was selected mainly because it had exhibited independent evidence of high metallic mercury content. All of the soils were processed as-received in their naturally moist state to preclude any possible changes in speciation as a result of drying or pulverizing. In addition, the steps pertaining to organic mercury in each method were skipped because of time constraints, previously determined very low concentrations, and the apparent absence of any discordancy among methods for the organic fraction. To test the effectiveness of each extraction scheme in recovering mercuric sulfide, one of the five soils was also spiked with metacinnabar and included as a sixth soil in the series.

Because of the discordancy between the results of the Revis method and the Environmental Monitoring Systems Laboratory method regarding the relative predominance of elemental mercury in EFPC soils, all 20 samples employed in the Environmental Monitoring Systems Laboratory and bioavailability studies were tested for the presence of headspace mercury vapor at room temperature [22 to 24°C (72 to 75°F)] and 50°C (122°F). Furthermore, each soil was heated to 150°C (302°F) for 5 d following the approximate protocol used by Revis et al. (1989c) to measure the metallic mercury content of EFPC soils. The Revis protocol for elemental mercury was modified for mass balance purposes to allow measurement of both the evolved mercury (collected on iodated charcoal) and the mercury lost from the soil (before and after treatment). These thermal treatments were conducted in closed vessels under an air purge (125-cc volume vessel purged every 2 min).

Results of the additional work were only recently completed at the time of this addendum. Results of these methodological comparisons are provided in Appendix A.

2.1.6 Conclusions

Widely divergent methods have shown that mercuric sulfide and metallic mercury are the likely dominant forms of mercury in the EFPC floodplain soils. Unequivocal test methods have confirmed the presence of mercuric sulfide, and quantitative elemental analysis and electron microscopy demonstrate that sulfur and mercury are co-located in the correct proportions to form mercuric sulfide. The EPA Environmental Monitoring Systems Laboratory sequential extraction method indicated that metallic mercury may be the more abundant mercury species and that detectable quantities of mercuric oxide, or mercury forms that behave like mercuric oxide, are present (although subordinate to mercuric sulfide). More importantly, the evidence is in

agreement that concentrations of both methyl mercury and mercuric chloride constitute a minor fraction of the total mercury content in the EFPC soils.

The Revis and EPA Environmental Monitoring Systems Laboratory studies obtained discordant results on the relative abundance of metallic mercury to mercuric sulfide. At the time of this report, the cause of the discordancies has not been completely determined. Nonetheless, both the Revis and the EPA Environmental Monitoring Systems Laboratory results support the primary conclusion that the mercury form that occurs in the EFPC floodplain is not readily available for biological uptake.

Regardless of the actual form, the solubility of the mercury in the EFPC floodplain soils, and hence the biological availability, is low. A test specifically designed to mimic dissolution of mercury from EFPC soils in the human digestive tract reveals that only minimal mercury (average of 3%) is made available for uptake.

The conclusions of the special studies and the data are used in Sect. 3.1 to support the use and selection of a bioavailability factor for the development of human health RGOs.

2.2 WETLANDS

2.2.1 Introduction

The special field investigation for wetland soil, biota, and woody plants was conducted by Science Applications International Corporation (SAIC) to supplement the ERA for the Lower EFPC RI Report and the FS. The investigation addressed the need for additional data on the uptake of contaminants in EFPC wetland soils by wetland biota and the uptake of contaminants by trees. Wetlands were inventoried by the U.S. Army Corps of Engineers after the Phase Ib sampling plan was already approved. Hence, wetlands were sampled during Phase I of the RI only when sample points fell, by coincidence, within wetland boundaries. Because wetlands are sensitive ecological resources and because biological processes may differ in very hydric soils, additional data were required to assess the potential effects of contaminants present in the EFPC floodplain.

The objectives of the sampling and analysis of wetland soil, biota, and woody plants were (1) to determine the presence of mercury and methyl mercury in wetland soils and biota, (2) to refine estimates of the transfer of mercury and methyl mercury from soil to wetland biota, and (3) to determine whether the mercury content of trees would affect the options for disposal of trees removed from the EFPC floodplain during remediation. In the absence of adequate monitoring data, the transfer of contaminants from wetland soil to biota must be calculated from transfer factors. The use of published factors often produces extremely conservative upper-bound estimates that do not reflect the true potential for exposure or accumulation. Therefore, site-specific information is needed to provide better informed and more reasonable values for wetland soil-biota transfer factors, which will, in turn, provide information needed for the development and refinement of RGOs.

Data collected during the sampling and analysis of wetland soils and biota are used in an exposure assessment for EFPC floodplain ecosystems. These data are also used in the FS to

3. DEVELOPMENT OF ALTERNATIVE RGOs

This section presents alternative RGOs for contaminants in environmental media of EFPC. These RGOs are derived based on an evaluation of risks to human health and ecological receptors and incorporate new information that has become available since the D2 draft of the RI Report (DOE 1994) was issued in January 1994. A full discussion of the development of RGOs and the methods for deriving these numbers is provided in Sect. 7 of the RI Report, Remediation Goal Options for EFPC. This addendum extends the previous work. The discussion in this addendum is limited to mercury.

3.1 RGOs FOR THE PROTECTION OF HUMAN HEALTH

The EFPC Risk Assessment Team derived a risk-based RGO for mercury in floodplain soil (See Sect. 7 of the RI Report). This RGO was developed to protect the most sensitive receptors (i.e., children) following long-term, inadvertent ingestion exposure and dermal contact with soil containing mercury. Consistent with the BRA, the derivation of the RGO assumed that mercury in floodplain soils was present in a highly toxic and mobile form (i.e., mercuric chloride). Based on the weight of evidence, however, it was hypothesized in the RI Report that less soluble and less toxic mercury species predominate. As discussed in Sect. 2.1 of this addendum, recent results of additional speciation and leaching/availability studies support the original hypothesis that less mobile and less bioavailable forms of mercury predominate in EFPC floodplain soils.

Given this new information, an additional RGO for mercury in soil for protection of human health has been derived based on the presence of mercuric sulfide and metallic mercury. EPA recognizes that development of alternate RGOs is a part of the RI/FS process that will occur as new supporting information becomes available. This section of the RI addendum provides the rationale and supporting material and presents the derivation of an additional RGO for mercury in soils. Note that at this point in the RI process, all RGOs are open for consideration. The final selection of remediation levels for mercury in EFPC soils will not occur until the FS is complete and the Record of Decision is prepared.

The new RGO for mercury (i.e., alternate value) derived in this section is presented as a conservative, risk-based, deterministic point estimate based on methods recommended by EPA. In addition to the point estimate, the EFPC Risk Assessment Team has conducted a quantitative uncertainty analysis to examine the uncertainty surrounding this additional RGO and the assumptions that form the basis of this estimate.

3.1.1 Overview of Absorption, Bioavailability, and Exposure Estimates

Bioavailability and absorption of mercury in soil directly influence exposure potential. Greater exposure potential will result in higher projected risk estimates and an associated lower value for the remediation goal (i.e., the higher the risk, the smaller the quantity of contaminant that is acceptable in soil). It is important at this point to review the concepts of absorption and bioavailability as they relate to human exposure to mercury species in soil.

Exposure estimates may be based on administered or absorbed dose. EPA defines *administered dose* as the mass of a substance that the receptor comes in contact with at an exchange boundary (e.g., gastrointestinal tract) per unit body weight per unit time (i.e., mg/kg-day). Alternatively, *absorbed dose* is the quantity of chemical crossing the exchange boundary following contact. Absorbed dose is expressed as mass absorbed into the body (e.g., into the blood stream) per unit body weight per unit time (i.e., mg/kg-day). Administered dose is referred to by EPA as "exposure intake" (not dose) and is not considered a true measure of the quantity of chemical experienced by the receptor at the target organ or target tissue (i.e., site of toxic action). Absorbed dose is considered by EPA to be a true dose estimate and may be used to quantify chemical concentration at the site of toxic action.

Absorbed dose is calculated from estimates of intake and is modified through application of a factor for *absorption efficiency*. Note, however, that only a fraction of the total contaminant concentration measured in soil is released from the soil matrix to the gastrointestinal environment and is available for absorption. The concept of *bioavailability* is important in this regard. Bioavailability is a function of both the compound-specific physical/chemical properties and the properties of the soil matrix itself. When soil is inadvertently ingested, not all of the contaminants in the ingested soil are available to be absorbed across the gut lining. In turn, only a percentage of the amount that is available within the gastrointestinal tract will be absorbed across the biological membrane. Absorption efficiency and bioavailability will differ as a function of the mercury species under evaluation.

To summarize, at least two important steps must be considered in understanding absorbed dose: (1) bioavailability of the chemical from the soil matrix and (2) chemical-specific absorption efficiency across the biological membrane. This is somewhat simplified, however, and true estimates of tissue or target organ dose would need to consider the pharmacokinetics and pharmacodynamics of the subject chemical. In addition to uptake or transport across the biological membrane, distribution, metabolism, and excretion are also important. However, for the purposes of this discussion, it is enough to understand that by not incorporating these considerations for the less mobile mercury species, the BRA for EFPC is likely to have overestimated intake/dose of contaminants from floodplain soils.

In *Risk Assessment Guidance for Superfund (RAGS)* (EPA 1989b), none of the recommended exposure equations incorporate a factor for absorption efficiency (or bioavailability), except the dermal contact pathway (EPA includes a factor for chemical-specific dermal permeability). EPA headquarters, by not specifying an efficiency factor in equations for other exposure pathways, is functionally assigning a value of 1.0 (or 100%) as the absorption efficiency.

The EFPC Risk Assessment Team began outlining the approach and methods for human health risk assessment for EFPC almost 2 years ago. The team prepared and distributed a formal methods document for review by EPA, DOE, the State of Tennessee, and ORNL before the risk assessment was initiated. In addition, the EFPC Risk Assessment Team gave a series of briefings regarding the proposed approach. At that time, the team raised the issue of the use of absorption or bioavailability factors in the EFPC risk assessment. Following a review of the literature and discussions with EPA Region IV, the team decided that such factors would only be used for the dermal contact pathway. EPA Region IV provided the EFPC Risk Assessment Team with default absorption factors for the dermal pathway. A default absorption factor of 1.0 was recommended by EPA Region IV for use for all other pathways (ingestion and inhalation). The decision to use

a default value of 1.0 was based in part on the lack of EPA-verified or accepted absorption or availability factors for the ingestion pathway.

Use of an oral bioavailability factor of 1.0 for chemicals in soils results in a conservative estimate of dose. A chemical that may be 90 to 100% available for uptake and absorption from drinking water may exhibit only a fraction of this availability from soil. This is the case for many metals in soil. In *RAGS* (EPA 1989b, Appendix A), EPA notes that some metals tend to be poorly absorbed (<5%) from the gastrointestinal tract. EPA indicates that a relatively conservative assumption for bioavailability and oral absorption of metals in the absence of appropriate information would be 5%.

3.1.2 Review of Oral Absorption and Bioavailability of Mercury Species

3.1.2.1 Literature review: oral absorption of mercury species

Published literature was reviewed to examine differences in the oral absorption of mercury for various mercury compounds. Although epidemiological, occupational, and clinical studies were reviewed, this discussion is largely drawn from experimental studies in animals.

Mercury absorption from the gastrointestinal tract is a complex process (Suzuki et al. 1990). The underlying pharmacokinetics of mercury absorption and distribution for the oral route indicate that aqueous solubility, ionic charge, nature of the matrix (e.g., food, soil), pH of the gut, and presence of complexing agents modify the absorption of mercury. The literature review focused mainly on the less soluble mercury species found in EFPC soils (i.e., mercuric sulfide, metallic mercury, and mercurous mercury) and indicates that four critical factors affect intestinal absorption: (1) the species of mercury, (2) the distribution of mercury, (3) the age of the receptor, and (4) the condition of the intestinal epithelium.

The first factor that affects the rate and extent of absorption from the gastrointestinal tract is the mercury species. Differences in the rates of absorption are the result of the ease with which less soluble mercury species convert to more soluble forms, resulting in increased rates of absorption. In general, metallic mercury, mercuric sulfide, and mercury-selenium complex are relatively less toxic forms of mercury for oral exposure than mercuric mercury and organic mercury. Metallic mercury is characterized by low aqueous solubility, greater lipophilicity, and an inert chargeless state. For these reasons, metallic mercury is less bioavailable and, consequently, less toxic by the oral route.

Table 3.1 summarizes the relative absorption of different forms of mercury (as a percent of the administered dose) reported in the literature for the oral route.

A review of the reported values for the absorption of mercury compounds indicates that absorption of mercury from the gastrointestinal tract is highly dependent on the form of mercury. For example, absorption from the gastrointestinal tract could range from 0.01% for metallic mercury to 80% for methyl mercury. Oral absorption of methyl mercury is high; up to 80% of the ingested dose was absorbed by this route in both humans and experimental animals. Other soluble compounds, such as mercuric chloride and mercuric nitrate, are generally absorbed at high levels (up to 79%) compared to less soluble species such as mercuric sulfide and mercuric acetate. Feeding studies in experimental animals indicated that, on average, only 0.5% of the

Table 3.1. Relative percent absorption of mercury compounds[a]

Mercury compound	Percent absorbed by the oral route
Metallic mercury	0.01
Mercurous mercury	
Mercurous chloride	2
Mercuric mercury	0.03 to 79[b]
Mercuric acetate	20
Mercuric chloride	2 to 79
Mercuric nitrate	15
Mercuric sulfide	0.03 to 2
Cinnabar	0.7
Methyl mercury	50 to 80

[a] Based on evidence from experimental animals and humans.
[b] 79% of the dose was retained 6 d after administration to the suckling rats. However, the literature appears to reflect a consensus for an average absorbed dose of 10% for mercuric mercury.

administered doses of pure mercuric sulfide (cinnabar) and soil samples containing mercuric sulfide was absorbed.

Experimental studies with animals, human volunteers, and occupational workers indicated that a negligible amount of metallic mercury is absorbed from the gastrointestinal tract. Although conversion of metallic mercury to organic forms in the gastrointestinal tract may result in increased absorption of mercury, there is no evidence of biotransformation of metallic mercury to organic species (particularly methyl mercury) in higher mammals, including humans.

Oxidation of metallic mercury to ionic species requires the participation of an enzyme called catalase, which, in the presence of hydrogen peroxide, converts metallic mercury to mercuric mercury. Conversion of metallic mercury to ionic forms is of minor significance for absorption by the oral route because this reaction occurs predominantly in the blood and not in the gut. Mercuric sulfide was shown to be poorly absorbed from the gastrointestinal tract. The percent absorbed ranged from 0.03 to 2% of the administered dose in experimental studies (Revis et al. 1990). Percent absorbed in the Revis study was measured in comparison with quantities excreted. The percent of mercury absorbed from cinnabar was reported to be up to 0.7% of the administered dose.

The second critical factor affecting absorption estimates is the manner in which mercury is distributed and retained in the body. Resecretion of mercury from the bile into the gastrointestinal tract is not accounted for in studies of oral absorption of mercury. This could

lead to an underestimation of the absorbed doses for less soluble (or more fat-soluble) mercury. Mercury is resecreted into the gastrointestinal tract by means of the bile secretions and then excreted through the feces. This is the primary mode of excretion of mercury. Some of the early ingestion studies with metallic mercury do not seem to have considered biliary resecretion in the calculation of metallic mercury absorbed from the gastrointestinal tract. This might result in an underestimation of absorption from the gastrointestinal tract. Some researchers are skeptical about feeding studies reporting low absorption of 0.01% based on only two sets of measurements (i.e., administered dose and fecal excretion) to estimate mercury retention in the body.

The third critical factor affecting absorption estimate is age-specific differences in the absorption of mercury from the gastrointestinal tract. In particular, increased absorption among the young has been reported. A recent report by Kargacin and Kostial (1990) on the retention of orally administered mercury (mercuric chloride) indicates that suckling rats retained as much as 80% of the administered dose, whereas older rats retained only 1% of the administered dose 6 d after the last day of administration.

There is some corroborating evidence that indicates suckling animals retain higher levels of mercury than adults (Jugo et al. 1975, Kostial et al. 1989). Researchers have implicated age-specific features such as immaturity of the kidneys and bile transport, differences in the binding affinities, and contents of metal carrier proteins as plausible reasons for the increased retention of mercury in the younger animals. Although these results are based on studies of mercuric chloride, the fate of mercuric sulfide and metallic mercury may be similar in younger age groups.

The fourth critical factor affecting the absorption estimates is the condition of the intestinal epithelium. Stripping of the epithelial cells of the intestinal lumen containing bound mercury may alter the mass balance calculations for the administered dose versus fecal excretion of mercury. Mercury retention (or elimination) from the gastrointestinal tract does not consider the effect of mercury binding to the lumen of the intestine. Stripping of the lumen will result in the release of bound mercury to the gastrointestinal tract and, consequently, in the feces. Epithelial stripping is a normal process that may confound measurements of excreted mercury. The effect depends on variations in the number of epithelial cells shed into the feces. Oral intubation studies interpreting mercury absorption based on administered dose and mercury recovered from the feces do not adequately account for the contribution of mercury from the stripping of the lumen. Moreover, it is not clear if the lumen-bound form is metallic or ionic mercury.

3.1.2.2 Recent studies: EPA Region IX and State of California

EPA regional offices have acknowledged that relative absorption factors (RAF) or bioavailability factors may be appropriately used if a chemical in the soil matrix is believed to be less available than the chemical present in solvent or water. EPA Region I defines the RAF as ". . . the ratio of the estimated absorption factor for the site-specific medium and route of exposure to the known or estimated absorption factor from the laboratory study from the which the cancer potency factor or the reference dose was derived." EPA Region I notes that use of this factor allows the risk assessor to make appropriate adjustments to dose estimates if the efficiency of absorption is known or expected to differ because of physiological, matrix, or vehicle effects. Note that the Region I RAF incorporates consideration of both bioavailability and absorption efficiency.

The EFPC Risk Assessment Team became aware of two waste sites under EPA Region IX jurisdiction at which mercury is the principal COC: Sulfur Bank and Carson River (*Preliminary Draft, Human Health Risk Assessment/Remedial Investigation Report, Carson River Mercury Site*, EPA, Region IX, San Francisco, California, April 1994; EPA 1994a). Mining was conducted at these sites and mercuric sulfide is one of the principal contaminants under investigation. The EFPC Risk Assessment Team contacted EPA Region IX to learn more regarding the Region's approach to risk assessment for mercury. EPA Region IX noted that the Agency had not derived alternate reference doses (RfDs) for different mercury species, but had adopted absorption factors for use in dose estimation.

EPA Region IX indicated that mercuric sulfide is the predominant form of mercury at the sites. Understanding that the EPA RfD for mercury is based on administration of mercuric chloride in test animals, and that the sulfide is much less mobile than the chloride species, EPA Region IX chose to use a factor to reflect the relative difference in absorption between the chloride and the sulfide forms. The decision to use the relative absorption factor was supported by the results of analysis of blood samples taken from residents living in the vicinity of the contaminated sites. EPA compared measured concentrations of mercury in blood with reference or background levels. They found no statistically significant difference and concluded that mercury exposure was being limited by the mobility of the sulfide species.

EPA Region IX had requested that the EPA Environmental Criteria and Assessment Office (ECAO) conduct a literature review of absorption factors for mercury species. The ECAO review identified an absorption factor of 15% for mercuric chloride via the oral route. Furthermore, ECAO indicated that absorption of mercuric sulfide by means of the oral route is 1/30th to 1/80th that of the chloride. EPA Region IX decided to use a conservative factor of 20% (1/5th) relative absorption of the sulfide versus the chloride. Based on this information, EPA Region IX adopted a relative absorption factor of 3.0% for use in estimating exposure of human receptors to mercuric sulfide at the Sulfur Bank and Carson River sites (15% x 0.20 = 3.0%).

Another recent example of the use of availability factors in risk assessment of exposure to mercury in soils is the Almaden Quicksilver County Park site in California. Almaden Quicksilver was the site of mercury mining for more than 100 years and is extensively contaminated with cinnabar. The State of California, Department of Health Services has oversight responsibility for the RI/FS of the facility. Mercuric sulfide is the predominant COC. In this BRA, an availability factor of 0.3 (30%) was used for the soil ingestion pathway to reflect the differences in bioavailability between the sulfide and chloride species of mercury.

In the studies discussed above, neither EPA Region IX nor the State of California adjusted the RfD value for mercury in association with use of an oral absorption factor. (Note that EPA specifies the need for comparability in toxicity and exposure estimates and both must be expressed either as absorbed or administered dose). In this regard, the "absorption factors" for mercury species used by EPA Region IX and the State of California might better be considered "bioavailability factors" that reflect the relative mobility of mercuric sulfide versus mercuric chloride from the soil matrix.

3.1.3 Selection of Bioavailability Factor for Mercury in EFPC Soils

In the BRA, the bioavailability factor for mercury in EFPC soils was set at 1.0 for the ingestion exposure route. As previously noted, this conservatively assumed that the bioavailability of mercury in EFPC soils was equivalent to that of mercuric chloride. Note that mercuric chloride is the mercury species that is the basis of the chronic oral RfD recommended by EPA for use in human health risk assessment. However, the available analytical data indicate that less mobile, less bioavailable mercury species predominate in EFPC soils. The weight of evidence points to mercuric sulfide and metallic mercury as the predominant forms.

On the basis of the available scientific data and the work conducted under authorization of EPA Region IX and the State of California, the EFPC Risk Assessment Team has selected a conservative bioavailability factor of 30% (0.3) for mercuric sulfide. Metallic mercury is considerably less mobile and bioavailable via the oral route than the sulfide species, and 30% is appropriate. The combined bioavailability of metallic mercury and mercuric sulfide from soil (oral route) is projected to be <30%. This conclusion is supported by the results of the solubility/leaching studies conducted by ORNL using EFPC soils (See Sect. 2.1 of this addendum).

3.1.4 Derivation of an Additional RGO for Mercury

RGOs were presented in the EFPC RI Report, addressing both human health effects (i.e., children/adult, residential/open land use, SLB worker) and ecological effects (i.e., various receptors in the food web). As previously noted, EPA recognizes that revision of RGOs is a part of the RI/FS process that will occur as new supporting information becomes available (EPA 1991). The new RGO presented here is a refinement arising from an improved understanding of mercury speciation and behavior in EFPC soil.

For human health assessment, the most important RGO is the one that will protect the most sensitive receptors (resident children) from adverse noncancer effects related to exposures to EFPC soils. The RGO is the concentration in soil below which the hazard index is likely to remain <1.0 over extended periods of time. Equation 1 was used to derive the RGOs for human health that are presented in the EFPC RI Report, and the same equation may be used to derive the revised RGO for resident children (as well as RGOs for less sensitive receptors).

$$RGO_{soil} = \frac{THQ \times BW \times AT \times RfD}{EF \times ED \times (CR_I \times B_I + CR_D \times ABS_D)} , \qquad (1)$$

where

RGO = remediation goal option for mercury in soils (mg/kg),
THQ = target hazard quotient (unitless),
BW = body weight (kg),
AT = averaging time (days),
RfD = reference dose for mercury [mg/(kg-day)],
EF = exposure frequency (days/year),
ED = exposure duration (years),

CR_I = contact rate for ingestion exposure to soil (mg/day),
CR_D = contact rate for dermal contact with soil (mg/day),
B_I = oral bioavailability factor for mercury (unitless),
ABS_D = percutaneous absorption factor for dermal contact with mercury.

RGOs developed from Equation 1 account for both oral ingestion and dermal contact with EFPC soil. The change in the RGOs is directly related to the revised bioavailability factor for oral ingestion, noted in Equation 1 as B_I. In the EFPC RI Report this value was set equal to 1.0. This assumes that the mercury in EFPC soils is as bioavailable as mercuric chloride, upon which the RfD for mercury is based. As noted in Sect. 3.1.3, the additional RGOs are based on a bioavailability factor (B_I) that is 30% of the bioavailability of mercuric chloride, so the revised B_I for EFPC soil is 0.3.

The newly derived RGO for resident children is 180 mg of mercury/kg of soil for residential and agricultural land use scenarios. The previously derived RGO (RI Report, Sect. 7) was 58 mg of mercury/kg of soil and was rounded down to 50 mg/kg. The new RGO is based on consideration of the reduced mobility and bioavailability (B_I) of mercury species in EFPC soils. No other factors or variables in Equation 1 have been changed. Note that the value of 180 mg of mercury/kg of soil remains protective and is an effective target concentration for human contact with EFPC soil. As derived, the value of 180 is based on a target hazard quotient of 1.0 and consideration of reduced bioavailability of mercury species in EFPC soils. Therefore, potential exposure of children to concentrations below 180 mg/kg will result in a hazard index <1.0 (i.e., within the target range established by EPA).

3.1.5 Quantitative Uncertainty Analysis of RGO

The uncertainty analysis presented in the EFPC BRA included a quantitative assessment of the sources of uncertainty in the input parameters and the relative influences on the results of risk assessment. In a similar manner, this section examines uncertainty in the derivation of the RGO for mercury in EFPC soils. The approach used incorporates consideration of the uncertainty surrounding estimates of bioavailability of mercury for the ingestion pathway.

In Sect. 3.1.4, the RGO was presented as a single number but should be viewed as an estimate that spans a range of possible values. Monte Carlo simulation was used to explore the uncertainty surrounding this value. Monte Carlo techniques were used to propagate the uncertainty in each exposure variable in Equation 1 and to create an output distribution (i.e., the RGO) that may be statistically evaluated. This analysis assists in evaluating the degree of "protection" or conservatism built into the value of 180 mg/kg of mercury in soil.

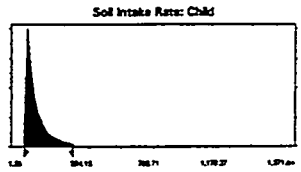
Monte Carlo simulation requires the development of a probability density function (PDF) for each uncertain variable. PDFs have been developed for the majority of the variables needed in deriving the RGO. These were presented in the BRA. A summary of the PDFs used in this evaluation is provided in Fig. 3.1. (See Sect. 5.5 of the RI Report for a more detailed discussion of the development of these distributions).

A new PDF had to be developed for bioavailability of mercury from EFPC soils. This distribution was developed using the experimental bioavailability data generated by ORNL (Sect. 2.1). As discussed previously, ORNL conducted a leaching/solubility study using EFPC

PDF for soil intake rate

Lognormal distribution with parameters:

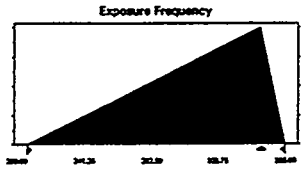
Mean	96.66
Standard Deviation	160.38



PDF for exposure frequency

Triangular distribution with parameters:

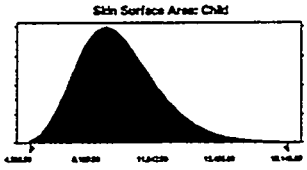
Minimum	200.00
Most Likely	350.00
Maximum	365.00



PDF for skin surface area - child

Lognormal distribution with parameters:

Mean	9,593.11
Standard Deviation	2,335.90



PDF for adherence factor

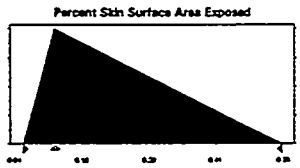
Triangular distribution with parameters:

Minimum	0.20
Most Likely	0.60
Maximum	1.20

PDF for skin surface area exposed

Triangular distribution with parameters:

Minimum	0.04
Most Likely	0.10
Maximum	0.53



PDF for body weight

Lognormal distribution with parameters:

Mean	26.52
Standard Deviation	9.28

Fig. 3.1. Probability density functions: exposure assumptions.

soil to simulate the bioavailability of mercury in the gastrointestinal tract. The bioavailability factor of 30% used to calculate the RGO of 180 mg/kg (point estimate) may be evaluated in light of the results of the ORNL study.

The results of the ORNL bioavailability study indicated that solubilities (percent mercury extraction from EFPC soils) are generally <10%. Values ranged from 0.3 to 45.9%. These data were used to develop a PDF for bioavailability of mercury, which was then used in the Monte Carlo simulation. To determine the shape and appropriate characteristics of the distribution (e.g., mean and standard deviation), a probability plot of the data was prepared and examined. The data did not clearly fit a normal distribution, and were transformed to a $\log_e$ scale, plotted, and examined for goodness-of-fit. The data also did not approximate a lognormal distribution. A "custom" distribution was then developed using the actual ORNL data.

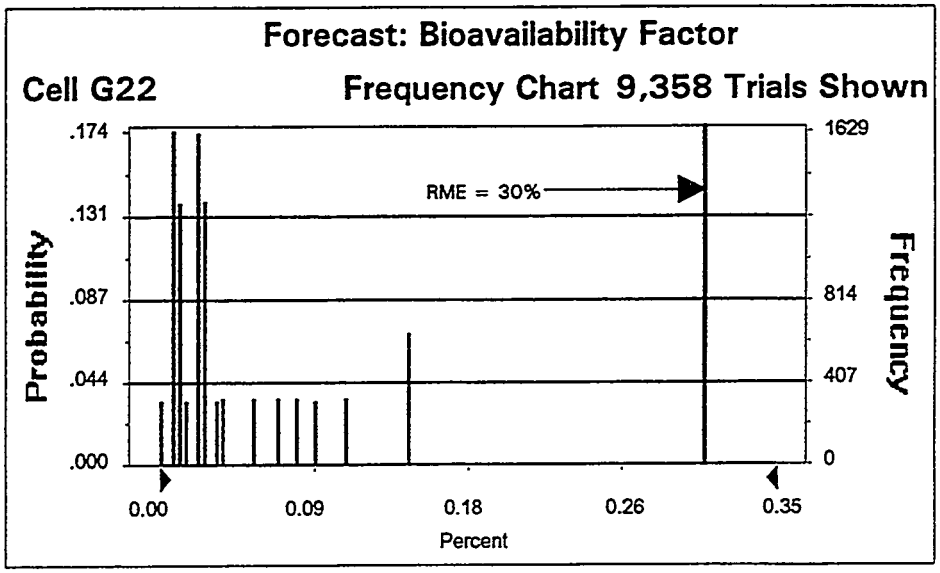
The custom distribution was developed using Crystal Ball® Version 3.01 for Microsoft® Excel (Decisioneering 1993). All of the solubilities were entered with an equal probability of occurrence. The resulting PDF was statistically evaluated and used to determine the percentile value associated with the point estimate of 30% used in Equation 1. The point estimate of 30% was plotted on the PDF for bioavailability (Fig. 3.2) and was found to fall above the 90th percentile. This supports the results of the literature survey and the work of EPA Region IX and the State of California indicating that 30% bioavailability is a conservative (and protective) estimate for mercuric sulfide and metallic mercury in EFPC soils.

Once the PDFs for each exposure variable had been generated and evaluated, Monte Carlo simulation was used to derive a probabilistic estimate of the RGO for mercury. (Note that no PDF was developed for the target hazard quotient; it was treated as a point estimate). The simulation consisted of 10,000 samples from each PDF and recalculation of the RGO. The 10,000 results were saved and aggregated as an output distribution. This output distribution for the RGO (Fig. 3.3) is depicted in the form of a reverse cumulative distribution. Figure 3.3 also presents the statistics and corresponding deciles of the forecast. As shown, 180 mg/kg of mercury in soil is the 98th percentile of the output distribution. In other words, given the uncertainty in the input variables, there is a 98% probability that the RGO is actually >180 mg/kg. Conversely, the likelihood that the selected RGO is not sufficiently conservative (i.e., has been overestimated) is only 2%.

As a final step, a sensitivity analysis was conducted to examine the influence of each uncertain variable (in Equation 1) on the RGO estimate. Crystal Ball® was used to compute the Spearman rank correlation coefficient between each uncertain input variable and the projected RGO estimate. The results of the sensitivity analysis are depicted in Fig. 3.4. The size of each bar is proportional to the measured rank correlation or influence of that variable on the magnitude of the RGO estimate. As illustrated, the bioavailability factor has the greatest influence on the RGO, followed by the other exposure variables (depicted in descending order of influence). The sign of the measured rank correlation coefficient (positive or negative) indicates the effect on the output forecast (RGO). For example, the negative correlation coefficients indicate that an increase in the magnitude of a given variable is associated with a corresponding decrease in the output estimate. This is evident in the decrease in the bioavailability factor for mercury from 100 to 30% and the resultant change in RGO values from 50 mg/kg to 180 mg/kg of mercury in soil.

Statistics:	Value
Trials	10000
Mean	0.06
Median (approx.)	0.02
Mode (approx.)	0.01
Standard Deviation	0.10
Variance	0.01
Skewness	2.90
Kurtosis	10.76
Coeff. of Variability	1.63
Range Minimum	0.00
Range Maximum	0.46
Range Width	0.46
Mean Std. Error	0.00

BASED ON TURNER DATA

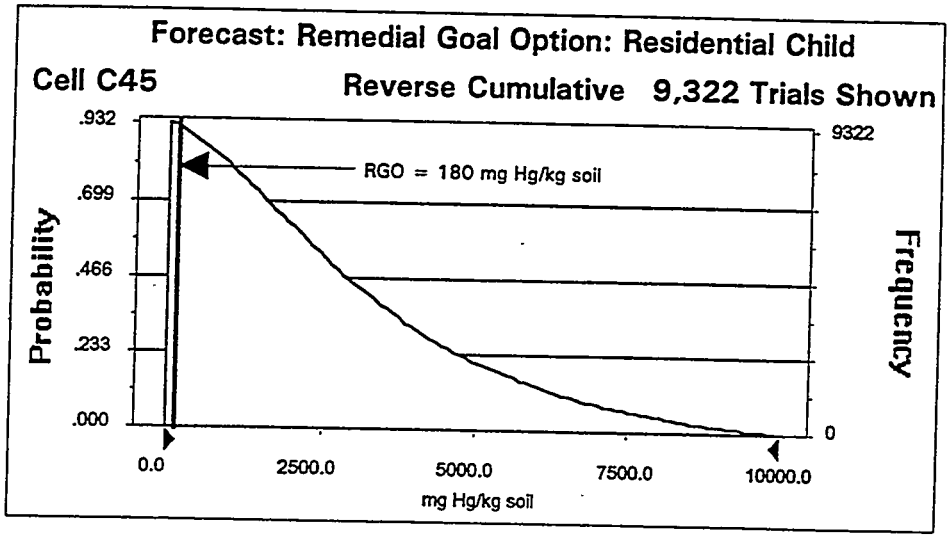


Percentiles:

Percentile	Percent (approx.)
0%	0.00
10%	0.01
20%	0.01
30%	0.01
40%	0.02
50%	0.02
60%	0.03
70%	0.04
80%	0.08
90%	0.14
100%	0.46

Fig. 3.2. Probability density functions: bioavailability of mercury in EFPC soils.

Statistics:	Value
Trials	10000
Mean	4128.1
Median (approx.)	3083.9
Mode (approx.)	1559.2
Standard Deviation	3837.1
Variance	14723666.2
Skewness	2.67
Kurtosis	15.41
Coeff. of Variability	0.93
Range Minimum	52.9
Range Maximum	43091.5
Range Width	43038.7
Mean Std. Error	38.37



Percentiles:

Percentile	mg Hg/kg soil (approx.)
100% 99.88% = 58 mg Hg/kg soil	52.9
90% 98.70% = 180 mg Hg/kg soil	790.7
80%	1384.3
70%	1934.9
60%	2470.5
50%	3083.9
40%	3811.4
30%	4726.8
20%	6091.9
10%	8544.0
0%	43091.5

Fig. 3.3. Probability distribution: RGO for mercury in EFPC soil.

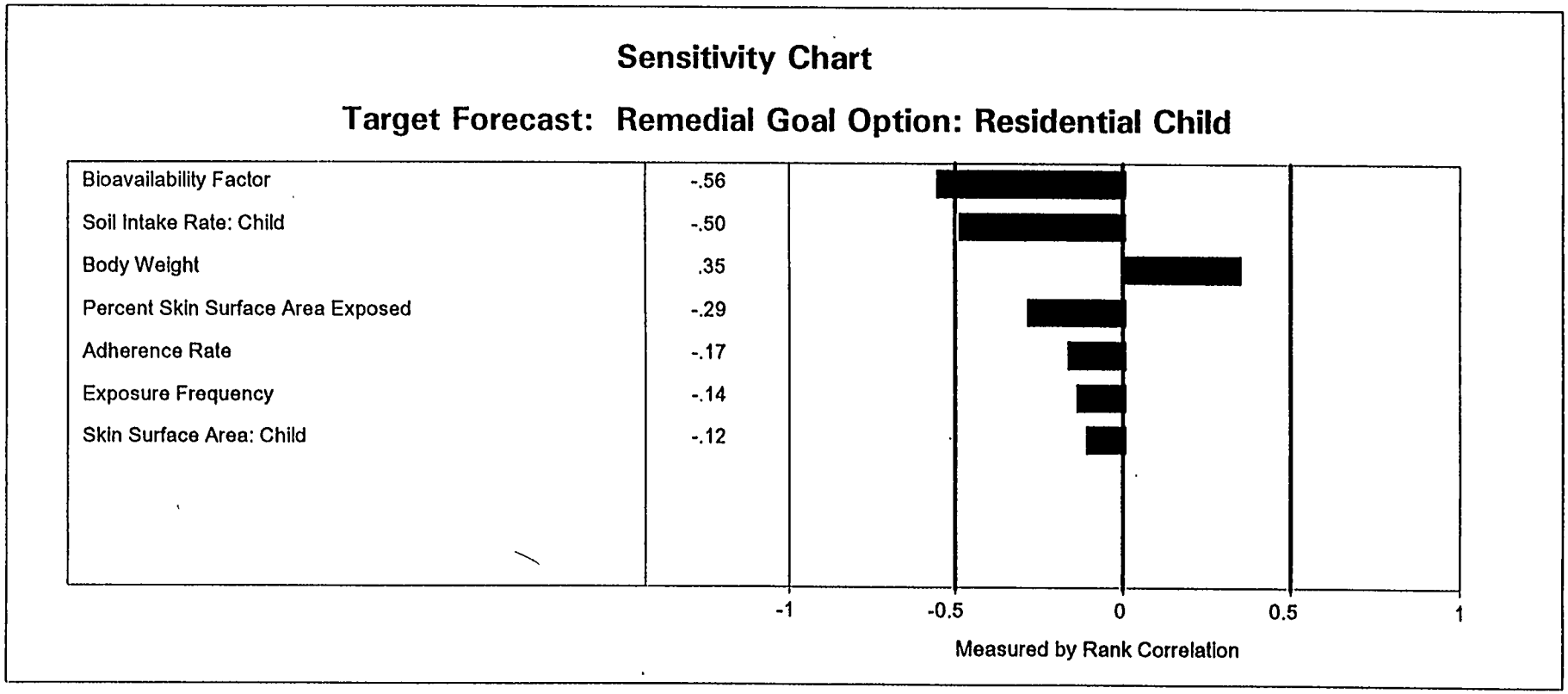


Fig. 3.4. Sensitivity analysis results.

3.1.6 Human Health Remediation Goal Option

The results of sampling and analysis indicate that the less soluble and less toxic mercury species predominate in EFPC soils. Recent results of additional speciation and leaching/availability studies using EFPC soils contribute to the weight of evidence that less mobile and bioavailable forms of mercury predominate. The weight of evidence points to mercuric sulfide and metallic mercury as the primary forms.

EPA recognizes that revision, or development of alternate RGOs is a normal part of the RI/FS process and will occur as new supporting information becomes available. In the BRA, the bioavailability factor for mercury in EFPC soils was set at 1.0 for the ingestion exposure route. In using a factor of 1.0, it was assumed that the bioavailability of mercury in EFPC soils was equivalent to that of mercuric chloride. (Note that mercuric chloride is the mercury species that is the basis of the chronic oral RfD recommended by EPA for use in human health risk assessment). In this addendum, the RGO for mercury in soil for protection of human health has been recalculated. This recalculation is based on inadvertent ingestion and dermal exposure of children to mercuric sulfide and metallic mercury in soil.

Based on the available scientific data and results of work conducted under authorization of EPA Region IX and the State of California, the EFPC Risk Assessment Team has selected a conservative bioavailability factor of 30% (0.3) for mercuric sulfide. Metallic mercury is considerably less mobile and bioavailable via the oral route than the sulfide species, and 30% is appropriate. The combined bioavailability of metallic mercury and mercuric sulfide from soil (oral route) is projected to be <30%. This conclusion is supported by the results of the solubility/leaching studies conducted by ORNL using EFPC soils.

A new RGO of 180 mg of mercury per kg of soil has been calculated using a bioavailability factor of 30%. This RGO is approximately three times greater than the RGO of 50 mg/kg of mercury in soil derived in the D2 draft of the RI Report. Both RGOs (along with those derived in the RI Report, Sect. 7) will be presented for consideration in the feasibility study. The new RGO of 180 mg/kg remains protective (i.e., due to consideration of reduced bioavailability) for human contact with EFPC soil for residential and agricultural land use scenarios. As derived, the value of 180 mg/kg is based on a target hazard quotient of 1.0. Therefore, potential exposure of children to concentrations below 180 mg/kg will result in a hazard index <1.0 (i.e, within the target range established by EPA). The change in bioavailability from 100 to 30% is applicable to the development of other human health RGOs for other land uses (e.g., the 331 mg/kg soil for commercial, DOE-owned, and other land uses would become 938 mg/kg).

3.2 DERIVATION OF ALTERNATIVE ECOLOGICALLY BASED RGOs FOR MERCURY

3.2.1 Introduction

Potential transport pathways and exposure routes for contaminants, especially mercury in EFPC and the EFPC floodplain, were described in the EFPC RI Report (DOE 1994). They are shown schematically in Figs. 6.38 (Y-12 Plant contaminants) and 6.39 (EFPC floodplain contaminants) in the RI Report. These figures indicate that some transport pathways and some

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DUPLICATE

#11

Revised
FINAL REPORT

An Assessment of Health Risk Associated with Mercury in Soil and Sediment from East Fork Poplar Creek, Oak Ridge, Tennessee

APR 6 2 45 PM '93

Nathaniel Revis, George Holdsworth,
Gene Bingham, Adolf King, James Elmore

Oak Ridge Research Institute
113 Union Valley Road
Oak Ridge, Tennessee

Work performed under contract No. DE-AC05-84OR21443 for the United States Department of Energy

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Table 20 Estimate of Dietary Intake of Mercury-Contaminated Soil Required by Female Mice to Achieve Kidney Levels of 25 µg Hg/g Wet Weight.....44

Table 21 Estimate of Dietary Intake of Mercury-Contaminated Soil Required by Male Mice to Achieve Kidney Levels of 25 µg Hg/g Wet Weight.....45

Table 22 Tumor Promotion and Initiation Study Using Soil Extracts.....49

Table 23 Mice Breeding Studies.....51

List of Figures

Figure 1 Protocol for Tumorigenesis Assay.....9

Figure 2 Map of Bear Creek and East Fork Poplar Creek.....12

Figures 3-28 Statistical Analysis of the Log of Liver and Kidney Mercury over the Log of Soil Mercury.....59-79

Table of Contents

Page

1 OBJECTIVE....1

2 INTRODUCTION....1

3 BACKGROUND....2
3.1 Mercury Metabolism....4
3.1.1 Absorption....4
3.1.1.1 Gastrointestinal....4
3.1.1.2 Pulmonary....4
3.1.1.3 Skin....4
3.1.2 Tissue Distribution and Half Life....4
3.2 Mercury Toxicity....5
3.3 Objective of the Health Risk Studies....6
3.3.1 Toxicity Assessments....7
3.3.2 Carcinogenicity Assessments....8
3.3.3 Teratogenicity Assessments....10

4 PROCEDURES AND METHODS....10
4.1 Procedures....10
4.1.1 Sample Collections....10
4.1.2 Chronic Feeding and Skin Painting Studies....13
4.1.2.1 Chronic Feeding Studies....13
4.1.2.2 Skin Painting Studies....14
4.2 Methods....14
4.2.1 Test Diet Preparation....14
4.2.2 Food Intake and Urinary Collection Studies....15
4.2.3 Soil Feeding Studies....15
4.2.4 Mercury Absorption Studies....15
4.2.5 Extracts for Skin Painting Studies....16
4.2.6 Skin Studies....16
4.2.7 Teratogenicity Studies....16
4.2.8 Histological Studies....16
4.2.9 Mercury Solubility Studies....17
4.2.10 EPA EP Toxicity Studies....17
4.2.11 Soil and Tissue Digestion....18
4.2.12 Chemical Analysis of Trace and Bulk Elements....18
4.2.13 Urine Analysis....18

5 RESULTS AND DISCUSSION.....19
5.1 Soil Studies.....19
5.2 Soil Feeding Studies.....26
5.2.1 Body Weights and Mortality.....31
5.2.2 Organ Distribution of Mercury.....36
5.2.3 Kidney Morphology and Function.....39
5.2.4 Dietary Intake of Soil or Sediment Required to Achieve Toxic Levels of Mercury in the Kidney.....40
5.2.5 Carcinogenicity and Teratogenicity Effects.....47

6 SUMMARY, CONCLUSIONS, and RECOMMENDATIONS.....52
6.1 Summary.....52
6.2 Conclusions.....53
6.3 Recommendations.....53

7 REFERENCES.....54

8 FIGURES 3 - 28.....59

APPENDIX (Raw Data)

List of Tables

Table 1 Specimens Collected.....11

Table 2 The Concentration of Selenium in Soil from East Fork Poplar Creek and Bear Creek.....20

Table 3 The Concentration of Zinc in Soil from East Fork Poplar Creek and Bear Creek.....21

Table 4 The Concentration of Lead in Soil from East Fork Poplar Creek and Bear Creek.....22

Table 5 The Concentration of Arsenic in Soil from East Fork Poplar Creek and Bear Creek.....23

Table 6 The Concentration of Cadmium in Soil from East Fork Poplar Creek and Bear Creek.....24

Table 7 The Concentration of Mercury in Soil from East Fork Poplar Creek and Bear Creek.....25

Table 8 The Solubility of Mercury from Soil and Sediment.....27

Table 9 The Solubility of Mercury from Soil and Sediment.....28

Table 10 The Solubility of Mercury from Soil and Sediment.....29

Table 11 EP Toxicity Test for Mercury.....30

Table 12 Recoverable Mercury in Mouse Feed.....32

Table 13 The Gastrointestinal Absorption of Mercury from Soil or Sediment in Mice.....33

Table 14 Mortality of Mice Exposed to Mercury Contamination.....34

Table 15 Mortality of Mice Continuously Exposed to Diets Containing Mercury-Contaminated Soil.....35

Table 16 Animals Killed During the Experimental Period Because of Trauma.....37

Table 17 Percent Mortality in Mice Exposed to Mercury-Contaminated Soil.....38

Table 18 Mercury Data Divided into Histology Classes.....41

Table 19 Partial Correlations (and P-values) for 12-Month Data.....43

1. OBJECTIVE

Heavy metal contamination of floodplain soil and sediment occurred following the release of water containing mercury and other heavy metals by a DOE facility in Oak Ridge, Tennessee. Because of the concern expressed by several regulatory agencies, experimental studies were performed to determine the potential health risk associated with the resulting contaminated soil and sediment. The objective of these studies, to determine the toxicity of the contaminated soil and sediment in experimental animals, was accomplished by feeding mice contaminated soil and sediment through the diet. Because the soil and sediment contained known carcinogens, skin tumor studies were also performed to determine the potential carcinogenicity of the contaminated soil and sediment. Results from the toxicity and carcinogenicity studies provide a basis for evaluating the health risk associated with human exposure to the contaminated soil and sediment.

2. INTRODUCTION

Mercury (Hg) is neither abundant nor widely distributed in the earth's crust.(1). It is estimated that the earth's crust contains 0.8 ppm Hg and that seawater contains 0.3 ppb (2). Mercury exists primarily in highly concentrated ores such as cinnabar, metacinnabar, and livingstonite. Elemental mercury, refined from its ores, finds extensive use in the electrical industry, electrolytic manufacture of chlorine and caustic soda, industrial control instruments, paint, pigments, and amalgam metallurgy. Mercury salts are used as catalysts in the manufacture of fungicides, herbicides, insecticides, bactericides, disinfectants, and antiseptics. Some Hg salts have been used as cathartics, diuretics, and antisyphilitics (calomel Hg_2); as a topical antiseptic and disinfectant ($HgCl_2$); and in the treatment of syphilis and gonorrhea (mercury benzoate).

As a result of the therapeutic use of and industrial exposure to Hg, the health profession became quite familiar with its toxic effects. However, as new drugs replaced the mercurial formulations, drug-induced signs of Hg poisoning became virtually unknown. At the same time, industrial demand for Hg was increasing, which resulted in an increase in human exposure to this metal. This exposure occurred not only through industrial activities but also through pollution of the environment (i.e., air, water, and soil). Environmental pollution may represent the greatest human health risk from Hg because a larger percent of the population may be exposed. For example, Hg contamination of drinking water following industrial contamination of surface and ground water would have a greater impact on the human population than would direct industrial exposure. Similarly, direct contamination of the water supply would present a greater health risk than would industrial exposure. Nevertheless, both industrial exposure and environmental pollution represent a risk to the environment and human health.

3. BACKGROUND

Mercury levels in the lithosphere have been reviewed by several expert groups. The National Science Foundation and the World Health Organization (3, 4) report that the concentration of Hg in sediment (i.e., soil) in nonpolluted rivers and lakes in the United States is less than 0.07 mg/kg of sediment. Recently, the Federal Drug Administration (FDA) recommended a guideline for sediment of 3 ppm total Hg (5). Similar levels have been recommended for soil. Mercury levels as great as 1000 ppm have been observed in sediments of polluted rivers and lakes in close proximity to chloralkali plants. Furthermore, freshwater fish and other aquatic life from polluted rivers and lakes usually contain higher-than-normal levels of Hg. The consumption of fish and/or sediment containing high levels of Hg may pose a health risk to humans, because Hg (in particular, methylmercury) has long been recognized as a toxic agent capable of inducing neurological and kidney disorders and death.

Effluents from the Y-12 Plant in Oak Ridge empty into the S-3 Ponds and New Hope Pond. These ponds are used to trap particulates from the effluents and to allow for biodegradation of the organics in the effluents. New Hope Pond drains into East Fork Poplar Creek, which eventually drains into Poplar Creek and the Clinch River. The S-3 Ponds drain into Bear Creek, which drains into East Fork Poplar Creek and Poplar Creek. Higher-than-normal levels of Hg and other pollutants (i.e., cadmium, lead, arsenic, organics, and radionuclides) have been observed in the soil and sediment from these holding ponds and in the creeks which drain from them. The S-3 Ponds also contain higher-than-normal levels of trichloroethylene, perchloroethylene, phenols, and chlorinated biphenyls (PCBs). Furthermore, higher-than-normal levels of radionuclides (i.e., uranium and thorium) have been observed in fish in the Clinch River near these creeks.

Because these pollutants present a potential human risk (6) for persons eating the sediment and/or soil associated with these sites, the studies described below were performed.

The toxicity associated with these pollutants has been established in both experimental and human studies (7-10), using a simple matrix such as water and/or organic solvents. The toxicity of these pollutants in a complex matrix, such as soil or sediment, has not yet been established. Therefore, in the studies reported here, we attempted to determine the toxicity of these pollutants in a complex matrix.

Mice were fed, via the diet, soil and sediment from various locations along East Fork Poplar Creek, and the toxicity and tissue distribution of the pollutants were determined at various time intervals. The tissue level relative to toxicity was used to determine the effect of a complex matrix on the gastrointestinal absorption and tissue distribution of these pollutants.

The level of each pollutant in the soil and sediment was known prior to feeding the mice. For some of these pollutants (on an individual basis), the amount absorbed per dose has been established. For example, cadmium absorption and tissue distribution have been established in mice and humans given daily doses of this element (11-14). The amount absorbed in the mouse per dose is assumed to be 2.8%, and 50% of this dose is distributed between the liver and kidney. Because of slow excretion (i.e., 0.1% of body burden), a

biological half-life of 200 days has been observed for cadmium (15). With this information, one can determine the effect of a complex matrix on cadmium absorption and tissue distribution.

Absorption and tissue distribution for lead, mercury, arsenic, uranium, and PCBs in mice have also been reported (16-20). This information is useful in establishing the effect of a complex matrix on absorption and tissue distribution for some of these pollutants. The results of the effect of this matrix on absorption and tissue distribution were used to assess the potential human health risk associated with humans eating soil or sediment from selected areas along East Fork Poplar Creek.

The toxicity of these pollutants on various organ systems was determined morphologically and physiologically. These determinations were based on previous results describing the toxicity of these pollutants, which is, in part, related to the tissue level. However, toxicity may be observed in an organ where the tissue level is below that predicted to induce toxicity. For example, in the kidney the critical concentration of cadmium required to induce renal pathology is 200 μg/g wet weight (21). If the kidney level of cadmium is below this value and renal pathology is observed, the synergism of other pollutants present may explain this effect.

Mercury, lead, and uranium have also been reported to induce renal pathology. Thus, the tissue level for each pollutant may not relate directly to the observed toxicity. Toxicity, therefore, is the most important index for assessing the health risk associated with soil and sediment contaminated with several environmental pollutants. For example, kidney toxicity may not be observed even though the concentrations of mercury, lead, cadmium, and arsenic in the kidney are elevated following exposure to contaminated soil and sediment. If toxicity is not observed [even though the levels of these metals in soil and sediment samples are above the Environmental Protection Agency (EPA) acceptable limits], we would suggest that these samples do not present a health risk at the dose used in these studies. The experiments described herein attempt to address this question.

The results in this report focus on the absorption and toxicity of Hg in soil and sediment samples, because the level of this element in most samples far exceeded the lead, cadmium, and arsenic levels. It was assumed, after measuring the levels of these metals in soil and sediment, that the predominant effects would be associated with Hg levels. However, as mentioned above, if toxicological changes are observed in the kidney following exposure to soil and sediment, attempts will be made to determine the relative importance of these metals in explaining this effect. The kidney was used as the target organ in these studies because the inorganic forms of mercury, lead, cadmium, and arsenic are associated with kidney toxicity.

The toxic effects of Hg have been reviewed (22). However, only in the last two decades have reports described the specific toxic effects of the mercuric salts (organic, mercurous, and elemental mercury). Furthermore, during this time reports in the literature have shown the metabolic fate of the various forms of Hg. Mercury metabolism and toxicity are described in Sections 3.1 and 3.2.

3.1 MERCURY METABOLISM

3.1.1 Absorption

3.1.1.1 Gastrointestinal

Three forms of Hg are absorbed through the intestinal tract, although there are differences in the rates of absorption between these forms. Organic Hg compounds are almost completely absorbed, whereas inorganic and elemental forms are absorbed at rates of 2 to 20% and 0.01 to 0.5% of the ingested dose, respectively (23-25). Several dietary factors have been shown to affect Hg absorption, including cysteine, selenium, and calcium. In humans, inorganic Hg is absorbed at rates of 7 to 15%, whereas in mice the rate is only 2% of the dose. However, these rates are dependent on the solubility of the mercuric compound. For example, since the solubility of mercuric chloride is greater than mercuric sulfide, we would anticipate differences in the absorption rates between these compounds.

3.1.1.2 Pulmonary

Three forms of Hg may enter the body through the lungs following inhalation. Mercury absorbed to particles and/or as a vapor (i.e., elemental and organic Hg compounds) readily diffuses from the lung into the bloodstream. Vapors of elemental and organic Hg readily diffuse from the inhaled air into the bloodstream and may cross the blood-brain barrier (26, 27). Unlike gastrointestinal absorption of elemental Hg, pulmonary absorption may exceed 45% of the inhaled dose (pulmonary absorption rates for inorganic Hg are similar to rates observed in the gastrointestinal tract). Differences in the absorption rate in the pulmonary tract between humans and mice have not been reported.

3.1.1.3 Skin

Mercury may be absorbed through the skin, but the amount depends on the form and carrier (28). Some investigators have observed an absorption rate of 6% for mercuric chloride (29). We assume that the absorption rate in skin would be significantly higher for organic mercurials than for inorganic Hg.

3.1.2 Tissue Distribution and Half-Life

Once absorbed, Hg enters the bloodstream and is transported to several organs. Elemental Hg, after entering the bloodstream, may be oxidized to the mercuric ion (30). In the blood, the mercuric ion quickly equilibrates with blood cells to give a plasma-to-cell ratio of approximately 1 (31). Elemental Hg, before oxidation, can cross the blood-brain barrier and accumulate in nervous tissue. Inorganic Hg entering the bloodstream equilibrates in blood cells to give a similar ratio, but does not appear to accumulate in nervous tissue. Organic Hg compounds, because of their lipid solubility, accumulate in blood cells and nervous tissue. However, unlike the mercuric ions, these compounds can concentrate in blood cells to give a plasma-to-cell Hg ratio approaching 108 (32). Furthermore, the

organic Hg compounds can cross the blood-brain barrier and accumulate in nervous tissue.

Mercury accumulates in all soft tissues by absorption from the blood. Inorganic Hg accumulates primarily in the kidney, followed, in descending order, by liver, spleen, and other tissues. Elemental Hg shows a similar distribution to that of inorganic Hg. However, following the inhalation of elemental Hg vapors, significant amounts are observed in brain tissue. Organic mercury compounds, in accordance with their particular chemical structure, differ considerably in patterns of tissue distribution. Virtually all organs accumulate significant amounts of the organic mercurials. Reports have indicated that the liver-to-kidney ratio may approach 1 after exposure to organic Hg, whereas 0.09 is observed for inorganic Hg. However, the kidney, liver, and brain contain the highest levels of Hg after exposure to methylmercury. Some of the short-chain alkyl Hg derivatives have been shown to accumulate in the fetus.

Mercury can enter cells of various organs by passive diffusion and/or active transport. In the cell, Hg binds to various macromolecules, primarily sulfhydryl-containing compounds. Turnover of cellular Hg is relatively slow; however, once released from the cell it is cleared from the body primarily via the bile, fecal, and urinary routes.

Inorganic and elemental Hg are excreted predominantly via the urine, while the organic mercurials are excreted primarily via the bile and feces (33, 34). Significant amounts of the organic mercurials are excreted via the bile into the intestinal tract and may be reabsorbed and/or excreted in the feces. Estimates for humans indicate a relatively long biological half-life for Hg of 42 to 70 days (35, 36), depending on the form of Hg in the body. The half-life for inorganic Hg is estimated to be 48 and 57 days for males and females, respectively (37). The half-life for organic mercurials in humans is estimated as 70 to 74 days (38). Shorter half-lives for inorganic and organic Hg are observed in experimental animals. For example, the half-lives for inorganic Hg and methylmercury in mice were 2 and 21 days, respectively (39, 40). The half-lives described above were determined for the whole body; it has been demonstrated that the half-life for different organs may be considerably different.

3.2 MERCURY TOXICITY

Toxic effects have been observed in humans and experimental animals following exposure to the three forms of Hg. Ingestion of inorganic Hg produced local necrotic changes in the gastrointestinal tract, circulatory collapse, and acute renal failure with aliguria or anuria. The chronic effects include renal tubular necrosis with proteinuria.

Chronic exposure to elemental Hg vapors occurs primarily in the workplace. Workers are frequently exposed to inorganic Hg through inhalation of dust particles. In clinical studies, attempts to separate the chronic effects of elemental Hg vapor from the effects of inorganic Hg have rarely been made. However, it is known that the respiratory absorption of elemental Hg exceeds that of inorganic Hg. Furthermore, it is known that elemental Hg, unlike the inorganic form, accumulates in brain tissue. This may explain the associated high incidence of central nervous system disorders frequently observed at the workplace (i.e., workers mining cinnabar). The toxic effects

associated with elemental Hg vapors include anorexia, loss of weight, asthenic-vegetative syndrome (i.e., micro-mercurialism), and renal failure. These symptoms are observed in the early stages of toxicity, whereas later stages are characterized by mercurial tremors, psychic disturbances, and changes in personality (erethismus mercurialis).

Animal studies have confirmed some of the effects associated with exposure to vapors of elemental Hg. These experimental studies also show significant levels of Hg in brain tissue following the exposure. Symptoms associated with the ingestion of elemental Hg are few, but include necrotic changes in the intestinal tract and proteinuria. These effects are only observed following acute exposure. Thus, exposure to elemental Hg vapors appears to be more toxic than exposure to equivalent amounts of elemental Hg via the gastrointestinal tract. This difference may be explained by the amount absorbed in the respiratory tract (i.e., 30 to 80% of the dose) versus the gastrointestinal tract (i.e., 0.01 to 0.5% of the dose). Furthermore, as mentioned above, elemental Hg accumulates in brain tissue, and thus an increase in the amount absorbed via the lungs will eventually affect the central nervous system.

Neurological and kidney disorders are observed in humans and mice exposed via pulmonary and/or intestinal routes to organic mercurials. The toxic effects of the organic mercurials are similar to the above descriptions for inorganic and elemental Hg. However, the organic mercurials are more toxic than dose equivalents of inorganic or elemental Hg because (1) the absorption rate (i.e., both pulmonary and intestinal) is greater; (2) they accumulate in nervous tissue; (3) they readily diffuse into cells; and (4) they have a longer half-life.

3.3 OBJECTIVE OF THE HEALTH RISK STUDIES

The overall objective of the studies described here was to determine experimentally the potential health risk associated with humans eating soil or sediment contaminated with Hg and heavy metals from East Fork Poplar Creek. This objective was accomplished by feeding mice a diet containing contaminated soil and sediment for 20 months and assessing the toxicity and organ levels of Hg at various time intervals. Because some of the soil and sediment samples contained known carcinogens, skin tumor studies were also performed to further evaluate potential health risks associated with these samples. Specific objectives were assessments of toxicity, carcinogenicity, and teratogenicity.

The toxicity assessments included (1) body weight changes over the experimental period, (2) liver and kidney weights over the experimental period, (3) a swim test to evaluate neurological changes, (4) levels of Hg in the liver and kidney at various time intervals during the exposure, (5) the ratio of liver-to-kidney Hg, (6) the morphology of the kidney, and (7) the functional physiology of the kidney. Other assessments included determining the levels of heavy metal contaminants in soil and sediment, the relative level of

water-soluble Hg in soil and sediment, and the exposure dose of Hg in the feed after the addition of soil and sediment. The carcinogenicity assessments included tumor promotion and tumor initiation. The teratogenicity assessments included determining litter size in the exposed animal.

3.3.1 Toxicity Assessments

Chronic feeding studies are frequently used in toxicology studies to assess the effect of a single chemical or a specific chemical in a complex matrix. The studies outlined here were designed to determine the amount of Hg absorbed into mice following the feeding of soil and sediments with known concentrations of Hg. These studies were also designed to determine the potential pathological effects of Hg in soil and sediment on the central nervous system and on the various organs described above. If the Hg in soil and sediment was mainly organic Hg (i.e., methylmercury), we would have anticipated changes in the ratio of liver-to-kidney Hg and effects on the central nervous system. However, if the Hg in soil and sediment was mainly in the inorganic form, we would have anticipated morphological and functional changes in the kidney. These pathological changes would, of course, depend on the levels of Hg in the soil and sediment and the amount of Hg absorbed via the intestinal tract. Thus, these chronic studies provide a method for assessing the potential health risk associated with the ingestion of Hg-contaminated soil and sediments. These studies also provide data necessary for determining the effect of soil constituents on Hg absorption and tissue distribution. For example, selenium has been shown to inhibit Hg absorption via the intestinal tract and to alter the tissue distribution and toxic effects of Hg.

The effect of other inorganics on organ systems in the mouse may be assessed by comparing the physiological and biochemical changes with the tissue level of these inorganics. For example, the following associations have been reported: (1) lead with a reduction in heme biosynthesis, (2) methylmercury and lead with neurological disorders, (3) mercury and cadmium with liver cell damage, (4) lead and uranium with bone abnormalities, (5) arsenic with cancer, (6) selenium with white muscle disease, and (7) lead, cadmium, uranium, and mercury with kidney disorders.

The tests outlined below provide a method for determining the toxicity of the various inorganics found in soil and sediments. For example, an increase in the level of lead (Pb) in the brain, kidney, and bone; neurological changes; and a decrease in the activity of aminolevulinic acid synthetase (ALAD) (the rate limiting enzyme in heme biosynthesis) would indicate that the level of Pb found in soil or sediment is toxic. However, if neurological disorders are observed along with increases in brain and kidney Hg, and if Pb in tissue and the activity of ALAD in plasma are both normal, we would assume, *a priori*, that the neurological disorders observed are associated with the level of Hg in the soil or sediments. It is also possible that neurological disorders may be observed when the tissue levels of both Hg and Pb are increased and the activity of ALAD is normal. In this case, we would assume that the neurological changes observed occurred as a result of the level of Pb and Hg in soil or sediments. Previously reported dose-response relationships (i.e., the relationship of tissue level of the above elements to pathological

changes in the mouse) will also be used to assess the potential toxicity of selected elements observed in soil and sediment samples.

The interaction of various elements in the soil sediment on absorption via the gastrointestinal tract was also assessed. By determining the tissue level of these elements relative to the dose, various interactions can be assessed. For example, changes in the calcium and selenium levels in the soil or sediments may affect the amount of cadmium and mercury absorbed, respectively. Interactions between calcium and lead on lead absorption and between zinc and cadmium on cadmium absorption have been previously reported (41). We thus anticipated that absorption and toxicity would be influenced by the various interactions of the elements found in soil or sediments. However, we note that these potential interactions are only assessed if toxicological effects are observed.

3.3.2 Carcinogenicity Assessments

Skin painting tests have frequently been used to determine comparative tumor-initiating activity of some complex mixtures in the Sencar mouse (42, 43). This mouse is used frequently for its increased sensitivity to two-stage carcinogenesis using 7,12-dimethylbenz(a)-anthracene (DMBA) as the initiator and 12-O-tetradecanoylphorbot-13-acetate (TPA) as the promotor. This system is also more sensitive to other polycyclic aromatic hydrocarbons (PAH) such as benzo(a)pyrene (B[a]P). In addition to its well-documented response to PAH, the mouse skin tumorigenesis bioassay system has identified many chemicals other than PAH as potential carcinogens. These chemicals represent a wide variety of structural classes, including aldehyde, carbamate, epoxide, haloalkyether, haloaromatic, haloalkylcarbonyl, hydroxylamine, lactone, nitrosamide, sulfonate, sulfone, and urea. The mouse skin tumorigenesis bioassay can also detect chemicals that cause tumors in the respiratory tract of animals. Of all known animal respiratory carcinogens, the mouse skin tumorigenesis system has, to date, detected PAH, quinolines, and carbamates. For highly suspect occupational respiratory carcinogens, the mouse skin tumorigenesis system has, to date, detected chloromethyl ethers and coke oven emissions. These results indicate that the mouse skin tumorigenesis bioassay can detect both dermal and nondermal carcinogens.

Two basic protocols frequently employed to detect chemical carcinogens in the mouse skin tumorigenesis assay are illustrated in Fig. 1. Multiple application of the test agent for up to 17 weeks will give rise to papillomas and/or malignant carcinomas of the skin. This protocol for complete carcinogens is a test for agents exhibiting both tumor-initiating and tumor-promoting activities. The bioassay protocol for tumor initiators is a double application of test agent followed by multiple applications of a potent tumor promoter 1 week later. Tumor initiation is one step in the multi-step carcinogenic process and involves the conversion of a normal cell into a preneoplastic one. In the case of chemical carcinogens, it involves the interaction of chemicals or their activated forms with cellular DNA. These initiated cells remain dormant for periods of up to 1 year or until they are stimulated to progress into hyperplastic or neoplastic lesions. This stimulation, called tumor promotion, is accomplished by applying croton oil or its most active component, TPA.

PROTOCOL FOR TUMORIGENESIS ASSAY

Tumor Initiation	Soil Extract (two applications)	TPA 2x Weekly
Weeks of treatment	1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17	
Tumor Promotion	DBMA (two applications)	Soil Extract 2x Weekly
Weeks of treatment	1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17	
Internal Control	DBMA	TPA 2x Weekly
Weeks of treatment	1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17	

At weekly intervals, the number of papillomas per mouse and the number of mice with papillomas will be recorded. For solvent controls, mice will be treated with acetone for 17 weeks. The number of mice per treatment group is 5 to 10 of either sex.

Figure 1

An initiated cell is, therefore, an irreversibly formed preneoplastic lesion that can be stimulated to express the transformed phenotype.

Relationships between tumor initiators and complete carcinogens have been previously described (44). Various structurally diverse chemicals are both complete carcinogens and tumor initiators in mouse skin from CD-1 and the genetically related Sencar mouse. Some agents, however, appear to have only tumor-initiating activities in mouse skin. The correlation between potencies as complete carcinogens and as tumor initiators is excellent for the 12 chemicals that show both kinds of activity. The relationship between the production of papillomas and the production of carcinomas in the same animals treated with the skin tumor initiator DMBA or B(a)P has been shown. The results indicate that the number of papillomas per mouse at 15 to 20 weeks correlates well with the number of malignant carcinomas formed at 50 weeks for animals treated with these two strong skin tumor initiators.

3.3.3 Teratogenicity Assessments

Teratology is the study of congenital malformations. Teratogenic effects are defined as alterations in the formation of cells, tissues, and organs resulting from physiologic and biochemical changes (i.e., generative changes). Thus, once the particular cells, tissues, physiological systems, or biochemical systems have been formed, a teratogenic change can no longer occur. The anatomical effect of teratogens thus occur very early in the fetal period. Teratogenic effects are often assessed by anatomical changes such as cleft palate, polydactyly, syndactyly, ectrodactyly, and hydrocephaly. Aborted fetuses and changes in litter size may occur as a result of exposure to teratogens. However, aborted fetuses and/or litter size changes may also occur as a result of chemical toxicity to the fetuses. Thus, if such changes do occur following the exposure to a chemical, it would be necessary to further evaluate the fetuses for congenital malformations. Identification of congenital malformations indicates that the material to which the animal was exposed is teratogenic.

4. PROCEDURES AND METHODS

4.1 PROCEDURES

4.1.1 Sample Collections

Sediment samples were taken with a stainless steel Jenkins sampler at a depth of 30 cm from the areas indicated in Table 1 and Fig. 2. The sampler was thoroughly washed (deionized water) after each sample to avoid contamination between samples. Soil and sediment samples for this study were collected from areas on a transect to include the floodplain and center of the creek bed. In this configuration, five samples were collected from the creek bed and five samples (i.e., five/side) from floodplains. Respective samples represent environmental conditions for aquatic as well as terrestrial systems. Sediment and soil samples within a transect were collected

TABLE 1
Specimens Collected*

Site (see Fig. 1)	Left flood-plain	Creek	Right flood-plain
Origin East Fork Poplar Creek (Y-12 area at guard house)	1	2	3
1.5 miles from New Hope Pond (near NOAA)	4	5	6
K-Mart	7	8	9
Bruners	10	11	12
Sewage Treatment Plant Oak Ridge Turnpike (7-8 miles from New Hope Pond)	13	14	15
Gaging Station Oak Ridge Turnpike (11.5 miles from New Hope Pond)	16	17	18
Fork where East Fork Poplar Creek enters Poplar Creek (14.3 miles from New Hope Pond)	19	20	21
Origin Bear Creek (Y-12 area near S-3 Ponds)	22	23	24
Fork of Bear Creek and East Fork Poplar Creek (8.8 miles from S-3 Ponds)	25	26	27
Origin Poplar Creek (100 ft below origin)	28	29	30

*Five 30-cm cores were taken at each of the above sites.

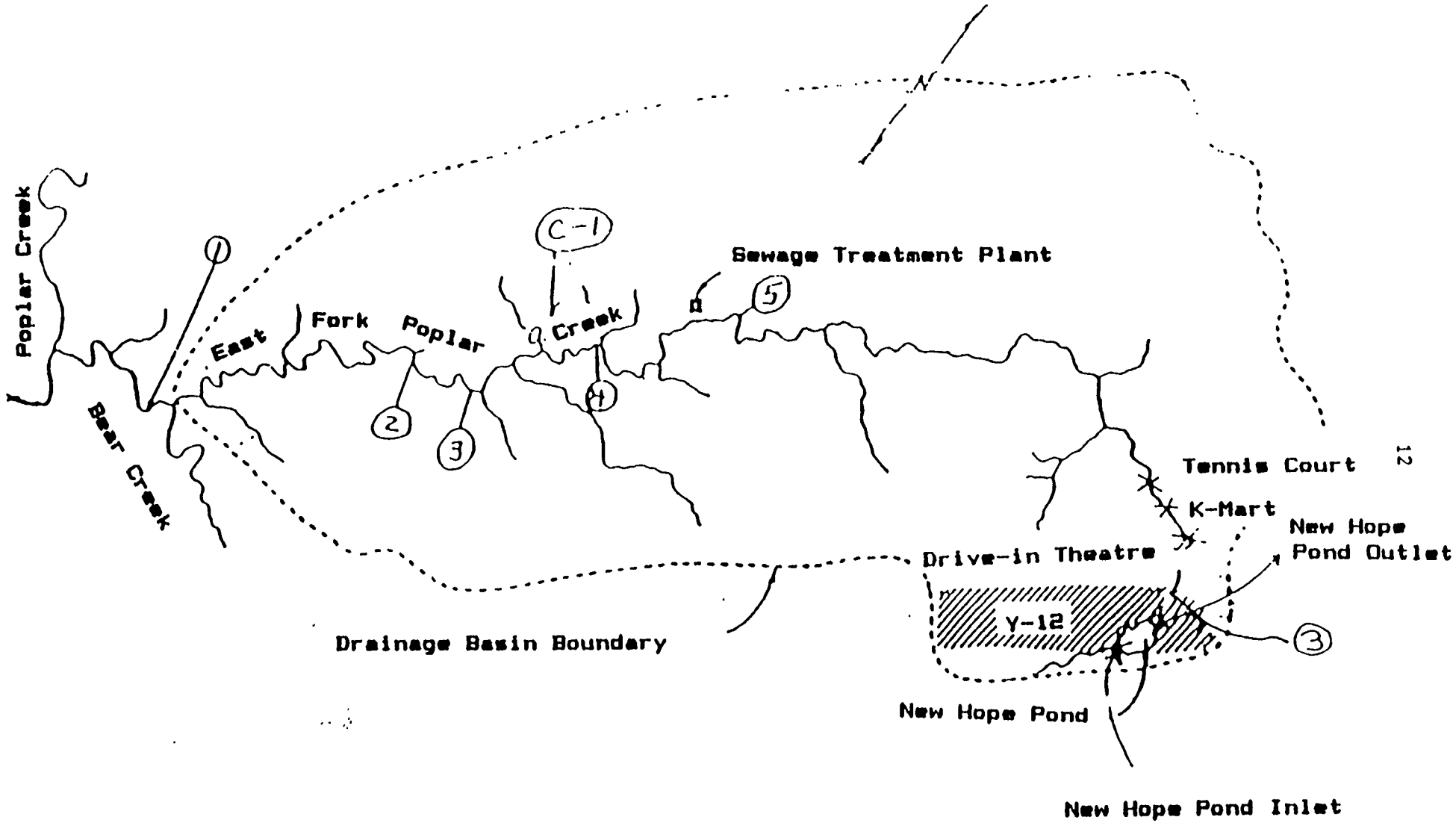
EAST FORK POPLAR CREEK DRAINAGE
Poplar Creek
Bear Creek
East Fork Poplar Creek
C-1
Sewage Treatment Plant
1
2
3
4
5
Tennis Court
K-Mart
New Hope Pond Outlet
Drive-in Theatre
Y-12
Drainage Basin Boundary
New Hope Pond
New Hope Pond Inlet
0
1
2
kilometer

Figure 2

from areas where particle size distribution is appropriate for the feeding experiments.

It is safe to assume that gross changes in the physical and chemical composition of the creek sediments can occur slowly over the period of the experiment, except at times of storm events, when scouring and filling redistribute sediment, and at times of accidental release of pollutants. To address these changes, soil and sediment samples were collected at 0 and 12 months (it is noted that the level of those inorganics measured did change over the experimental period).

Pollutants released into the creek may be partitioned into several fractions, which may be transported from the system at variable rates, with a portion trapped in the sediments and alluvium within the channel and floodplains, respectively. Although pollutants trapped in the sediments and alluvium may be exposed to some physicochemical changes through time, the long-term effects can be assessed through the described feeding experiments. Pollutants most likely to be associated with creek sediments are distributed according to stream morphology and flow dynamics at baseline and storm events.

The collected samples were frozen, cut in half vertically, and analyzed for specified inorganics at 10-cm intervals. The top 10 cm was lyophilized to constant weight and used for feeding and skin painting studies or stored frozen at -60°C. The top 10 cm of the core samples was selected for the animal studies, because it represents the approximate depth at which pollutants may exchange with water in the creek basin. A similar exchange would be expected in the floodplains. The top 10 cm was also selected for these studies because it contained the highest concentration of those inorganics measured.

4.1.2 Chronic Feeding and Skin Painting Studies

4.1.2.1 Chronic Feeding Studies

The chronic feeding studies were performed to assess the potential health risk which may be associated with the various pollutants observed in soil and sediment samples. Soil and sediment samples were obtained as described above. After mixing and lyophilizing the soil and sediments, the dried soil or sediment was added to powdered Purina mouse chow, mixed, and feed pellets were prepared by Ralston Purina. The soil or sediment concentration in the feed was 5%; animals (i.e, 2,700 male and female Swiss mice) were fed these modified diets over a 20-month period. At 6, 12, and 20 months of exposure, 5 to 10 mice from each group were analyzed for neurological changes and muscle coordination by measuring their ability to swim in a tank of water for 100 sec. This simple swimming test has been used frequently to assess central nervous function in rats and mice (45). After this measurement, final body weights were determined, the animals were sacrificed, and the following analyses performed: (1) Hg levels in the liver and kidney, and (2) histological evaluations of the kidney for morphological changes. Some of the mice in this main group were also used in the food intake and urinary collection studies described below.

In some studies, mercuric chloride was added to the dried soil and sediment and mixed with the powdered mouse chow. Animals were fed these diets for 20 months, except for groups 31 and 32. These latter two groups were fed these diets for only 6 months. The groups fed the

the final concentrations of Hg in the each diet were 2,000, 1,000, 500, 4,000, and 8,000 ppm, respectively. Animals in groups 2, 14, and 27 were treated the same as described for the other experimental groups. In groups 31 and 32, body weights, liver and kidney Hg levels, and the morphology of the kidney were determined following 6 months of exposure.

The number of mice used in this study were as follows:

(1) Chronic feeding studies: 2,700 male and female Swiss mice 3 months of age.
(2) Chronic exposure to feed containing mercuric chloride: 80 mice (40 males and 40 females) for groups 2, 14, and 27, and 40 mice (20 males and 20 females) all 3 months of age.
(3) Food consumption and mercury absorption studies: 300 male and female Swiss mice who were previously exposed to the above diets for 6 and 12 months (i.e., groups 1 through 30).
(4) Urinary studies: 150 male and female Swiss mice who were previously exposed to the above diets for 12 months (i.e., groups 1 through 30).
(5) Teratogenicity studies: 30 male and 60 female Swiss mice who were previously exposed for 6 months to the above diets (i.e., groups 1 through 30.
(6) Skin painting studies: 1,060 male and female Sencar mice, 3 months of age.

4.1.2.2 Skin Painting Studies

The procedure used in these studies is shown in Fig. 1. Briefly, the organics in soil or sediment were extracted into chloroform-methanol (2:1 v/v) at 4°C and the samples dried with nitrogen. The dried samples were diluted in acetone and applied to the shaven backs of female and male Sencar mice. The period of application and the volume applied are shown in Fig. 1. At 1-week intervals, the number of papillomas (those greater than 2 mm in diameter) per mouse and the number of mice with papillomas were recorded. Papillomas were removed randomly for histological verification. A total of 1,060 female and male Sencar mice 10 weeks of age were used in this study.

4.2 METHODS

4.2.1 Test Diet Preparation

Thirty test diets were made using soil and sediment samples collected at the beginning of the study and 12 months after the study was in progress. Soil and sediment samples (top 10-cm layer) taken from the sites shown in Table 1 were thoroughly mixed and dried to a constant weight by lyophilization. The powdered soil and sediment samples were maintained at -60°C until required for preparation of the test diets. The soil and sediment samples were pulverized to a fine powder and uniformly mixed with a powder form of Purina mouse chow. This mixture was made into 15-cm pellets by Ralston Purina at 0, 10, and 15 months. Mice were fed this mixture ad libitum for 20 months. The soil and sediment from the 30 sites were added individually to the Purina mouse chow to give a total concentration of 5%. Thus, each

diet contained 5% soil or sediment. A total of 80 Swiss mice (i.e., 40 males and 40 females) were fed these diet for 20 months. A total of 2,700 male and female mice 3 months of age were fed these diets for 20 months.

After preparation of the test diet samples, aliquots were taken for chemical analysis, which included the measurement of mercury, cadmium, arsenic, lead, and selenium. These measurements were made to determine the dose exposure levels.

4.2.2 Food Intake and Urinary Collection Studies

Food intake was measured in male and female mice exposed for 6 and 12 months to diets containing Hg-contaminated soil. The animals (five per experimental group per sex) were placed individually into metabolic cages and acclimatized. After 16 hours, a weighed amount (13 to 16 g) of feed was given to each mouse. After 24 hr, the residual amount of feed was determined. The measurement of food intake was repeated twice, with an overnight interval between measurements, and the results averaged. It is noted that data obtained are subject to a standard deviation of 10 to 18% because, while the animals were eating, some of the feed (small particles) was observed at the bottom of the cage. A total of 300 males and females from the main chronic feeding studies were used to measure food intake and, after these measurements, the animals were returned to the main study. It is noted that food intake was determined in animals fed the respective modified diets. After 12 months, urine samples were collected from mice (five per group per sex) exposed to the modified diet. The animals were placed individually into metabolic cages and acclimatized for approximately 56 hours. Urine samples were then collected over a period of 24 hr. Three collections were made at 8, 10, and 24 hr, and the samples were combined and analyzed for protein, gamma GT, and creatinine. A total of 150 males and females from the main chronic feeding studies were used to collect urine. During the period of urine collection, the animals were fed the respective modified diets.

4.2.3 Soil Feeding Studies

Germ-free 3-month-old Swiss mice were obtained from Jackson Laboratories (Bar Harbor, Maine). For the 30 experimental groups, 80 mice (40 males and 40 females) were used. Animals were fed the test diet *ad libitum*. At 6, 12, and 20 months of exposure, 5 to 10 mice were removed from the study and the various tests and analyses were performed.

4.2.4 Mercury Absorption Studies

The absorption of mercury through the gastrointestinal tract was determined in 3-month-old male and female Swiss mice. Animals (five per experimental group per sex) were placed individually into metabolic cages and fasted overnight. After fasting they were fed the test diet for 24 hr, and their food intake measured. After the 24-hr exposure, they were fed a normal diet and fecal samples were collected over a 96-hr period. Samples were then dried to a constant weight, digested in nitric acid, and Hg determined in the digest. A total of

90 Swiss mice (both male and female) were used in these absorption studies.

4.2.5 Extracts for Skin Painting Studies

Soil and sediment extracts were prepared as described above. Extracts in acetone were used in all studies. A 0.2-mL sample was applied to the backs of Sencar mice for both the initiation and promotion studies.

4.2.6 Skin Studies

Figure 1 outlines the protocol used in the initiation/promotion studies. Briefly, 5 to 15 Sencar mice (male and female) per treatment group were initiated with 0.2 mL of extract (2x) and then promoted with TPA twice weekly (1 µg per application) for 17 weeks. In the promotion studies, animals were initiated with DMBA (two 5-nM applications) and promoted with 0.2 mL of soil extract twice weekly for 17 weeks. The negative controls were treated with 0.2 mL of acetone, DMBA, or TPA for 17 weeks. The positive controls were initiated with DMBA (two applications of 5 nM each) and promoted with TPA twice weekly (1 µg/ application) for 17 weeks. Both TPA and DMBA were dissolved in acetone.

After applying the above mixtures to the skin, the number of animals with tumors and the number of tumors per animal were recorded. Weekly counts of cumulative skin tumors (i.e., tumors that were observed for 3 consecutive weeks) were made for the 17-week exposure period. Results are expressed as the total cumulative number of skin tumors per mouse per treatment group.

4.2.7 Teratogenicity Studies

Litter size has been frequently used to determine the potential teratogenicity of various chemicals (46). To determine the potential teratogenicity of the contaminated soil and sediment samples, male and female Swiss mice were exposed for 6 months to the test diets described above. One male and two females from each group were then time-mated. At 24 and 96 hr postdelivery, the litter size was determined in each group. A total of 30 males and 60 females were used in these teratogenicity studies.

4.2.8 Histological Studies

Mice were exposed to the experimental diets for 20 months. At 6, 12, and 20 months, 5 to 10 animals were sacrificed and a gross necropsy performed. After visible lesions had been recorded, the liver and kidney were removed for further analysis. A section of kidney was removed for microscopic evaluation and the remainder was used for elemental analysis. The kidney section was fixed in a buffered formaldehyde solution. After dehydration and embedding, 10-µ sections were stained with hematoxylin-eosin. The stained sections were examined under a light microscope for necrotic epithelial cells of the proximal convoluted tubules and/or glomerulus, diffuse necrosis, or fibrosis. Epithelial necrosis was defined as karyoklasis and cytoplasmic granulation.

Thirty fields were examined for each kidney section for necrosis and fibrosis. After examination each section was ranked as follows:

0 = normal kidney
1 = 0-2 fields showing one or more necrotic proximal tubules
2 = 2-4 fields showing one or more necrotic proximal tubules
3 = 4-6 fields showing one or more necrotic proximal tubules
4 = 8-12 fields showing one or more necrotic proximal tubules
5 = 12-20 fields showing one or more necrotic proximal tubules and necrotic glomerulus
6 = diffuse necrosis with fibrosis.

4.2.9 Mercury Solubility Studies

The solubility of mercury from soil and sediment samples was determined by adding 10 g of soil to 160 mL of deionized water, benzene, or concentrated nitric acid. This mixture was agitated for 24 hr, and an aliquot of the liquid phase was removed and filtered through a 0.45-μ filter. The mercury level was then determined on the filtrate. In studies using benzene, samples were agitated for 24 hr, then centrifuged at 21,000 rpm for 90 min, and an aliquot of supernatant was removed and the Hg determined. The Hg levels in these samples were determined using the method of addition (i.e., using methylmercury, mercury chloride, and elemental mercury to determine the accuracy of these extraction methods). It is noted that the above species of mercury were all soluble in nitric acid, whereas elemental mercury was sparingly soluble in benzene and deionized water (i.e., 20 μg/L in deionized water and 4 μg/L in benzene), and mercuric chloride was soluble in deionized water and sparingly soluble in benzene (i.e., 6 μg/L).

The factors associated with the different species of mercury in soil samples from East Fork Poplar Creek can be both biological and chemical. For example, chemical and bacterial systems have been identified that convert inorganic mercury Hg^{2+} to elemental mercury Hg^{o}, bacterial systems that convert Hg^{2+} to methylmercury, and systems that convert Hg^{2+} to mercuric sulfide HgS. Methylmercury, elemental Hg, and mercuric sulfide have been identified in soil from East Fork Poplar Creek. The importance of the solubility of the various species of mercury for these feeding studies is discussed in detail in Section 5.1.

4.2.10 EPA EP Toxicity Studies

Soil and sediments obtained from the sites in Table 1 were mixed and weighed. A 100-g sample was placed into a 3-L flask and 1.6 L of deionized water added. This mixture was agitated for 30 min and the pH determined. The pH was adjusted to 5.0 by the addition of 0.5 N acetic acid. During the first 8 hr, the pH was adjusted (if required) at half-hour intervals. Thereafter, pH adjustment occurred every 6 hr. The total incubation period was 24 hr, and the amount of acid added during this period did not exceed 4 mL/g of soil. During the incubation period, samples were agitated and the temperature maintained at 25°C (±5).

After incubation, deionized water was added to the sample using the equation outlined by EPA (i.e., V = (20)W - 14(W) - A (A = mL of [illegible] the volume, a 100-mL aliquot was

filtered through a 0.45-μ filter and the filtrate analyzed for mercury, lead, cadmium, and arsenic.

4.2.11 Soil and Tissue Digestion

Soil and sediment samples were digested as described by Feldman (47). Briefly, a 1-g sample was added to a 250-mL volumetric flask, and 5 mL of (ultrapure) H_2SO_4 plus 10 mL of (ultrapure) HNO_3 were added. A condenser (wrapped with asbestos tape and further insulated with teflon tape) was placed on top of the flask, and the flask was placed on a hotplate set at 350°C. The sample was heated until all the HNO_3 evaporated (usually 4 hr). A 10-mL aliquot of $HClO_4$ was added to the flask and the temperature increased to 450°C. The sample was digested for 15 hr (or until the $HClO_4$ evaporated) and then allowed to cool before rinsing the condenser with distilled water. After the digestion volume was adjusted to 250 mL with distilled H_2O, the above metals were analyzed.

A 1-g sample of liver, kidney, or brain was lyophilized to a constant weight. An aliquot of the dried sample (100 to 200 mg) was placed in HNO_3-washed vials and 4 mL of HNO_3 added. The sample was heated at 300°C for 8 hr (or until the tissue was completely digested), diluted to 4 mL with deionized water, and the above metals analyzed after further dilution. For both the soil and tissue samples, aliquots of the above metals were added to the sample to determine the percentage of recovery.

4.2.12 Chemical Analysis of Trace and Bulk Elements

The following elements were analyzed using the following methods. Ca^{2+}, AA flame; Zn^{2+}, AA flame; Pb^{2+}, Cd^{2+}, and Se^{2+}, AA graphite furnace; Hg^{2+} and hydride, cold vapor; and As^{2+}, U, ICP.

4.2.13 Urine Analysis

Urine was analyzed for urinary protein, urinary gamma, and creatinine. Protein was measured according to the method of Lowry et al. (48), using bovine serum albumin as standard. Suitable dilutions of albumin and samples were reacted with Folin-Ciocalteau reagent in the presence of alkaline mixtures of sodium carbonate and citrate. The resulting blue color, proportional to the amounts of protein, was read for absorbance at 750 mu, against a similarly treated water blank. Gamma GT was measured in urine by the kinetic procedure described in Sigma Applications Note 418. In this procedure, the enzyme converts L-γ-glutamyl-p-nitroanilide to p-nitroaniline in the presence of glycylglycine. The change in optical density at 405 mu, after exactly 5 min of incubation of sample with reagent mixture, is proportional to the enzyme activity. Each assay run was standardized by the inclusion of Acutrol chemistry controls (Sigma) containing accurately known levels of normal or elevated Gamma GT activities.

Creatinine was measured in urine samples using Sigma Diagnostic Kit, No. 555. Samples of urine and of accurately determined standard solutions of creatinine were reacted with picric acid solution to form

a yellow-orange color whose absorbance was measured at 500 mu. Addition of acetic acid solution resulted in a loss of color intensity whose absorbance was measured again at 500 mu after 5 min. The change in optical density was proportional to the creatinine concentration.

5. RESULTS AND DISCUSSION

5.1 SOIL STUDIES

It has been reported that children may eat up to 1 g of soil per day during normal activity (49). Also, in the southeastern United States, there are adults who reportedly eat various quantities of soil per day. Thus, a human health risk may exist in persons ingesting soil with known toxic pollutants. However, human ingestion of soil is not considered an important pathway for human exposure to toxic pollutants. Nevertheless, it is a pathway that requires further examination, as discussed below.

The environmental release of and human exposure to toxic pollutants are regulated by state and federal government agencies because of their potentially harmful effect(s) on environmental and mammalian systems (i.e., humans). These effects have been established under defined experimental conditions which include determining the dose-response relationships for specific chemicals in a simple matrix. However, under normal circumstances humans are exposed to these toxic chemicals in a complex matrix (i.e., foods and/or liquids) which may contain several toxic pollutants. Determining the dose-response relationship for these chemicals in a complex matrix would seem to be a realistic approach in establishing limits for human intake. The objective of the present studies was to determine experimentally the dose-response relationship of toxic inorganic pollutants in soil and sediment (i.e., a complex matrix) on various organ systems. This objective was accomplished by feeding to mice, via the diet, soil or sediment containing a variety of toxic inorganics.

The soil and sediment used in these studies were obtained from an area previously contaminated with arsenic, lead, cadmium, and mercury. These metals were released into a creek, resulting in contamination of the creek basin and the associated floodplains. Samples of soil and sediment were taken from the floodplains and creek basin along this contaminated area. The sites were selected randomly but included areas with relatively high levels of the above-mentioned inorganics. The sites selected are described in Table 1.

Five 30-cm cores were taken from the sites, and sections (10 cm) from the cores were mixed and lyophilized to a constant weight. After acid digestion, aliquots of the dried soil were analyzed for lead, mercury, cadmium, arsenic, selenium, and zinc. The top 10 cm was used in the feeding studies because (1) it represents the approximate depth at which these inorganics may exchange with water, (2) it reasonably represents the depth to which soil-eaters and playing children would be exposed, and (3) the highest level of the above inorganics was observed at this depth. The concentrations of these metals from the various sites are shown in Tables 2 through 7.

Significant site-to-site variations were observed for all the metals studied, but the greatest variation was observed for Hg.

TABLE 2
The Concentration of Selenium in Soil
from East Fork Poplar Creek and Bear Creek

Site	Site number		Selenium mean ± SD (ppm)
Guard-house	1	Left	129.57 ± 39
	2	Middle	190.99 ± 18
	3	Right	197.05 ± 50
NOAA	4	Left	271.51 ± 40
	5	Middle	231.15 ± 38
	6	Right	100.71 ± 27
K-Mart	7	Left	191.47 ± 39
	8	Middle	162.55 ± 53
	9	Right	128.98 ± 16
Bruners	10	Left	190.52 ± 45
	11	Middle	124.68 ± 38
	12	Right	209.11 ± 25
Sewage Plant	13	Left	126.16 ± 18
	14	Middle	59.86 ± 12
	15	Right	69.81 ± 16
East Fork Turnpike Building	16	Left	168.98 ± 13
	17	Middle	90.09 ± 19
	18	Right	142.95 ± 40
Poplar Creek Perimeter Road	19	Left	99.10 ± 16
	20	Middle	73.00 ± 20
	21	Right	93.61 ± 17
Bear Creek	22	Left	111.25 ± 13
	23	Middle	101.29 ± 16
	24	Right	99.27 ± 17
Bear Creek	25	Left	100.69 ± 19
	26	Middle	107.74 ± 13
	27	Right	195.09 ± 14
Bear Creek	28	Left	127.04 ± 16
	29	Middle	101.78 ± 20
	30	Right	122.52 ± 23

TABLE 3
The Concentration of Zinc in Soil
from East Fork Poplar Creek and Bear Creek

Site	Site number		Zinc mean ± SD (ppm)
Guard-house	1	Left	51.40 ± 5
	2	Middle	97.05 ± 8
	3	Right	248.53 ± 23
NOAA	4	Left	294.12 ± 36
	5	Middle	213.78 ± 40
	6	Right	146.48 ± 25
K-Mart	7	Left	91.58 ± 10
	8	Middle	50.93 ± 7
	9	Right	83.83 ± 8
Bruners	10	Left	242.07 ± 20
	11	Middle	78.09 ± 10
	12	Right	190.71 ± 13
Sewage Plant	13	Left	86.97 ± 7
	14	Middle	59.68 ± 8
	15	Right	141.61 ± 24
East Fork Turnpike Building	16	Left	109.83 ± 13
	17	Middle	58.16 ± 4
	18	Right	90.50 ± 8
Poplar Creek Perimeter Road	19	Left	85.66 ± 10
	20	Middle	86.26 ± 5
	21	Right	73.34 ± 11
Bear Creek	22	Left	52.95 ± 6
	23	Middle	59.85 ± 4
	24	Right	86.64 ± 9
Bear Creek	25	Left	68.30 ± 11
	26	Middle	55.44 ± 8
	27	Right	56.46 ± 10
Bear Creek	28	Left	64.20 ± 7
	29	Middle	38.79 ± 5
	30	Right	66.73 ± 5

TABLE 4
The Concentration of Lead in Soil
from East Fork Poplar Creek and Bear Creek

Site	Site number		Lead mean ± SD (ppm)
Guard-house	1	Left	5.65 ± 1.3
	2	Middle	9.85 ± 1.7
	3	Right	10.67 ± 1.3
NOAA	4	Left	17.29 ± 2.1
	5	Middle	12.48 ± 1.3
	6	Right	12.92 ± 2.0
K-Mart	7	Left	5.61 ± 2.1
	8	Middle	7.46 ± 0.9
	9	Right	7.63 ± 1.1
Bruners	10	Left	17.41 ± 3.2
	11	Middle	7.61 ± 1.2
	12	Right	9.57 ± 0.6
Sewage Plant	13	Left	22.17 ± 1.8
	14	Middle	5.84 ± 0.8
	15	Right	11.29 ± 1.0
East Fork Turnpike Building	16	Left	6.55 ± 0.8
	17	Middle	3.86 ± 0.9
	18	Right	5.67 ± 0.7
Poplar Creek Perimeter Road	19	Left	3.95 ± 0.3
	20	Middle	5.58 ± 0.6
	21	Right	5.82 ± 0.9
Bear Creek	22	Left	2.41 ± 0.15
	23	Middle	6.26 ± 0.36
	24	Right	7.04 ± 0.83
Bear Creek	25	Left	5.07 ± 0.22
	26	Middle	3.78 ± 0.74
	27	Right	5.37 ± 0.63
Bear Creek	28	Left	4.43 ± 0.74
	29	Middle	4.23 ± 0.83
	30	Right	4.48 ± 0.63

TABLE 5
The Concentration of Arsenic in Soil
from East Fork Poplar Creek and Bear Creek

Site	Site number		Arsenic mean ± SD (ppm)
Guard-house	1	Left	.59 ± 0.13
	2	Middle	.73 ± 0.06
	3	Right	5.49 ± 0.52
NOAA	4	Left	5.69 ± 0.74
	5	Middle	3.18 ± 0.20
	6	Right	4.32 ± 1.0
K-Mart	7	Left	.58 ± 0.06
	8	Middle	1.32 ± 0.16
	9	Right	2.57 ± 0.38
Bruners	10	Left	6.10 ± 0.27
	11	Middle	3.40 ± 0.68
	12	Right	6.93 ± 0.19
Sewage Plant	13	Left	4.64 ± 0.77
	14	Middle	2.63 ± 0.69
	15	Right	4.17 ± 0.90
East Fork Turnpike Building	16	Left	2.83 ± 0.30
	17	Middle	.57 ± 0.09
	18	Right	2.11 ± 0.11
Poplar Creek Perimeter Road	19	Left	1.79 ± 0.15
	20	Middle	.75 ± 0.06
	21	Right	3.88 ± 0.29
Bear Creek	22	Left	1.32 ± 0.46
	23	Middle	1.90 ± 0.88
	24	Right	2.20 ± 0.71
Bear Creek	25	Left	7.08 ± 0.93
	26	Middle	4.38 ± 0.76
	27	Right	2.37 ± 0.29
Bear Creek	28	Left	4.34 ± 0.85
	29	Middle	2.53 ± 0.46
	30	Right	2.77 ± 0.53

TABLE 6
The Concentration of Cadmium in Soil
from East Fork Poplar Creek and Bear Creek

Site	Site number		Cadmium mean ± SD (ppm)
Guard-house	1	Left	.45 ± 0.03
	2	Middle	12.4 ± 1.2
	3	Right	8.17 ± 1.1
NOAA	4	Left	92.9 ± 18
	5	Middle	2.67 ± 0.63
	6	Right	12.8 ± 1.9
K-Mart	7	Left	26.8 ± 3.7
	8	Middle	6.1 ± 0.63
	9	Right	9.5 ± 1.1
Bruners	10	Left	11.5 ± 2.1
	11	Middle	8.9 ± 0.48
	12	Right	13.2 ± 0.99
Sewage Plant	13	Left	26.1 ± 1.9
	14	Middle	3.3 ± 0.65
	15	Right	35.5 ± 3.9
East Fork Turnpike Building	16	Left	10.7 ± 1.8
	17	Middle	12.2 ± 0.9
	18	Right	9.8 ± 1.6
Poplar Creek Perimeter Road	19	Left	7.3 ± 0.8
	20	Middle	13.6 ± 1.9
	21	Right	7.6 ± 0.8
Bear Creek	22	Left	11.2 ± 2.3
	23	Middle	16.2 ± 1.7
	24	Right	12.3 ± 2.1
Bear Creek	25	Left	1.8 ± 0.7
	26	Middle	5.5 ± 0.9
	27	Right	3.8 ± 0.4
Bear Creek	28	Left	1.13 ± 0.3
	29	Middle	9.5 ± 1.3
	30	Right	1.11 ± 0.2

TABLE 7

The Concentration of Mercury* in Soil from East Fork Poplar Creek and Bear Creek

Site	Site number		Mercury mean ± SD (ppm)	
Guard-house	1	Left	1.97 ± 0.6	
	2	Middle	12.19 ± 1.3	(2000)**
	3	Right	507.55 ± 40.1	
NOAA	4	Left	974.83 ± 63.6	
	5	Middle	49.03 ± 5.9	
	6	Right	97.57 ± 8.6	
K-Mart	7	Left	61.06 ± 7.1	
	8	Middle	16.16 ± 2.3	
	9	Right	48.50 ± 1.7	
Bruners	10	Left	1799.36 ± 88.9	
	11	Middle	17.91 ± 3.2	
	12	Right	987.94 ± 70.6	
Sewage Plant	13	Left	43.52 ± 5.1	
	14	Middle	33.18 ± 5.4	(1000)**
	15	Right	220.85 ± 33.6	
East Fork Turnpike Building	16	Left	78.84 ± 7.9	
	17	Middle	1.33 ± 0.5	
	18	Right	65.43 ± 4.1	
Poplar Creek Perimeter Road	19	Left	22.79 ± 3.6	
	20	Middle	12.51 ± 1.3	
	21	Right	6.13 ± 0.6	
Bear Creek	22	Left	11.23 ± 1.1	
	23	Middle	3.47 ± 0.6	
	24	Right	34.62 ± 2.1	
Bear Creek	25	Left	6.61 ± 1.3	
	26	Middle	.77 ± 0.06	
	27	Right	2.64 ± 0.93	(500)**
Bear Creek	28	Left	.59 ± 0.09	
	29	Middle	.72 ± 0.10	
	30	Right	13.07 ± 1.7	

* Normal soil levels 3 ppm.

**Mercuric chloride was added to soil samples to levels indicated in parenthesis (ppm).

floodplains and creek basin and from site to site. Contamination of the creek basin for all the metals analyzed was significantly less than that observed for the floodplains. Thus, the major heavy-metal contamination was observed in the floodplains.

The intestinal absorption of significant amounts of the above metals from ingested soil would occur only if these metals are soluble in the digestive fluid. Studies were thus performed with the contaminated soil to determine its solubility in various media and also because the different forms of inorganic Hg show various solubilities in water. Furthermore, the different species of Hg (inorganic, organic, and elemental) vary considerably in solubility in liquid media. For example, in contrast to inorganic forms, organic mercurials are completely soluble in organic media (i.e., benzene), and elemental Hg is completely soluble in nitric acid and sparingly soluble in water (i.e., 20 µg/L). As shown in Tables 8 and 9, the solubility of soil and sediment Hg in water and in benzene was less than 1%. In contrast, the solubility of soil and sediment Hg in nitric acid was much greater (Table 10), ranging from 11 to 100%.

These results suggest that the predominant species of Hg in the selected soil and sediment samples are the elemental and inorganic forms. Significant concentrations of organic mercurials do not appear to be present in the soil and sediment samples. The fact that Hg was not completely soluble in nitric acid from some of the samples may suggest that different inorganic compounds of Hg (such as HgS) exist in these samples. This form is sparingly soluble in nitric acid. Based on these data, we suggest that the intestinal absorption of Hg from the ingested soil and sediment will depend on (1) the binding coefficient of Hg to soil constituents, (2) the relative concentration of the different species of Hg, and (3) the relative concentration of various inorganic forms of Hg in these samples.

In support of the solubility studies are results for the EPA-recommended EP toxicity test. This test is a measure of the solubility and, thus, bioavailability of various pollutants in solid media such as soil. The results of these studies (Table 11) show that the EPA acceptable Hg limit of 200 µg/L was never achieved using this soil. These data suggest, as previously mentioned, that the Hg in soil is not readily bioavailable. Although 23 of the 30 sites had soil Hg levels above normal (3 ppm); only 5 sites had water solubility levels greater than 10 µg/L, which is 20 times below the acceptable limit. It is noted that the soil and sediment Hg solubility studies described above were performed using methods similar to the EP toxicity test, except for the use of three solvents (water, benzene, and concentrated nitric acid). As shown above, the solubility of soil Hg was more apparent in concentrated nitric acid than in the 0.5 N acetic acid, which would be anticipated.

5.2 SOIL FEEDING STUDIES

To determine the long-term toxic effects of contaminated soil and sediment on various organ systems, mice were fed a diet containing 5% soil or sediment for 20 months. At 6, 12, and 20 months of exposure, various tests were performed to determine the toxicity of these inorganic contaminants. A total of 2400 male and female mice were divided into 30 groups (40 males and 40 females) and exposed to the soil and sediment collected from the 30 sites shown in Table 1.

H_2O

TABLE 8
The Solubility of Mercury from Soil and Sediment*

Site	Site number		Mercury ppm in soil	Mercury ppm soluble in water	Percent of total mercury soluble in water
Guard-house	1	Left	1.97 ± 0.6	0 ±	0
	2	Middle	12.19 ± 1.3	0.054 ± 0.016	.44
	3	Right	507.55 ± 40.1	0.973 ± 0.035	.19
NOAA	4	Left	974.83 ± 63.6	0.919 ± 0.026	.09
	5	Middle	49.03 ± 5.9	0.031 ± 0.009	.06
	6	Right	97.57 ± 8.6	0.293 ± 0.035	.30
K-Mart	7	Left	61.06 ± 7.1	0.027 ± 0.009	.04
	8	Middle	16.16 ± 2.3	0.190 ± 0.016	.01
	9	Right	48.50 ± 1.7	0.050 ± 0.009	.10
Bruners	10	Left	1799.36 ± 88.9	0.252 ± 0.025	.01
	11	Middle	17.91 ± 3.2	0.035 ± 0.007	.01
	12	Right	987.94 ± 70.6	0.392 ± 0.014	.03
Sewage Plant	13	Left	43.52 ± 5.1	0.032 ± 0.004	.07
	14	Middle	33.18 ± 5.4	0.146 ± 0.008	.004
	15	Right	220.85 ± 33.6	0.288 ± 0.017	.13
East Fork Turnpike Building	16	Left	78.84 ± 7.9	0.008 ± 0	.01
	17	Middle	13.33 ± 0.5	0 ± 0	0
	18	Right	65.43 ± 4.1	0.012 ± 0	.01
Poplar Creek Perimeter Road	19	Left	22.79 ± 3.6	0.636 ± 0.047	.03
	20	Middle	12.51 ± 1.3	0.392 ± 0.085	.03
	21	Right	6.13 ± 0.6	0.130 ± 0.011	.04
Bear Creek	22	Left	11.23 ± 1.1	0.098 ± 0.006	.009
	23	Middle	13.47 ± 0.6	0.136 ± 0.015	0
	24	Right	34.62 ± 2.1	0.463 ± 0.080	0
Bear Creek	25	Left	6.61 ± 1.3	0.007 ± 0	.005
	26	Middle	.77 ± 0.06	0 ± 0	0
	27	Right	2.64 ± 0.93	0.13 ± 0.003	.05
Bear Creek	28	Left	.59 ± 0.09	0 ± 0	0
	29	Middle	.72 ± 0.10	0 ± 0	0
	30	Right	1.3 ± 0.7	0.003 ± 0.008	.25

*Soil samples obtained from East Fork Poplar Creek and Bear Creek were extracted into distilled water overnight, filtered, and mercury determined by the cold vapor technique. Mean ± SD for triplicate analysis.

Organics

TABLE 9

The Solubility of Mercury from Soil and Sediment*

Site	Site number		Mercury ppm in soil	Mercury ppm soluble in organics	Percent of total mercury soluble in organics
iuard- ouse	1	Left	1.97 ± 0.6	0	0
	2	Middle	12.19 ± 1.3	0.031 ±	.25
	3	Right	507.55 ± 40.1	0.280 ±	.05
NOAA	4	Left	974.83 ± 63.6	0.225 ±	.02
	5	Middle	49.03 ± 5.9	0.066 ±	.13
	6	Right	97.57 ± 8.6	0.056 ±	.05
-Mart	7	Left	61.06 ± 7.1	0.097 ±	.15
	8	Middle	16.16 ± 2.3	0	0
	9	Right	48.50 ± 1.7	0.042 ±	.08
runers	10	Left	1799.36 ± 88.9	0.314 ±	.01
	11	Middle	17.91 ± 3.2	0	0
	12	Right	987.94 ± 70.6	0.479 ±	.04
ewage	13	Left	43.52 ± 5.1	0.031 ±	.07
lant	14	Middle	33.18 ± 5.4		0
	15	Right	220.85 ± 33.6	0.039 ±	.01
ast Fork	16	Left	78.84 ± 7.9	0.027 ±	.03
urnpike	17	Middle	13.33 ± 0.5	0 ±	0
uilding	18	Right	65.43 ± 4.1	0.025 ±	.03
oplar reek erimeter oad	19	Left	22.79 ± 3.6	0 ±	0
	20	Middle	12.51 ± 1.3	0.012 ±	.09
	21	Right	6.13 ± 0.6	0 ±	0
ear reek	22	Left	11.23 ± 1.1	0 ±	0
	23	Middle	13.47 ± 0.6	0 ±	0
	24	Right	34.62 ± 2.1	0 ±	0
ear reek	25	Left	6.61 ± 1.3	0 ±	0
	26	Middle	.77 ± 0.06	0 ±	0
	27	Right	2.64 ± 0.93	0 ±	0
lear reek	28	Left	.59 ± 0.09	0 ±	0
	29	Middle	.72 ± 0.10	0 ±	0
	30	Right	1.3 ± 0.7	0 ±	0

*Soil samples obtained from East Fork Poplar Creek and Bear Creek were extracted overnight with benzene, filtered, and mercury determined by the cold vapor technique. Mean ± SD of triplicate analysis.

HNO_3

TABLE 10

The Solubility of Mercury from Soil and Sediment*

.te	Site number		Mercury ppm in soil	Mercury ppm soluble in HNO_3	Percent of total mercury soluble in HNO_3
ıard-	1	Left	1.97 ± 0.6	1.81 ± 0.19	91.88
ouse	2	Middle	12.19 ± 1.3	2.71 ± 0.63	22.23
	3	Right	507.55 ± 40.1	326.66 ± 38	64.36
JAA	4	Left	974.83 ± 63.6	253.81 ± 45	26.04
	5	Middle	49.03 ± 5.9	36.17 ± 7	73.77
	6	Right	97.57 ± 8.6	33.29 ± 3	34.12
-Mart	7	Left	61.06 ± 7.1	14.25 ± 2	23.34
	8	Middle	16.16 ± 2.3	4.78 ± 0.6	29.58
	9	Right	48.50 ± 1.7	23.93 ± 1.1	49.34
runers	10	Left	1799.36 ± 88.9	528.34 ± 69	29.36
	11	Middle	17.91 ± 3.2	11.48 ± 1.3	64.10
	12	Right	987.94 ± 70.6	118.42 ± 12	11.99
;wage	13	Left	43.52 ± 5.1	16.47 ± 0.88	37.84
.ant	14	Middle	33.18 ± 5.4	8.71 ± 1.21	26.25
	15	Right	220.85 ± 33.6	44.21 ± 3.66	20.02
ıst Fork	16	Left	78.84 ± 7.9	37.51 ± 4.0	47.58
urnpike	17	Middle	13.33 ± 0.5	4.5 ± 0.13	33.83
uilding	18	Right	65.43 ± 4.1	41.08 ± 4.8	62.78
)plar					
reek	19	Left	22.79 ± 3.6	13.05 ± 0.69	57.26
-rimeter	20	Middle	12.51 ± 1.3	8.30 ± 1.20	66.35
)ad	21	Right	6.13 ± 0.6	2.30 ± 0.11	37.52
ear	22	Left	11.23 ± 1.1	12.12 ± 1.3	100+
·eek	23	Middle	13.47 ± 0.6	1.98 ± 0.4	57.06
	24	Right	34.62 ± 2.1	30.59 ± 1.9	88.36
;ar	25	Left	6.61 ± 1.3	3.42 ± 0.49	51.74
·eek	26	Middle	.77 ± 0.06	.32 ± 0.11	41.56
	27	Right	2.64 ± 0.93	2.12 ± 0.35	80.30
;ar	28	Left	.59 ± 0.09	.39 ± 0.14	66.10
·eek	29	Middle	.72 ± 0.10	.52 ± 0.35	72.22
	30	Right	1.3 ± 1.7	0.81 ± 1.0	62.28

;oil samples obtained from East Fork Poplar Creek and Bear Creek were extracted into concentrated nitric acid, filtered, and mercury determined by the cold vapor technique. Mean ± SD of triplicate analysis of soil.

TABLE 11
EP Toxicity Test for Mercury (RCRA MCL 200 µg/L)

Site	Soil and sediment mg/kg	EP toxicity test µg/L
1	1.97 ± 0.6	2 ± 1.5*
2	12.19 ± 1.3	3 ± 1.8*
3	507.55 ± 40.1	17 ± 2.6
4	974.55 ± 63.6	18 ± 3.4
5	49.03 ± 5.9	10 ± 1.3
6	97.57 ± 8.6	8 ± 1.8
7	61.06 ± 7.1	9 ± 0.9
8	16.16 ± 2.3	4 ± 1.1*
9	48.50 ± 1.7	8 ± 0.6
10	1799.36 ± 88.9	33 ± 5.2
11	17.91 ± 3.2	5 ± 0.6
12	987.94 ± 70.6	23 ± 4
13	43.52 ± 5.1	7 ± 2
14	33.18 ± 5.4	3 ± 1.6*
15	220.85 ± 33.6	13 ± 0.8
16	78.84 ± 7.9	9 ± 1.4
17	13.33 ± 0.5	3 ± 1.4*
18	65.43 ± 4.1	5 ± 1.0
19	22.79 ± 3.6	7 ± 1.9
20	12.51 ± 1.3	7 ± 2.3
21	6.13 ± 0.6	4 ± 1.6
22	11.23 ± 1.1	7 ± 1.3
23	3.47 ± 0.6	6 ± 0.9
24	34.62 ± 2.1	9 ± 2
25	6.61 ± 1.3	0
26	.77 ± 0.06	0
27	2.64 ± 0.93	3 ± 2*
28	.59 ± 0.09	0
29	.72 ± 0.10	0
30	1.3 ± 1.7	0

*Values obtained at the limit of detection.

Soil and sediment from the respective sites were added to the feed, mixed, and made into feed pellets. From each diet, samples of the pellets were digested and Hg concentrations determined. The results in Table 12 outline the Hg recovery following the addition of Hg-contaminated soil and sediment to the diet. The Hg recovery ranged from 73 to 127%. In only two samples was the recovery less than 85%, and in these samples recoveries of 73 and 81% (sites 21 and 25, respectively) were observed. These differences of 15% are within the experimental error in these studies. It is noted that, with some samples, recoveries were greater than 100%. The increased recovery (0 to 27%) is assumed to be associated with experimental error.

To determine the absorption rate for Hg in soil and sediment samples, mice were exposed to the experimental diets for 24 hr. After exposure, fecal samples were collected for 96 hr and Hg determined. The results (Table 13) show that the absorption of Hg from soil and sediment samples varied between 5 and 16%. The factors associated with this variation may be (1) differences in the relative amounts of the various species of Hg, (2) differences in the relative amounts of the various forms of inorganic Hg, and (3) the binding coefficient of Hg for soil particulates. It is difficult to speculate at this time which factor is responsible for the observed variation. However, it is known that the soluble forms of inorganic Hg are absorbed in the intestinal tract of mice at rates of 1.5 to 2.0% (50).

Several tests were used to determine the toxicity of the contaminated soil and sediment. These included the tissue level for the various inorganics, body weights over the experimental period, kidney function tests, morphology of the kidney, and the swim test to determine neurological effects. The raw data and means (± SD) are shown in the appendix. The data from these studies (summarized in the tables and figures) were analyzed using SAS ANOVA on an IBM AT. Results from the data are summarized below.

5.2.1 Body Weights and Mortality

In chronic toxicity studies, body weight changes are frequently used to assess toxicity of ingested compounds. Body weights were measured at 0, 6, 12, and 20 months for both females and males in the 30 experimental groups. In all groups, body weight increased, on average, by 17.5 g in males and 16.3 g in females after a 6-month exposure to the experimental diet. After 6 to 12 month's exposure, the increases were 2.3 and 3.3 g for males and females, respectively. From 12 to 20 months, body weights remained constant for males and increased by 2 g for females (results shown in the appendix). These observed changes in body weight of the Swiss mouse would be anticipated based on previous studies by this investigator and reports in the literature.

Analyses of variance for body weights did not show significant differences between the 30 groups at the various time intervals. This suggests that the experimental diets did not significantly affect growth and, thus, may not be toxic to the exposed animals. Similar conclusions were reached for the organ weights of the liver and kidney. The toxicity of a compound may also be measured by the number of animals that died following ingestion of that compound. Table 14 shows the total mortality data for the study. Table 15 shows mortality in the 30 groups following the exposure to the diets for 6, 12, and 20 months. Apparent differences in mortality were observed

TABLE 12
Recoverable Mercury in Mouse Feed

Site	Site number		ppm in soil	µg Hg added to 95 g of feed	µg Hg recovered in 100 g of feed	Percent difference
Guard-house	1	Left	1.97	9.9	10.6 ± 1.3	+ 7
	2	Middle	2000	10,000	9,999 ± 8	- 1
	3	Right	507.55	2,538	2,502 ± 75	- 1
NOAA	4	Left	974.83	4,874	4,183 ± 279	-15
	5	Middle	49.03	245	283 ± 3	+17
	6	Right	97.57	485	425 ± 7	-12
K-Mart	7	Left	61.06	305	315 ± 6	+ 3
	8	Middle	16.16	81	83 ± 4	+ 2
	9	Right	48.50	245	263 ± 5	+ 7
Bruners	10	Left	1799.36	8,997	9,315 ± 100	+ 4
	11	Middle	17.91	89	74 ± 8	+17
	12	Right	987.94	4,940	5,213 ± 10	- 6
Sewage Plant	13	Left	43.52	220	210 ± 3	- 5
	14	Middle	1000	166	166 ± 9	0
	15	Right	220.85	1,104	1,004 ± 18	- 9
East Fork Turnpike Building	16	Left	78.84	394	349 ± 10	-11
	17	Middle	13.33	65	59 ± 0.6	-10
	18	Right	65.43	325	322 ± 4	- 1
Poplar Creek Perimeter Road	19	Left	22.79	114	116 ± 8	+ 2
	20	Middle	12.51	65	61 ± 3	- 6
	21	Right	6.13	31	25 ± 1.3	-19
Bear Creek	22	Left	11.23	58	50 ± 3	-14
	23	Middle	3.47	17	15 ± 2	+12
	24	Right	34.62	175	175 ± 9	0
Bear Creek	25	Left	6.61	30	22 ± 3	-27
	26	Middle	.77	3.9	4.6 ± 1.0	+18
	27	Right	500	2500	2410 ± 0.7	- 4
Bear Creek	28	Left	.59	3.3	3.0 ± 0.4	- 9
	29	Middle	.72	3.6	3.9 ± 0.13	+ 8
	30	Right	1.3	6.5	6.4 ± 5	- 1

TABLE 13
The Gastrointestinal Absorption of Mercury
from Soil or Sediment in Mice

Site	Sex	Total intake per 24 hours: Feed, g**	Soil, g	Hg, µg	Total Hg recovered in feces,* µg**	Percent absorption from GI tract
2	F	3.3 ±0.4	0.165	1.9	1.8 ±0.2	5
	M	4.1 ±0.6	0.210	2.5	2.3 ±0.3	8
4	F	2.7 ±0.5	0.135	132	125 ±15	5
	M	3.8 ±0.4	0.190	185	170 ±22	8
8	F	2.9 ±0.1	0.145	2.32	1.95 ±0.4	16
	M	3.4 ±0.8	0.170	2.72	2.39 ±0.8	12
10	F	2.4 ±0.3	0.12	216	203 ±18	6
	M	4.3 ±0.8	0.220	396	380 ±16	4
15	F	3.1 ±0.5	0.155	34	30 ±1.3	9
	M	3.9 ±0.3	0.196	43	41 ±4.6	5
18	F	2.5 ±0.3	0.125	8.2	2 ±0.8	12
	M	3.9 ±0.9	0.22	13.1	11 ±2.0	16
24	F	2.2 ±0.3	0.110	3.8	3.3 ±0.4	14
	M	4.4 ±0.6	0.220	7.6	7.2 ±0.8	5
30	F	2.8 ±0.6	0.140	1.83	1.60 ±0.22	13
	M	4.0 ±0.9	0.200	2.6	2.2 ±0.3	15
?	F	2.3 ±0.4	0.115	230	200 ±18	13
	M	3.8 0.1	0.190	380	335 ±40	12

*96 hours post-feeding.
**Mean ± SD for five animals per experimental group.

[illegible] 3.96 ± 0.29
[illegible] 0.69 ± 0.37 S.D

TABLE 14
Mortality of Mice Exposed to Mercury Contamination

Group	No. mice exposed		No. mice tested		No. mice died		No. mice surviving		No. mice killed because of trauma	
	M	F	M	F	M	F	M	F	M	F
1	40	40	17	22	23	5	0	13	0	0
2	40	40	19	22	12	8	1	10	3	0
3	40	40	22	22	14	8	1	10	3	0
4	40	40	18	22	12	4	0	12	10	2
5	40	40	22	22	16	4	0	11	2	3
6	40	40	22	22	13	5	0	13	5	0
7	40	40	22	22	11	6	0	12	7	0
8	40	40	22	22	15	7	0	11	3	0
9	40	40	19	22	15	3	1	15	5	0
10	40	40	21	22	13	4	1	12	5	2
11	40	40	22	22	13	8	0	10	5	0
12	40	40	22	22	12	3	0	12	7	3
13	40	40	19	22	11	7	0	11	10	0
14	40	40	22	22	12	6	0	12	6	0
15	40	40	20	22	11	4	0	14	9	0
16	40	40	21	22	12	6	1	12	6	0
17	40	40	19	22	12	8	3	10	6	0
18	40	40	18	22	10	5	0	13	12	0
19	40	40	19	22	12	5	0	13	9	0
20	40	40	22	22	6	5	2	13	10	0
21	40	40	19	22	17	5	0	12	4	1
22	40	40	22	22	9	11	3	7	6	0
23	40	40	18	22	15	6	0	12	7	0
24	40	40	16	22	17	3	0	15	7	0
25	40	40	20	22	10	7	0	10	10	1
26	40	40	22	22	7	2	3	16	8	0
27	40	40	18	22	19	6	0	12	3	0
28	40	40	22	22	13	3	0	12	5	3
29	40	40	19	22	15	7	0	11	6	0
30	40	40	22	22	10	8	4	7	4	3
TOTAL	1200	1200	605	660	387	169	19	353	189	18

TABLE 15
Mortality of Mice Continuously Exposed to Diets
Containing Mercury-Contaminated Soil

	Intake µg Hg/day		Exposure time							
			6 Mo.		12 Mo.		20 Mo.		Total	
Group	M	F	M	F	M	F	M	F	M	F
1	0.34	0.27	40/1*	40/0	43/9	35/3	18/13	25/2	40/23	40/5
2	392.0	282.0	40/2	40/0	27/2	35/2	15/8	26/2	40/12	40/8
3	104.0	74.0	40/3	40/0	29/0	35/0	22/11	28/8	40/14	40/8
4	176.0	146.0	40/2	40/0	26/2	33/1	14/8	25/3	40/12	40/4
5	9.3	7.8	40/9	40/1	24/3	32/2	14/4	22/1	40/16	40/4
6	15.0	13.0	40/1	40/0	29/0	35/1	22/12	27/4	40/13	40/5
7	11.9	8.6	40/0	40/0	31/2	35/2	19/9	26/4	40/11	40/6
8	3.1	2.3	40/1	40/1	31/1	34/1	23/13	25/5	40/15	40/7
9	8.7	8.0	40/4	40/0	27/1	35/0	17/10	25/3	40/15	40/3
10	360.0	261.0	40/2	40/0	28/4	33/2	18/7	24/2	40/13	40/4
11	3.5	2.3	40/6	40/2	29/1	33/2	17/6	24/4	40/13	40/8
12	173.0	128.0	40/0	40/0	31/3	35/0	18/9	25/3	40/12	40/3
13	10.4	6.1	40/0	40/1	29/1	34/0	17/10	27/6	40/11	40/7
14	186.0	145.0	40/2	40/0	33/0	35/0	20/10	28/6	40/12	40/6
15	42.0	34.0	40/0	40/0	28/4	35/1	15/7	27/3	40/11	40/4
16	15.0	13.0	40/2	40/3	27/1	33/0	18/9	26/3	40/12	40/6
17	0.24	0.17	40/0	40/1	33/3	34/3	19/9	24/4	40/12	40/8
18	12.0	8.0	40/1	40/1	28/2	34/1	13/7	26/3	40/10	40/5
19	4.2	3.7	40/3	40/0	25/3	35/0	13/6	28/5	40/12	40/5
20	2.6	1.6	40/0	40/0	25/2	35/2	16/4	26/3	40/6	40/5
21	1.2	0.7	40/2	40/0	32/1	34/1	21/14	26/4	40/17	40/5
22	2.3	1.7	40/1	40/1	28/4	34/4	17/4	23/6	40/9	40/11
23	0.68	0.50	40/3	40/0	27/1	32/3	17/11	22/3	40/15	40/6
24	5.2	4.7	40/4	40/1	24/4	34/1	13/9	26/1	40/17	40/3
25	0.99	1.6	40/2	40/1	24/1	33/1	15/7	25/5	40/10	40/7
26	0.14	0.08	40/1	40/0	26/2	35/1	16/4	27/1	40/7	40/2
27	98.0	70.0	40/2	40/0	33/0	35/0	23/17	28/6	40/19	40/6
28	0.11	0.07	40/1	40/0	32/2	33/0	20/10	25/3	40/13	40/3
29	0.13	0.08	40/4	40/1	31/2	34/1	16/9	26/5	40/15	40/7
30	2.6	1.4	40/0	40/1	31/2	32/1	22/8	24/6	40/10	40/8

*No. of exposed animals/No. of animals that died.

between the males and females, but the differences did not appear to be related to the dietary level of toxic metals. High mortality is frequently observed in males housed together because of wounds inflicted by other mice. We therefore suggest that these observed mortality differences were related to wounds inflicted by males on each other. Table 16 shows the number of animals killed during the experimental period because of trauma.

Apparent differences in mortality were observed between the experimental diets for males and females. For example, cumulative mean mortality data (calculated from the data in the appendix) were 5.6 for females and 12.9 for males. One group of females (i.e., 22) and four groups of males (i.e. 1, 21, 24, and 27) exceeded the mean values. Mortality levels in four groups of females (9, 24, 26, and 28) and two groups of males (20 and 26) were lower than the respective means. However, these differences did not appear to be related to Hg or any other toxic metal. Those groups receiving the highest level of Hg showed mortality levels similar to the cumulative mean. Statistical analysis of the data did not show a significant correlation between mortality and Hg intake, which suggests that the ingestion of soil containing Hg at levels approaching 2000 ppm did not affect mortality. Table 17 shows the percent mortality of mice continuously exposed to mercury-contaminated soil.

5.2.2 Organ Distribution of Mercury

Several reports have shown that the critical organs for Hg accumulation are the liver and kidney (51). These studies have also observed that these organs may represent up to 50% of the total body burden following the intestinal absorption of inorganic or elemental mercury (52). The distribution of Hg in the body following exposure to organic mercurials is more evenly distributed throughout these organs (53, 54). Thus, the liver and kidney may not represent 50% of the total body burden in animals exposed to the organic mercurials. We would anticipate in the present studies that, if Hg in the soil and sediment was primarily in the inorganic and/or elemental forms, liver and kidney Hg should represent 50% of the total body burden. However, if Hg was in the organic form in these samples, we would anticipate a somewhat even distribution between the liver and kidney. As shown in the appendix, the Hg level in the liver and kidney appeared to increase as the concentration of soil Hg increased. In general, the concentration of Hg in the kidney was far in excess of the levels observed in the liver. Furthermore, in general, the Hg in the liver and kidney of males exceeded the levels observed in females.

Data in the appendix also show that the ratio of liver to kidney Hg was similar to reports showing this ratio in animals exposed to inorganic Hg. This ratio shows great variation between sex and exposure levels. This ratio was determined because several investigators have observed, in animals exposed to inorganic Hg, a liver-to-kidney ratio of 0.01 to 0.21 (wet weight basis) (32, 55). In contrast, the ratio observed in animals exposed to methylmercury ranged from 0.6 to 0.9 (wet weight basis). As shown in the appendix, this ratio on a wet weight basis following a 6-month exposure ranged from 0.06 to 0.34. However, there were exceptions: group 5 (0.89, F; 0.48, M), group 23 (0.91, F; 0.41, M), and group 24 (0.31, F; 0.41, M). This high ratio in females from groups 5 and 23 may require

TABLE 16
Animals Killed During the Experimental Period Because of Trauma*

Group	Exposure time, months			
	0 to 6		6 to 12	
	M	F	M	F
1	0	0	0	0
2	0	0	3	0
3	3	0	0	0
4	7	2	3	0
5	2	2	0	1
6	5	0	0	0
7	4	0	3	0
8	3	0	0	0
9	5	0	0	0
10	5	2	0	0
11	1	0	4	0
12	4	0	3	3
13	6	0	4	0
14	6	0	0	0
15	7	0	2	0
16	6	0	0	0
17	2	0	4	0
18	6	0	6	0
19	7	0	2	0
20	10	0	0	0
21	1	1	3	0
22	6	0	0	0
23	5	0	2	0
24	7	0	0	0
25	9	1	1	0
26	8	0	0	0
27	0	0	3	0
28	2	2	3	1
29	0	0	6	0
30	4	3	0	0

*Trauma inflicted by other mice within the cage.

TABLE 17
Percent Mortality in Mice Exposed to
Mercury-Contaminated Soil

	Exposure period in months							
	6		12		20		Total	
Group	M	F	M	F	M	F	M	F
1	3	0	26	9	72	8	58	13
2	5	0	7	6	53	23	30	30
3	8	0	0	0	50	29	35	20
4	5	0	8	3	57	12	30	10
5	23	3	13	6	29	5	40	10
6	3	0	0	3	55	15	33	13
7	0	0	7	6	47	15	28	15
8	3	3	3	3	57	20	38	18
9	10	0	4	0	59	12	38	8
10	5	0	14	6	39	8	33	10
11	15	5	4	6	35	17	32	20
12	0	0	10	0	50	12	30	8
13	0	3	4	0	59	22	28	18
14	5	0	0	0	50	21	30	15
15	0	0	14	3	47	11	28	10
16	5	8	4	0	50	12	30	15
17	0	3	9	9	47	17	30	20
18	3	3	7	3	54	12	25	13
19	8	0	12	0	46	18	30	13
20	0	0	8	6	25	12	15	13
21	5	5	3	3	67	15	43	13
22	3	3	14	12	24	26	23	28
23	8	0	4	9	65	14	38	15
24	10	3	17	3	69	4	43	8
25	5	3	4	3	47	20	25	18
26	3	0	8	3	25	4	18	5
27	5	0	0	0	74	21	48	15
28	3	0	6	0	50	12	33	8
29	10	3	6	3	56	19	38	18
30	0	3	6	3	36	25	25	20

additional study to determine the possible factors. Mercury sediment levels in groups 5 and 23 were 49 and 13 ppm, respectively. Extraction studies showed that less than 1% of the total sediment Hg was soluble in water and benzene. Thus, it appears that groups 5 and 23 were exposed to sediment containing primarily inorganic and elemental Hg. Additional studies are in progress to explain this result. It is noted that groups 5 and 23 are the only sediment samples which showed this high ratio.

An important factor in predicting the possible long-term effect of Hg in soil is to determine the dose-response relationship of intake versus liver or kidney Hg. In classical toxicological studies to determine a dose-response relationship, an animal is exposed to a single compound and the level of intake compared to the organ level of that compound. Frequently, these studies provide information necessary for predicting minimal and maximal acceptable doses for specific compounds. Again, these studies are performed using a single compound in a simple matrix (i.e., water). In the present studies, attempts were made to determine the dose-response relationship of soil Hg to liver and kidney Hg. Results showing the dose-response effects (Figs. 3 through 28; p. 59) indicate, in some cases, a significant dose-response relationship. Thus, the exposure dose is correlated with the level of Hg in the liver and kidney. However, it is difficult to determine the minimal and maximal acceptable dose from these data because the dose-response relationships were not linear.

Factors that may be responsible for this observation include (1) the binding coefficient of Hg to soil and sediment, and (2) the various species and inorganic forms of Hg in soil and sediment. For example, the solubility of elemental Hg in water is significantly less than that observed for inorganic Hg. Thus, we would anticipate different absorption rates for the different Hg species. The absorption of inorganic Hg (i.e., $HgCl_2$) in mice has been reported to be 1.5 to 2.0% of the dose, whereas elemental Hg is absorbed at rates of 0.01 to 0.5% (50, 56). Variations in the absorption of mercuric compounds which appear to be associated with the solubilities of the compound have also been reported (57). Mercuric chloride and nitrate are absorbed at significantly higher rates than mercuric sulfide and oxides. To determine the effect of the binding coefficient on Hg absorption, $HgCl_2$ was added to soil at levels ranging from 500 to 8000 ppm. Results of these studies are shown in Figs. 15 through 28. In contrast to the data shown in Figs. 3 through 14, a linear dose-response relationship of intake versus kidney Hg was observed in animals exposed to soil containing the $HgCl_2$. This result suggests that possible binding coefficients of Hg to soil may not be responsible for the lack of linearity observed in Figs. 3 through 14. Thus, the species and/or inorganic form of Hg may be the factors explaining the nonlinear response.

5.2.3 Kidney Morphology and Function

The kidney is the critical organ for Hg toxicity. Renal damage and a decrease in kidney function (i.e., proteinuria) have been observed following chronic exposure to inorganic, organic, and elemental Hg. The observed effects include renal tubular (i.e., proximal tubule) and glomerular damage, proteinuria, and renal failure (58). These changes in kidney morphology and function have been observed when the mercury level in the kidney exceeds 10 to 49 µg/g

wet weight (59-62). To determine the effect of Hg in soil on kidney morphology and function, light microscope studies and urinary analyses were performed. Sections of kidney from all the exposed groups were made, stained with H&E, and evaluated for renal tubular and glomeruli damage and kidney fibrosis. Each section was scored according to the criteria outlined in the methods. Kidney function was determined by measuring the level of protein, creatinine, and gamma glutamyl transferase in the urine.

Table 18 describes the histological results. The histological data were ranked into different classes according to the level of damage observed. Results showed that 87% of females and 85% of males exposed for 6 months to the Hg-contaminated soil did not develop significant morphological changes in the kidney. The groups showing morphological changes were those exposed for only 6 months to soil to which $HgCl_2$ was added to levels of 4000 and 8000 ppm. At 12 months of exposure, the kidneys in 94% of the females and 90% of the males appeared relatively normal. Similar results were observed following 20 months of exposure. Results also showed approximately 15% proximal tubule damage occurred when the mean log kidney Hg exceeded values of 7.1 (i.e., class number 3). The exception was observed in groups exposed to 4000 and 8000 ppm $HgCl_2$. In these groups, four females and two males had mean log Hg levels of 8.4 and 9.2, respectively, and approximately 11% proximal tubule damage.

Although proximal tubule damage was occasionally observed in groups exposed to soil containing relatively high levels of Hg, significant correlations between kidney Hg and changes in three urinary constituents were not observed (Table 19). Partial correlation analysis following 12 months of exposure showed that kidney Hg was not significantly associated with urine protein, gamma glutamyl transferase, and creatinine. Thus, the increase in kidney Hg was not significantly paralleled by increases in urinary protein, glutamyl transferase, or creatinine. This suggests that although slight renal damage was observed, kidney function remained normal. These data imply that soil Hg levels as high as 2000 ppm in East Fork Poplar Creek may not be associated with health effects for those persons eating the contaminated soil.

As discussed above, renal tubule damage with a decrease in kidney function has been observed when the Hg level in this organ exceeds 20 to 40 µg/g wet weight. This relationship has been observed in both human and experimental animal studies. In the present studies, slight proximal renal tubular damage was observed even though the level of Hg in the kidney did not exceed 20 µg/g wet weight. However, this damage was not associated with an impairment to kidney function.

5.2.4 Dietary Intake of Soil or Sediment Required to Achieve Toxic Levels of Mercury in the Kidney

We have estimated the dietary intake of Hg-contaminated soil and sediment from the various sites along East Fork Poplar Creek required to achieve 25 µg/g wet weight of kidney in both female and male mice (Tables 20 and 21). This estimate assumes a daily intake at the levels shown for 6 months. These intake amounts were calculated from the level of kidney Hg observed in female and male mice exposed to soil and sediment from East Fork Poplar Creek for 6 months. It was assumed that at 6 months the intake and excretion of Hg had reached a steady state. If this assumption is correct, the level of Hg in the

TABLE 18
Mercury Data Divided into Histology Classes

Histology No.*	Females			Males		
	No. of mice	Mean log Hg	SE	No. of mice	Mean log Hg	SE
			Six months			
Kidney						
0	126	4.4	.10	111	4.9	.08
1	22	6.4	.23	34	6.8	.23
2	4	8.4	.60	2	9.2	.40
3	3	9.4	.26	4	10.2	.18
4	13	9.6	.10	10	10.5	.10
5	1	9.8	-	3	10.7	.14
6	1	10.3	-	1	11.1	-
Liver						
0	126	3.9	.08	111	4.0	.07
1	22	5.8	.18	34	5.8	.15
2	4	7.3	.54	8	7.2	.35
3	3	8.3	.54	4	8.5	.15
4	13	8.6	.13	10	9.1	.15
5	1	8.9	-	3	9.0	.19
6	1	9.1	-	1	9.3	-
			Twelve months			
Kidney						
0	162	4.4	.08	149	4.9	.07
1	36	6.1	.15	40	6.6	.20
2	12	6.8	.26	12	6.4	.16
Liver						
0	162	4.0	.05	149	4.1	.06
1	35	5.5	.16	40	5.8	.13
2	12	6.4	.16	21	6.3	.16

Table continued on next page.

TABLE 18 (cont'd)
Mercury Data Divided into Histology Classes

Histology No.*	Females			Males		
	No. of mice	Mean log Hg	SE	No. of mice	Mean log Hg	SE
		Twenty months				
Kidney						
0	129	3.8	.08	82	3.8	.11
1	136	4.4	.11	123	4.8	.13
2	26	6.0	.28	34	6.6	.23
3	4	7.1	.42	7	8.1	.18
Liver						
0	129	4.1	.06	83	3.8	.07
1	136	4.4	.08	121	4.3	.08
2	26	5.5	.25	34	5.0	.16
3	4	6.5	.40	7	6.1	.20

* 0 = normal kidney.
1 = 0 to 2 fields showing one or more necrotic proximal tubules.
2 = 2 to 4 fields showing one or more necrotic proximal tubules.
3 = 4 to 6 fields showing one or more necrotic proximal tubules.
4 = 8 to 12 fields showing one or more necrotic proximal tubules.
5 = 12 to 20 fields showing one or more necrotic proximal tubules and necrotic glomorulus.
6 = diffuse necrosis with fibrosis.

TABLE 19
Partial Correlations (and P-values) for 12-Month Data

Correlation of/ With	Kidney Hg	Liver Hg	Urine Protein	Urine -gt	Urine Creatinine
Kidney Hg	1	.08/.27	.03/.70	.00/.99	-.05/.54
Liver Hg		1	-11/14	.08/.26	-166/.03
Urine protein			1	.00/.97	.82/.0001
Urine -gt				1	-/02/.92
Urine creatinine					1

TABLE 20
Estimate of Dietary Intake of Mercury-Contaminated Soil Required by Female Mice to Achieve Kidney Levels of 25 µg Hg/g Wet Weight*

Site (conc., ppm)	Soil intake day/kgBW g/kg BW/day	Hg intake day/kgBW mg/kg BW/day	Hg/g wet wt. kidney ng/g	Soil intake** day/kgBW kg	Hg intake** day/kgBW mg
1 (2)	7.8	0.015	77	2.4	4.8
2 (2000)	7.0	14	1822	0.096	192
3 (508)	7.8	4.1	111	1.8	920
4 (975)	8.4	8.2	1144	0.18	179
5 (49)	8.9	0.44	396	0.57	28
6 (98)	6.5	0.65	434	0.38	37
7 (61)	7.8	0.48	230	0.85	52
8 (16)	7.8	0.13	734	0.28	4.4
9 (48)	9.5	0.45	414	0.57	27
10 (1799)	7.0	13	4924	0.037	66
11 (18)	6.5	0.12	334	0.5	8.9
12 (988)	7.0	6.9	5806	0.03	32
13 (44)	7.8	0.34	811	0.24	11
14 (1000)	7.8	8.1	2958	0.068	68
15 (221)	8.4	1.9	489	0.44	97
16 (79)	8.9	0.73	228	0.10	80
17 (13)	6.5	0.085	228	0.72	9
18 (65)	6.7	0.45	119	1.5	95
19 (23)	8.9	0.20	69	3.2	72
20 (13)	6.5	0.08	105	1.5	19
21 (6)	5.7	0.04	67	2.5	15
22 (11)	7.8	0.09	82	2.5	25
23 (13)	7.3	0.03	44	1.3	17
24 (35)	7.8	0.26	204	0.91	32
25 (7)	8.4	0.06	1705	0.13	1.0
26 (.8)	6.2	0.05	75	10	8.0
27 (500)	7.8	3.9	1788	0.11	55
28 (.6)	6.0	0.04	302	5.5	3.3
29 (72)	6.7	0.05	77	2	15
30 (.3)	6.2	0.08	99	36	11

*Results are expressed as mean from mice exposed to mercury-contaminated soil for 6 months.
**Intake required to achieve 25 µg Hg/g wet weight of kidney in the mouse.

TABLE 21
Estimate of Dietary Intake of Mercury-Contaminated Soil Required by Male Mice to Achieve Kidney Levels of 25 µg Hg/g Wet Weight*

Site (conc., ppm)	Soil intake day/kgBW g/kg BW/day	Hg intake day/kgBW mg/kgBW/day	Hg/g wet wt. kidney ng/g	Soil intake** day/kgBW kg	Hg intake** day/kgBW mg
1 (2)	7.6	0.015	137	1.4	2.7
2 (2000)	8.4	17	5003	0.43	85
3 (508)	8.4	4.3	155	1.3	694
4 (975)	8.3	8.1	768	0.27	264
5 (49)	8.7	0.43	468	0.50	23
6 (98)	6.3	0.63	414	0.38	38
7 (61)	8.4	0.51	149	1.4	90
8 (16)	7.9	0.13	548	0.4	6
9 (48)	8.3	0.40	430	0.48	23
10 (1799)	8.4	15	8948	0.039	70
11 (17)	7.8	0.14	263	0.78	13
12 (988)	6.8	6.6	5208	0.032	32
13 (44)	9.3	0.39	598	0.37	16
14 (1000)	7.0	7.3	5181	0.035	35
15 (221)	7.1	1.6	358	0.51	112
16 (79)	7.8	0.59	197	0.95	75
17 (13)	7.0	0.09	316	0.55	7
18 (65)	7.4	0.47	92	2.0	128
19 (23)	7.8	0.17	133	1.4	32
20 (13)	8.0	0.10	264	0.73	9.5
21 (6)	7.2	0.04	67	2.5	14.9
22 (11)	7.2	0.08	189	0.96	11
23 (13)	7.4	0.03	107	0.54	7
24 (35)	5.6	0.19	170	0.79	28
25 (7)	9.1	0.06	1483	0.14	1
26 (.8)	5.9	0.05	153	10	8
27 (500)	8.4	4.1	981	0.21	105
28 (.6)	7.0	0.04	362	4.6	2.8
29 (72)	6.7	0.05	115	0.15	11
30 (.3)	7.2	0.09	207	36	11

*Results are expressed as mean from mice exposed to mercury-contaminated soil for 6 months.
**Intake required to achieve 25 µg Hg/g wet weight of kidney in the mouse.

kidney at 6 months should remain constant if the intake remained the same. The calculations used in Tables 20 and 21 are as follows:

(1) Soil intake in the mouse was determined by measuring the intake of feed containing 5% soil (data shown in the appendix).

Soil intake/body weight x 1000 = soil intake/kg body weight

(2) Mercury intake was determined from the data on soil intake and the analysis of soil for Hg.

Soil intake/kg body weight x soil Hg concentration = Hg intake/kg body weight

(3) Kidney mercury concentrations were determined in the kidneys of mice exposed for 6 months to the diet containing 5% Hg-contaminated soil (data shown in the appendix).

(4) Mercury intake to give a kidney concentration of 25 mg/kg:

25 mg/kg x Hg intake in the mouse (Hg intake/kg body weight)/Hg concentration in the mouse kidney (ng/g) x 1000 = µg of Hg intake/day

(5) Soil intake to give a kidney Hg concentration of 25 mg/kg:

µg of Hg intake/soil Hg concentration = soil intake g/kg body weight/day

The soil and sediment intake would be associated with the level of Hg in this matrix. As shown, the intake of soil/day by mice to give 25 mg Hg/kg body weight varied between 0.03 to 36 kg in females and 0.04 to 36 kg in males. If the mice data were extrapolated to humans, the following intakes of soil would be necessary to give 25 mg Hg/kg body weight: young child weighing 25 kg, 0.75 to 900 kg for females and 1.0 to 900 kg for males; adult weighing 70 kg, 2.25 to 2,520 kg for females and 2.8 to 2,520 kg for males. If one accepts the 1 g/day intake levels reported by CDC for children, the estimated intake levels reported here to achieve toxicity would range from 750 to 900,000 times the CDC estimate. However, this estimates assumes that the absorption rate and the biological half-life in mice and humans are the same. In mice, the absorption rate and biological half-life have been estimated at 2% and 4 days, respectively. In humans, the absorption rate and half-life are estimated at 15% and 40 days, respectively. As compared to the mouse, humans absorb 7.5 times more Hg and have a half-life which is 10 times longer. This suggests that humans are 75 times more sensitive than the mouse and, thus, would require less Hg-contaminated soil to reach the 25 µg limit. The estimated amount required in humans to reach this limit would range from 5.6 to 6,750 g/day for 6 months. These amounts are still higher than the 1 g/day estimate of CDC. A young child would have to eat 5.6 g of soil with Hg levels of 2000 ppm or 6,750 g with soil Hg levels of 0.3 ppm for 6 months in order to reach the 25 µg limit. We thus suggest that the Hg observed in the soil and sediment from East Fork Poplar Creek may not be a health risk to children playing and/or soil

observed in soil and sediment. If the Hg observed in the soil and sediment is significantly converted to the organic mercurials, then further evaluation of these sites may be required.

In addition the neurological evaluation using the swim test following 6, 12, and 20 months of exposure to these diets showed no difference between the 30 experimental groups. Two observations may be made from these results: (1) the swim test will not discern subtle neurological changes and may be only useful in showing acute neurological effects, and (2) neurological effects are not associated with these soil diets. In the absence of any effect, we suggest that this test may not discern those neurological effects that are observed in humans exposed to methylmercury and/or methylmercury may be too low to induce an effect. We note that in our studies, methyl mercury levels were less than 0.01% of total mercury present in the soil and sediment samples.

5.2.5 Carcinogenicity and Teratogenicity Effects

The soil and sediment feeding studies were supplemented by carcinogenicity and teratogenicity experiments. Results for these studies are shown in Tables 22 and 23. Skin tumors were not observed in the tumor promotion studies. However, several sites were associated with tumor initiation. Statistical analysis showed a significant correlation between Hg levels in soil and sediment and the number of tumors. Although these increases in the number of tumors were small, they occurred at sites with the highest levels of Hg. When the number of tumors at these sites is compared to the positive controls (i.e., the DMBA/TPA-treated mice), it is clear that the soil and sediment would be considered only as a weak tumor initiator. For example, in the positive controls tumors were observed for all mice, and the average number of tumors per mice was 7.9; the experimental data showed a high value of one tumor per mouse. We suggest that the samples used in these studies are, at best, weak tumor initiators. However, we recommend additional studies to determine the biological factors that may be associated with observed initiation of tumors in those sites with high levels of Hg.

We also suggest that the observed tumor initiation effect is not directly related to inorganic and elemental Hg because these metals would not be expected to extract into the organic solvent. Thus, an organic metabolite associated with Hg and/or an organic pollutant associated with these sites may be responsible for the observed tumor initiation effect.

To test the potential teratogenicity of pollutants in soil and sediment from the 30 experimental sites, mice breeding studies were performed. As shown in Table 23, litter size was not significantly correlated to the Hg levels in the soil and sediment. Furthermore, changes in the number of digits of the hand and foot, apparent neurological changes, and cleft palate were not observed in the litters. These data suggest that the level of Hg and other heavy metals in these samples may not be sufficient to induce a change in litter size and congenital abnormalities. We thus suggest that the soil and sediment from these sites would not be considered as teratogens.

TABLE 22
Tumor Promotion and Initiation Study Using Soil Extracts

Site	Tumor Promotion*		Tumor Initiation**	
	Female	Male	Female	Male
1	5-0	5-0	5-0	2-3/3-0
2	5-0	5-0	5-0	2-3/3-0
3	5-0	5-0	2-2/3-0	2-3/3-0
4	5-0	5-0	6-12/9-0	6-11/9-0
5	5-0	5-0	6-9/9-0	6-7/8-0
6	5-0	5-0	5-0	5-0
7	5-0	5-0	5-0	5-0
8	5-0	5-0	1-1/4-0	2-2/3-0
9	5-0	5-0	5-0	5-0
10	5-0	5-0	8-10/7-0	1-1/14-0
11	5-0	5-0	7-9/8-0	5-6/8-0
12	5-0	5-0	9-10/6-0	9-15/6-0
13	5-0	5-0	1-1/4-0	2-2/3-0
14	5-0	5-0	5-0	5-0
15	5-0	5-0	5-0	5-0
16	5-0	5-0	5-0	5-0
17	5-0	5-0	5-0	5-0
18	5-0	5-0	15-0	1-1/14-0
19	5-0	5-0	5-0	5-0
20	5-0	5-0	5-5/10-0	4-5/10-0
21	5-0	5-0	5-0	5-0
22	5-0	5-0	5-0	5-0
23	5-0	5-0	5-0	5-0
24	5-0	5-0	5-0	5-0
25	5-0	5-0	5-0	5-0

Table continued on next page.

TABLE 22 (cont'd)
Tumor Promotion and Initiation Study Using Soil Extracts

Site	Tumor Promotion* Female	Tumor Promotion* Male	Tumor Initiation** Female	Tumor Initiation** Male
26	5-0	5-0	5-0	5-0
27	5-0	5-0	5-0	5-0
28	5-0	5-0	5-0	5-0
29	5-0	5-0	5-5/9-0	2-3/13-0
30	5-0	5-0	5-0	5-0

Controls	Females	Males
TPA***	1-1/9-0	1-1/9-0
DMBA+	3-3/7-0	4-4/6-0
DMBA + TPA	10-73	10-84
Acetone	5-5/10-0	6-6/9-0

* Initiate with DMBA (10 nm) and promote with soil extract (0.2 mL) twice weekly for 17 weeks.
**Initiate with 0.2 mL soil extract (X2) and promote with TPA twice weekly (1 µg) for 17 weeks.
*** TPA = Phorbol 12 Myristate 13 Acetate.
+ DMBA = 7,12-dimethylbenz (a,h) anthracene.

TABLE 23
Mice Breeding Studies*

Site	Litter Size**
1	6 ± 1
2	9 ± 3
3	5 ± .6
4	6 ± 1
5	7 ± 1
6	8 ± 2
7	8 ± 1
8	8 ± .5
9	7 ± 1
10	6 ± .6
11	8 ± 1
12	7 ± 1
13	7 ± 2
14	6 ± 1
15	8 ± 2
16	7 ± 1
17	6 ± .5
18	7 ± 1
19	5 ± .5
20	6 ± .6
21	10 ± 3
22	8 ± 2
23	6 ± 4
24	6 ± .5
25	8 ± 1
26	5 ± .5
27	7 ± 2
28	6 ± 1
29	7 ± 1
30	8 ± 3

$\bar{X} = 6.9$
$A = 1.2$
$\mu.s = 9.3$

*Mice were exposed to soil and sediment from the sites for 6 months. After exposure, animals were time-mated in pairs of three from each site. The size of each litter was determined within 24 hr and again at 96 hr after delivery

**Mean ± SD for three breeding pairs.

6. SUMMARY, CONCLUSIONS, AND RECOMMENDATIONS

6.1 SUMMARY

Several investigators have established that Hg and other heavy metals are absorbed through the intestinal tract and bioaccumulate in various organs. The organs primarily associated with the bioaccumulation include the liver and kidney. The brain is also associated with heavy metal accumulation. However, reports show that brain accumulation of heavy metals occurs after exposure to metals in the organic and/or elemental form. Furthermore, reports in the literature indicate differences in the organ distribution of metal following the exposure to metals in the organic form versus metals in the inorganic form. For example, after exposure to inorganic Hg, the liver-to-kidney ratio for Hg ranged from 0.01 to 0.31. In contrast, this ratio ranged from 0.6 to 0.9 following the exposure to the organic mercurials. Because metals in the organic form accumulate in brain tissue, toxicity differences are observed between the inorganic and organic metals. Thus, differences in the toxicity and the organ distribution of metals provide a method for determining the relative level of metals in the inorganic and organic forms. These reports provide a means of assessing (1) the toxicity of Hg in a complex mixture, and (2) the interaction of constituents in a complex mixture on the organ distribution of Hg. Because Hg and other heavy metals accumulate in the kidney, this would be used to assess the toxicity of a mixture contaminated with heavy metals. The assessment of interactions may be determined by comparing results showing the organ distribution of Hg following exposure to this metal in a simple matrix versus exposure to this metal in a complex matrix.

The present studies were performed to determine the effect of soil and sediment containing Hg and other heavy metals on (1) distribution of Hg in the liver and kidney, (2) morphology of the kidney, (3) functional physiology of the kidney, (4) animal mortality, and (5) body weight. Other toxicological assessments of soil and sediment included determinations of carcinogenicity and teratogenicity.

The observed results showed:

1. A significant correlation between level of Hg in soil and sediment and Hg in the kidney.
2. A ratio of Hg in the liver and kidney ranging from 0.06 to 0.34 (with the exception of groups 5, 23, and 24).
3. Nonsignificant morphological changes in the kidney in groups exposed for 6, 12, and 20 months.
4. Nonsignificant functional changes in the kidney following 12 months of exposure.
5. A nonsignificant correlation between the level of Hg in soil and sediment and mortality.
6. A nonsignificant correlation between the level of Hg in soil and sediment and teratogenicity effects.
7. A nonsignificant correlation between the level of Hg in soil and sediment and changes in body weight.
8. A significant correlation between Hg in soil and sediment and skin tumor initiation in sites 4, 5, 6, 10, 11, and 12.

6.2 CONCLUSIONS

Based on the above results the following conclusions were reached.

1. Mercury in soil and sediment is absorbed through the intestinal tract and bioaccumulates in the liver and kidney.

2. A significant dose-response relationship was observed, but this relationship was not linear. The factors that may have affected the linear response include the binding coefficient of Hg to soil, the relative level of the various species and inorganic forms of Hg in the different soils, and the effect of various soil constituents on Hg absorption.

3. Mercury was absorbed through the intestinal tract and bioaccumulated in the kidney, but no significant morphological and physiological changes in this organ were observed. If this animal model is accepted as a predictor of the potential health effects of humans eating contaminated soil and sediment, we suggest that no significant health effects associated with heavy metal toxicity should occur in humans exposed to the soil and sediment from East Fork Poplar Creek. This suggestion is supported by the results showing that significant correlations were not observed between the level of Hg in soil and sediment and mortality, body weight, or litter size.

4. The skin tumor initiation and promotion studies showed a significant correlation between Hg in soil and sediment and tumor initiation. Since extracts of soil and sediment were used in these studies, we suggest that an organic pollutant(s) associated with Hg and/or these sites initiates tumors. However, results show that only two areas (of 30 studied) account for the significant increase in tumors. Furthermore, the Hg levels in several sites in these areas which show a significant correlation were below levels observed in sites from other areas in which the tumor incidence was below the calculated mean for all sites. It may be suggested from these data that the areas which show a significant increase in tumors have other organic contaminants that initiate tumors. This suggestion will require additional experiments.

6.3 RECOMMENDATIONS

The three recommendations suggested by the present studies include the following.

1. Determine, in soil and sediment from East Fork Poplar Creek, the levels of elemental, inorganic, and organic mercury as well as the level of mercury sulfide.

2. Perform additional studies to establish the relationship between mercury and tumor initiation.

3. Perform additional statistical analyses of the dose-response data to determine if several levels of response (i.e., kidney mercury) can be discerned. This information will help establish minimal and maximal intake levels for soil and sediment.

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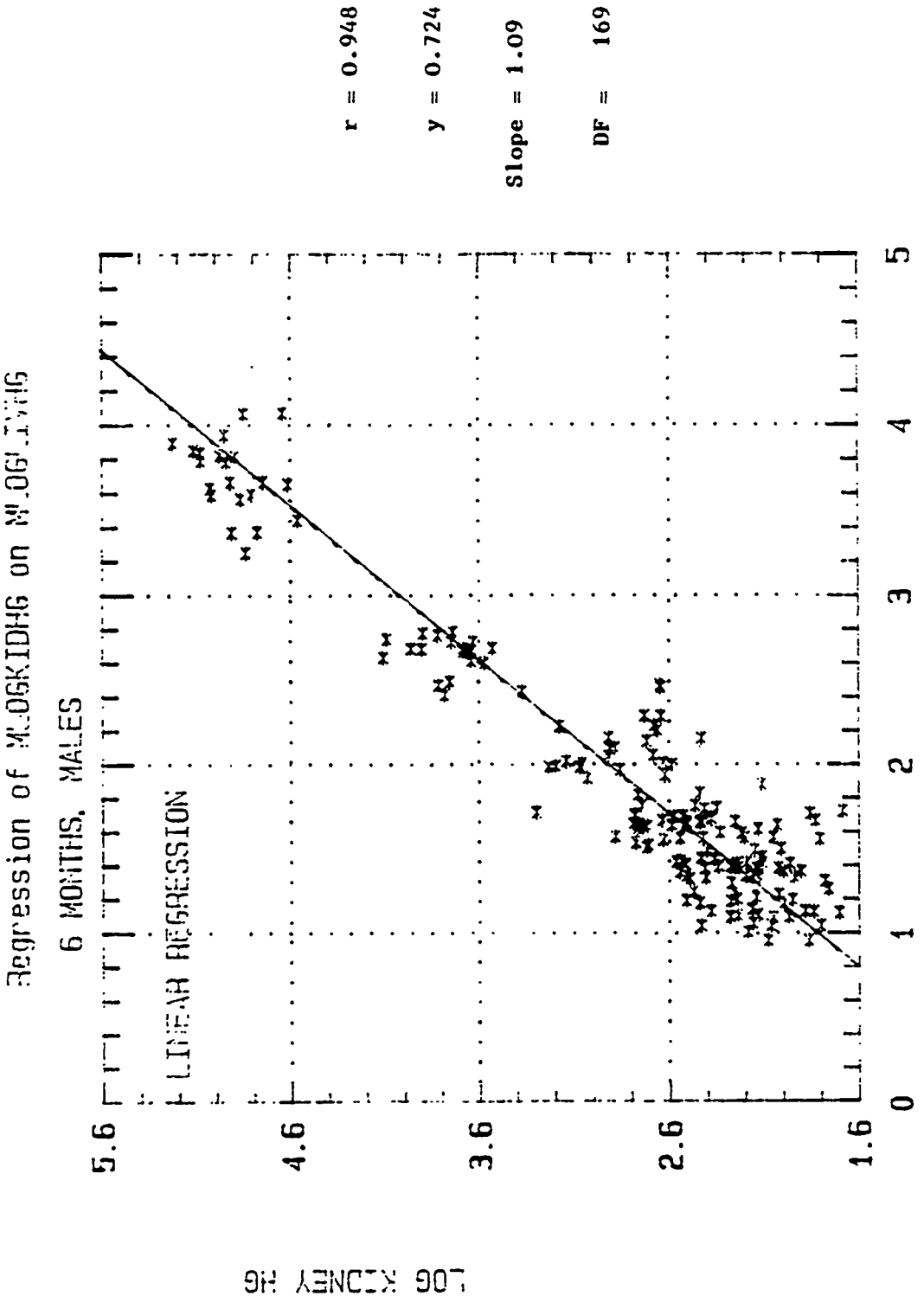
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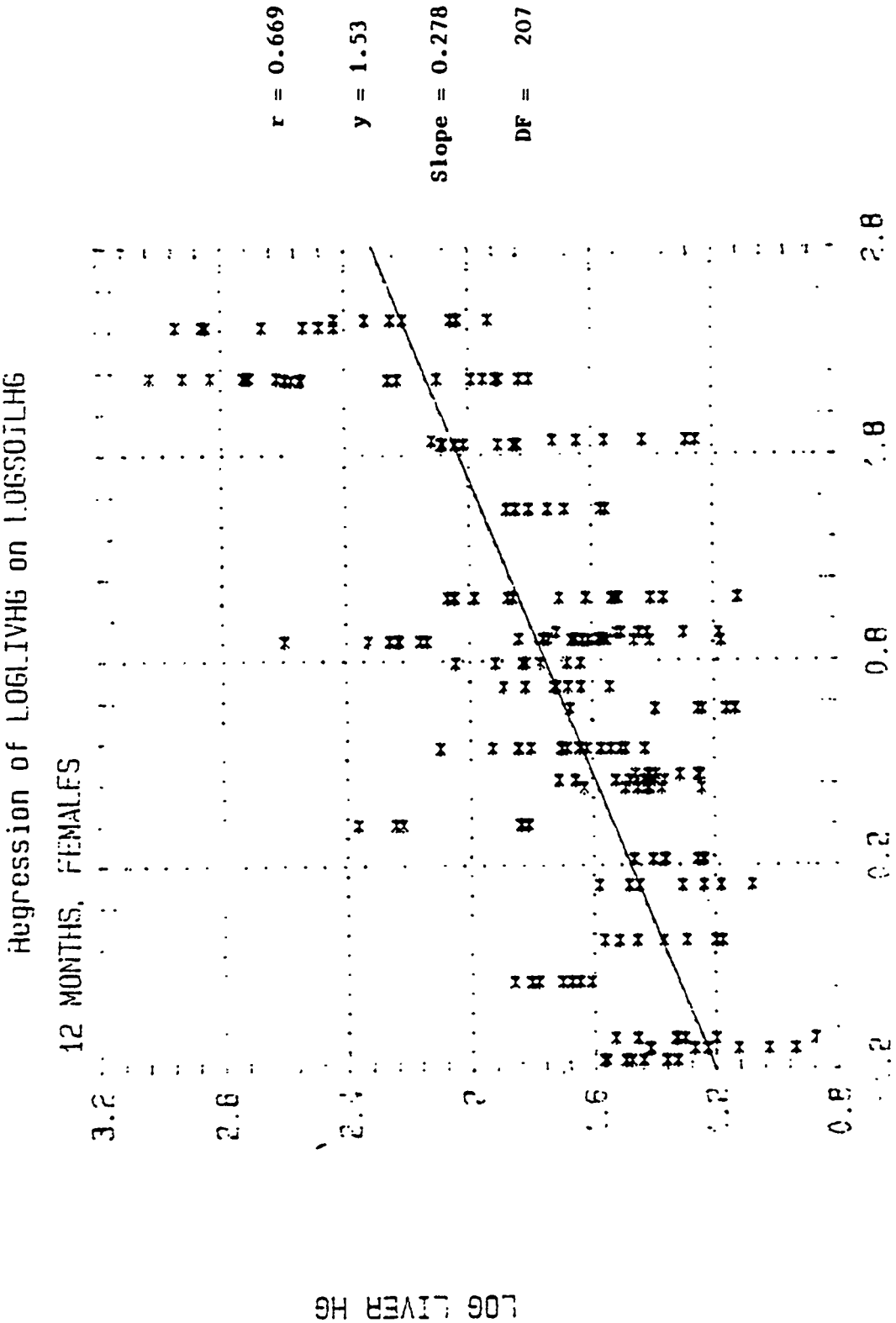
Regression of KIDNEYS on LIVERS
6 MONTHS, MALES
LINEAR REGRESSION
LOG KIDNEY HG
LOG LIVER HG
5.6
4.6
3.6
2.6
1.6
0
1
2
3
4
5

Figure 8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Regression of LOGLIVHG on LOGSOILHG
12 MONTHS, FEMALES
LOG LIVER HG
LOG SOIL HG

Figure 9

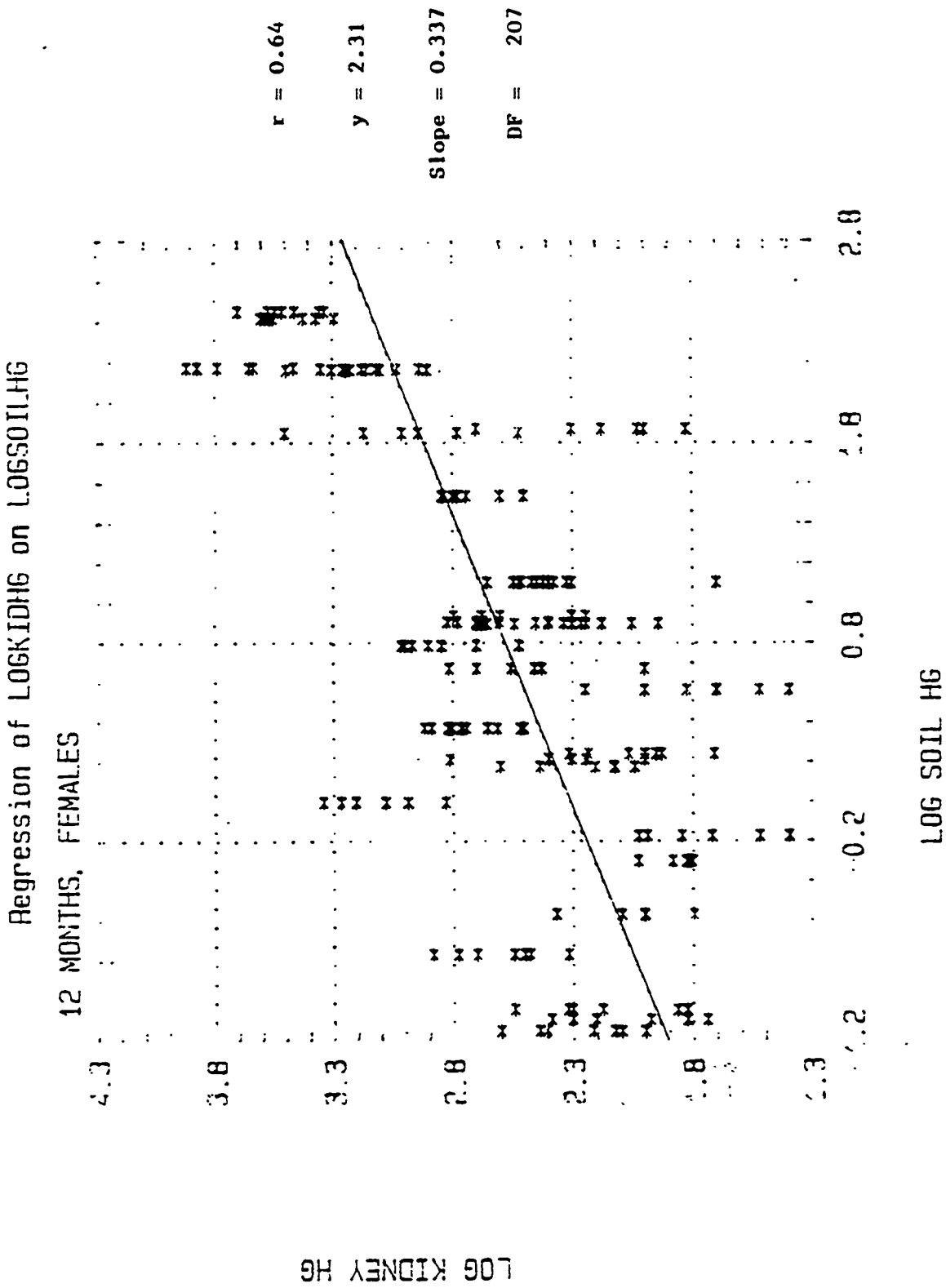

Regression of LOGKIDHG on LOGSOILHG
12 MONTHS, FEMALES
r = 0.64
y = 2.31
Slope = 0.337
DF = 207
LOG SOIL HG
LOG KIDNEY HG

Figure 10

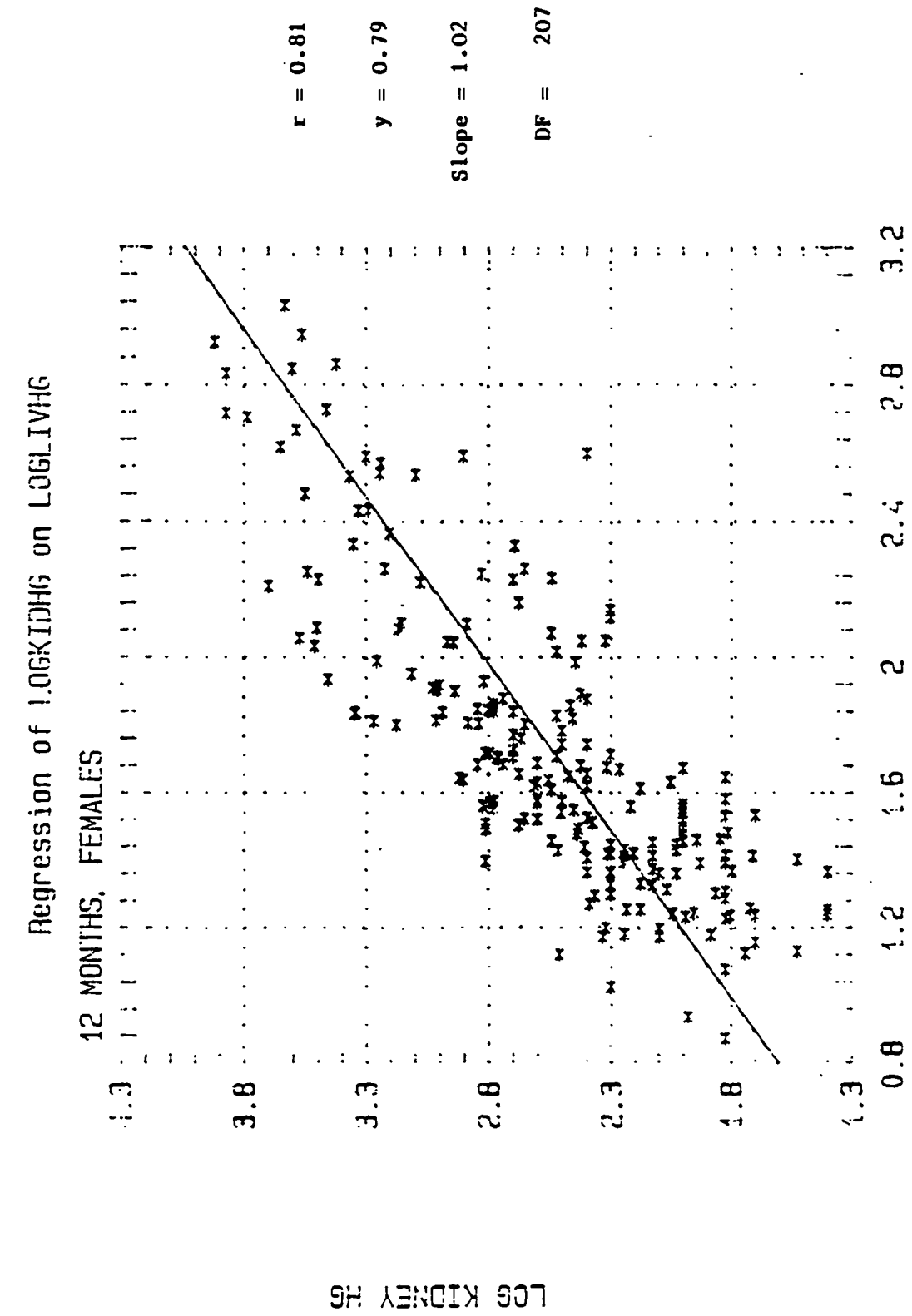

Regression of LOGKIDHG on LOGLIVHG
12 MONTHS, FEMALES
r = 0.81
y = 0.79
Slope = 1.02
DF = 207
LOG KIDNEY HG
LOG LIVER HG
4.3
3.8
3.3
2.8
2.3
1.8
1.3
0.8
1.2
1.6
2
2.4
2.8
3.2

Figure 11

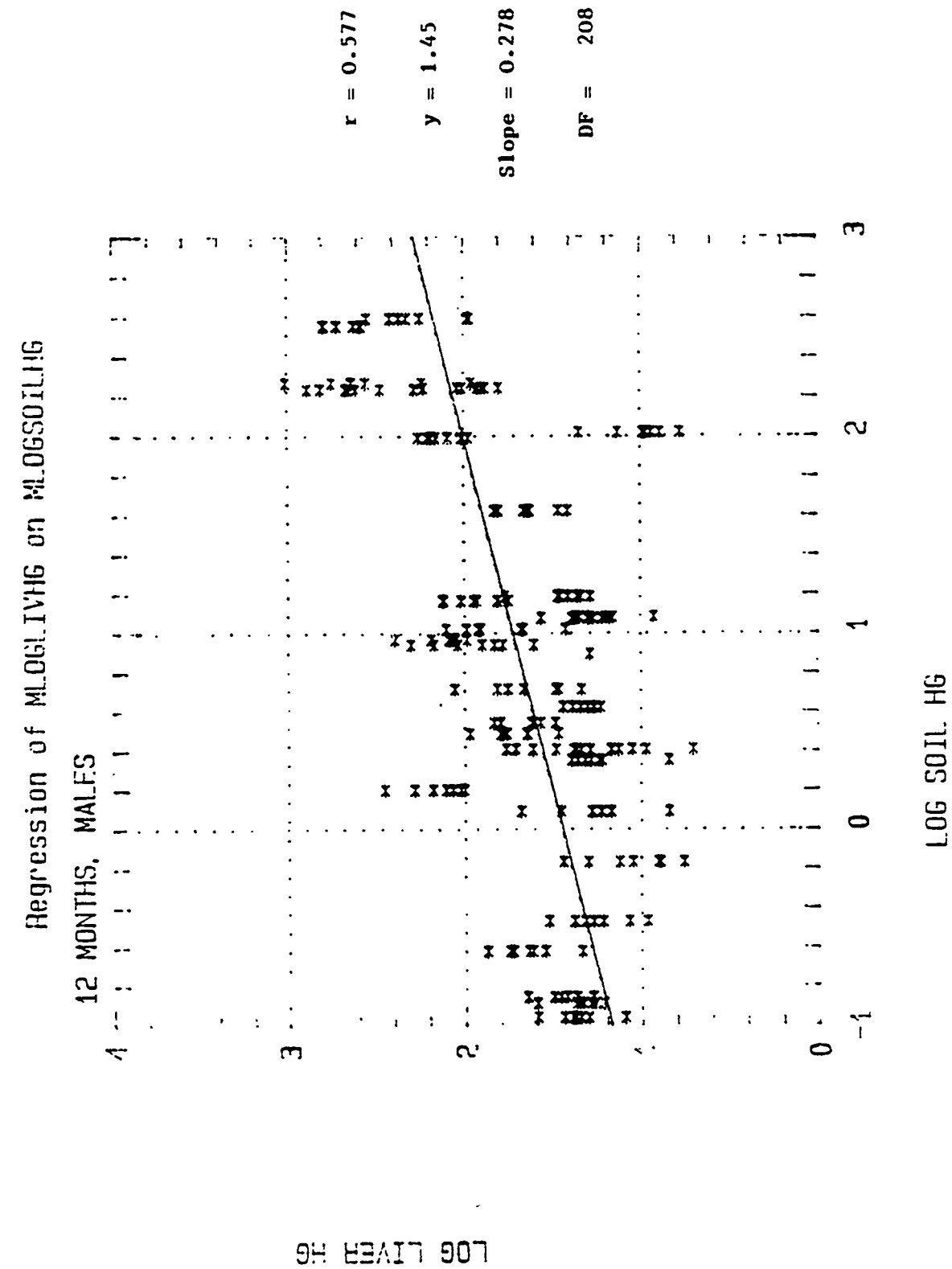
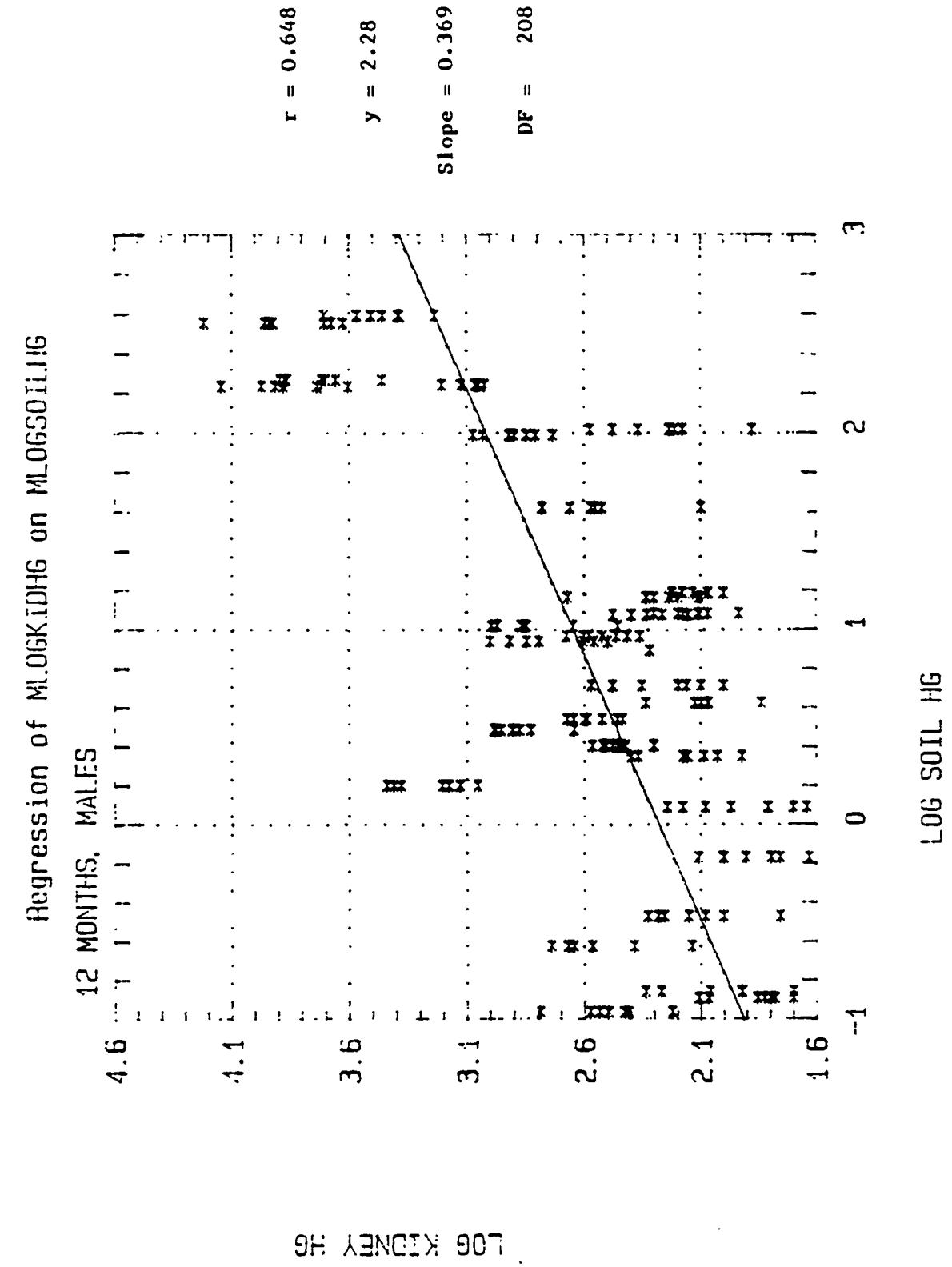

r = 0.577
y = 1.45
Slope = 0.278
DF = 208
Regression of MLOGLIVHG on MLOGSOILHG
12 MONTHS, MALES
LOG LIVER HG
LOG SOIL HG
4
3
2
1
0
-1
0
1
2
3

Figure 12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y = 2.28
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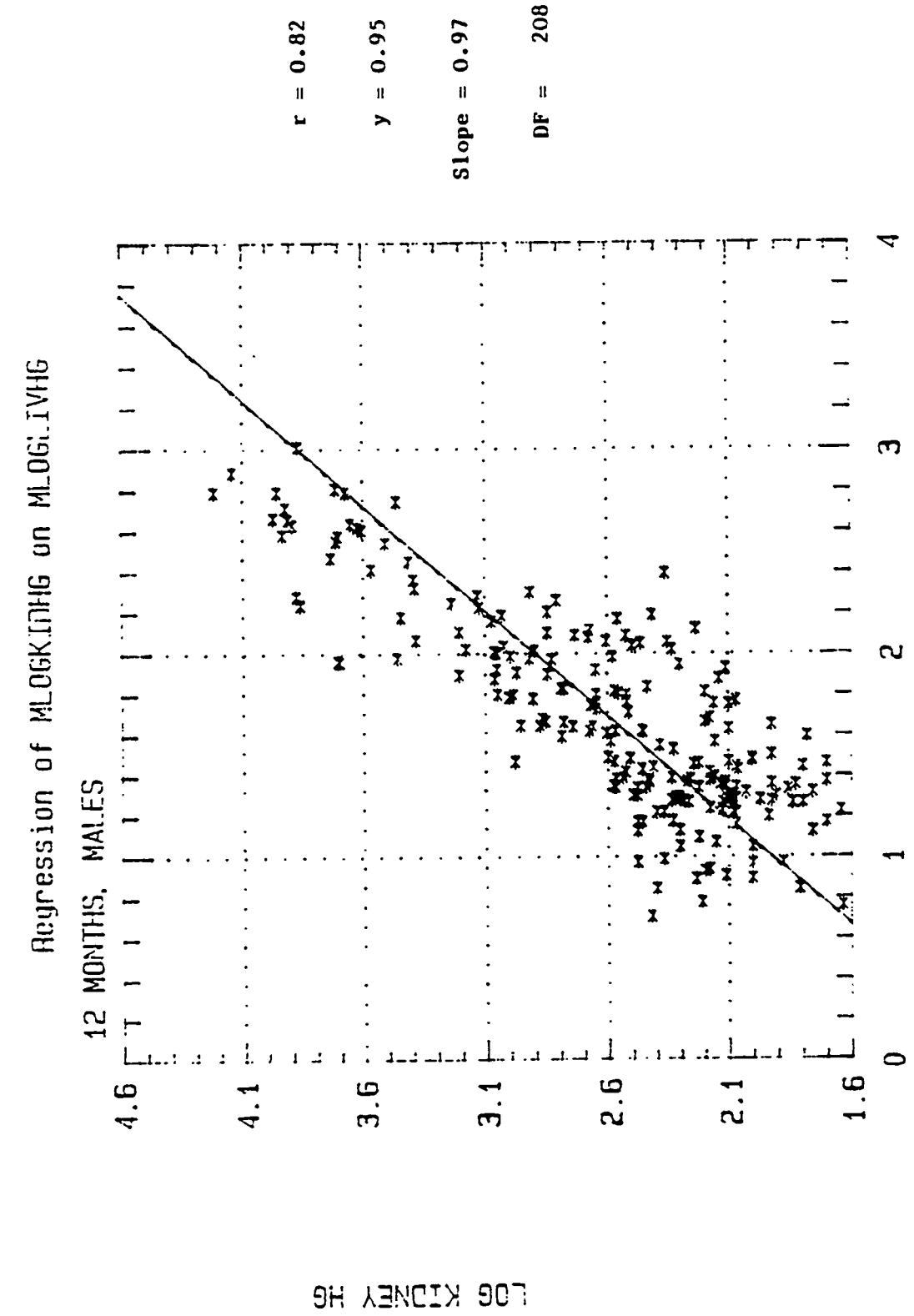
Regression of MLOGKIDHG on MLOGSOILHG
12 MONTHS, MALES
LOG KIDNEY HG
LOG SOIL HG
4.6
4.1
3.6
3.1
2.6
2.1
1.6
3
2
1
0
-1

Figure 13


Regression of MLOGKIDHG on MLOGLIVHG
12 MONTHS, MALES
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y = 0.95
Slope = 0.97
DF = 208
LOG KIDNEY HG
LOG LIVER HG
4.6
4.1
3.6
3.1
2.6
2.1
1.6
0
1
2
3
4

Figure 14

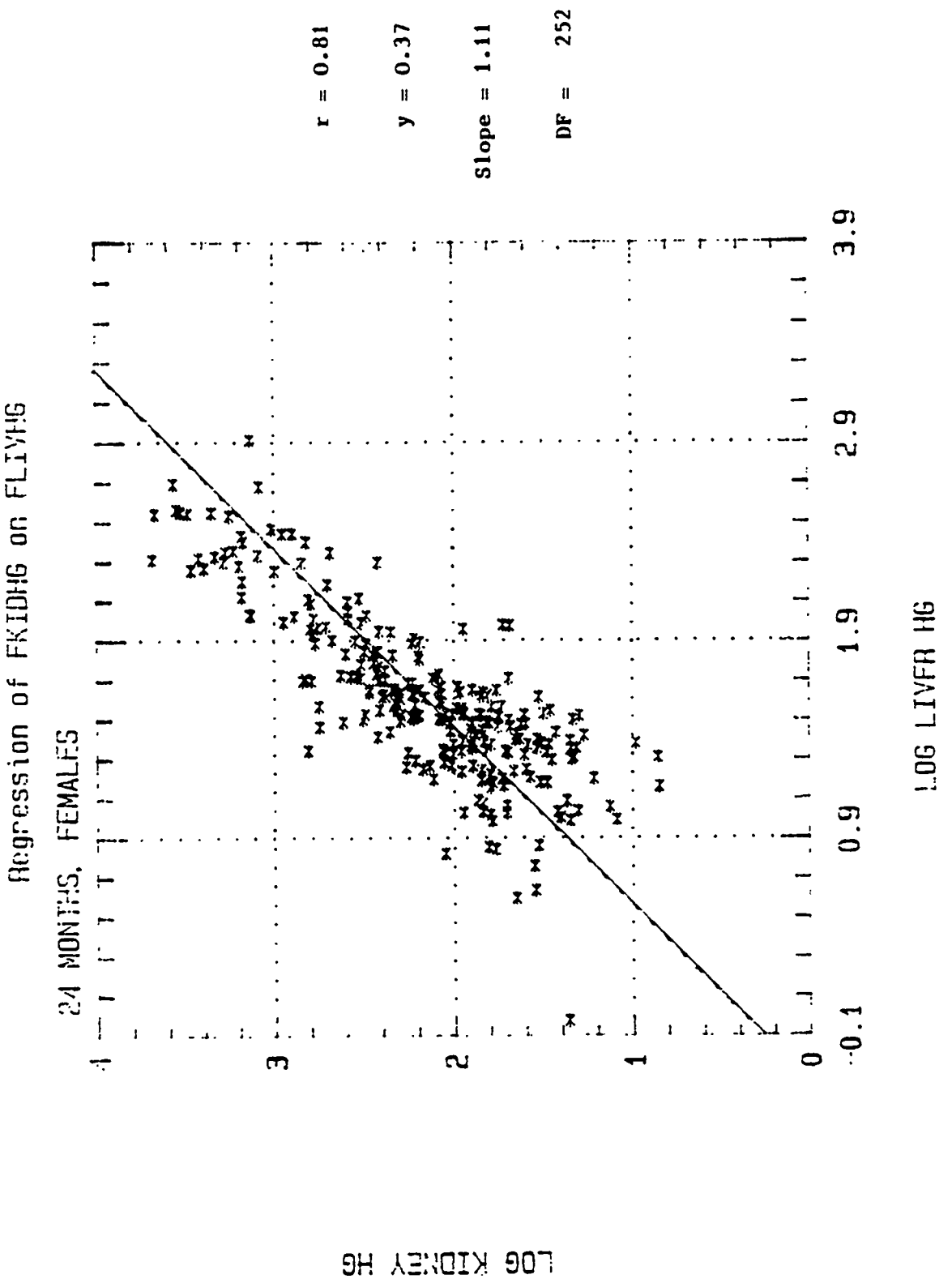

Regression of KIDNG on FLIVNG
24 MONTHS, FEMALES
r = 0.81
y = 0.37
Slope = 1.11
DF = 252
LOG KIDNEY HG
LOG LIVER HG
-0.1
0.9
1.9
2.9
3.9
0
1
2
3
4

Figure 15

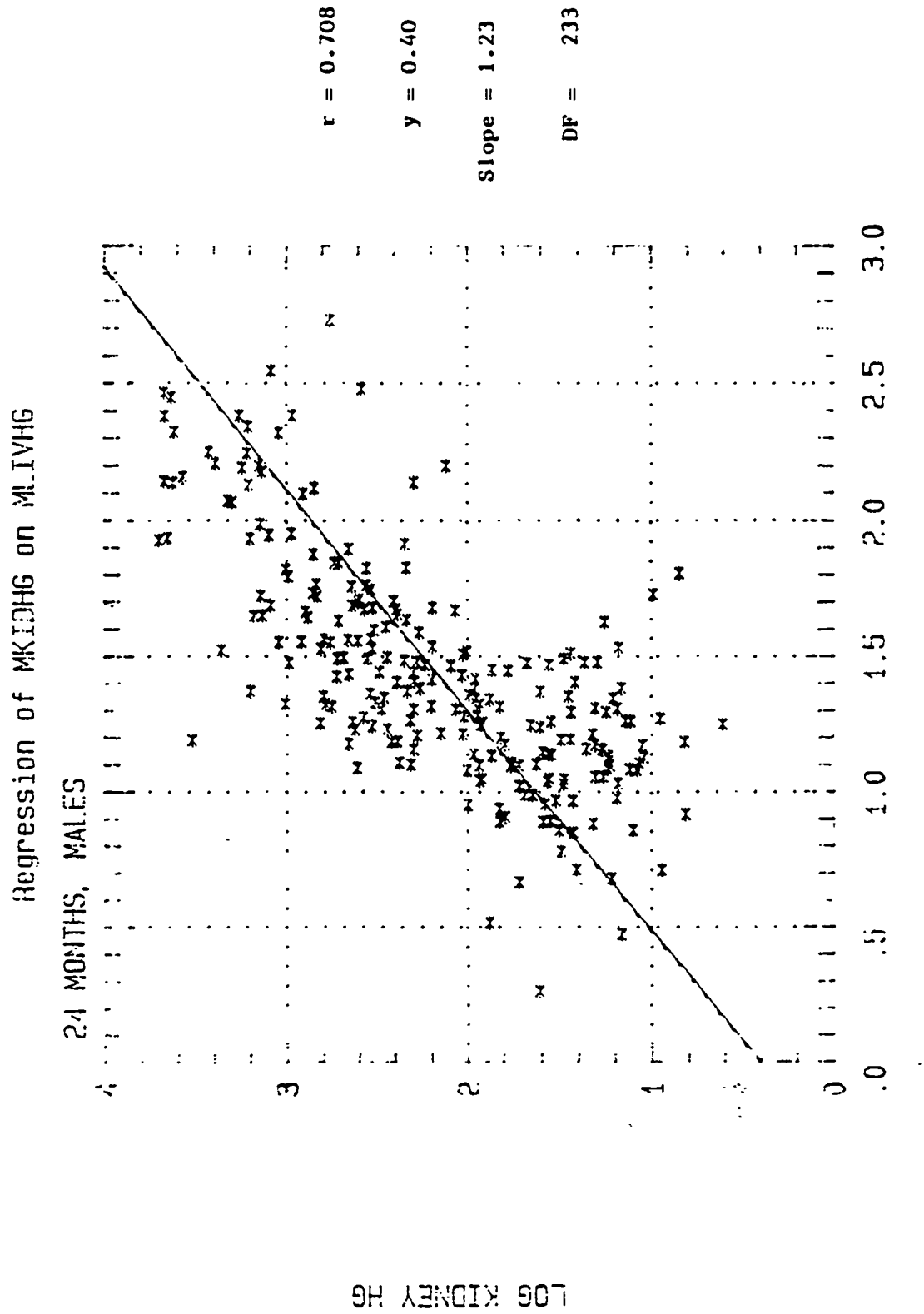

Regression of MKIDHG on MLIVHG
24 MONTHS, MALES
r = 0.708
y = 0.40
Slope = 1.23
DF = 233
LOG KIDNEY HG
LOG LIVER HG
.0
.5
1.0
1.5
2.0
2.5
3.0
1
2
3

Figure 16

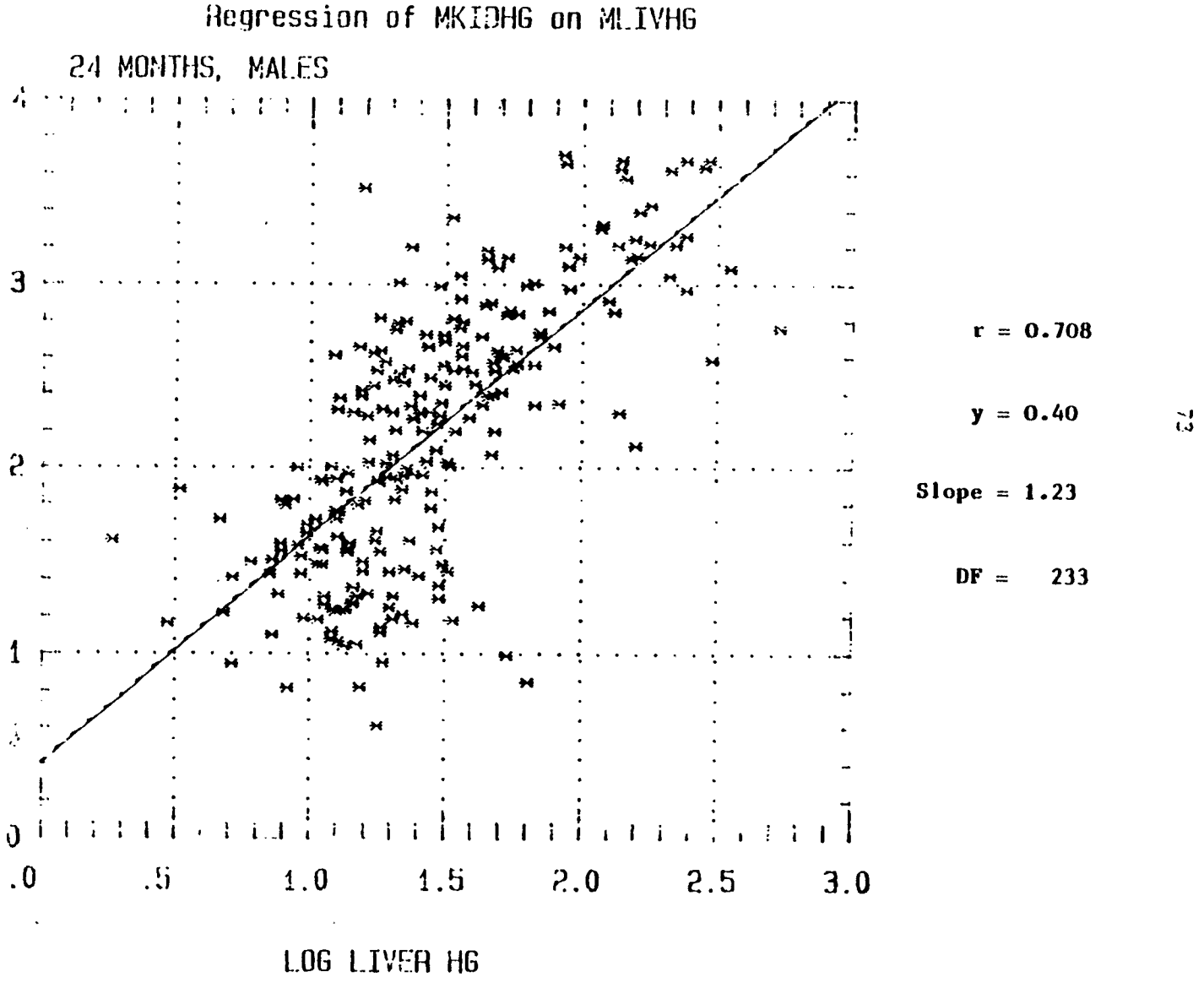
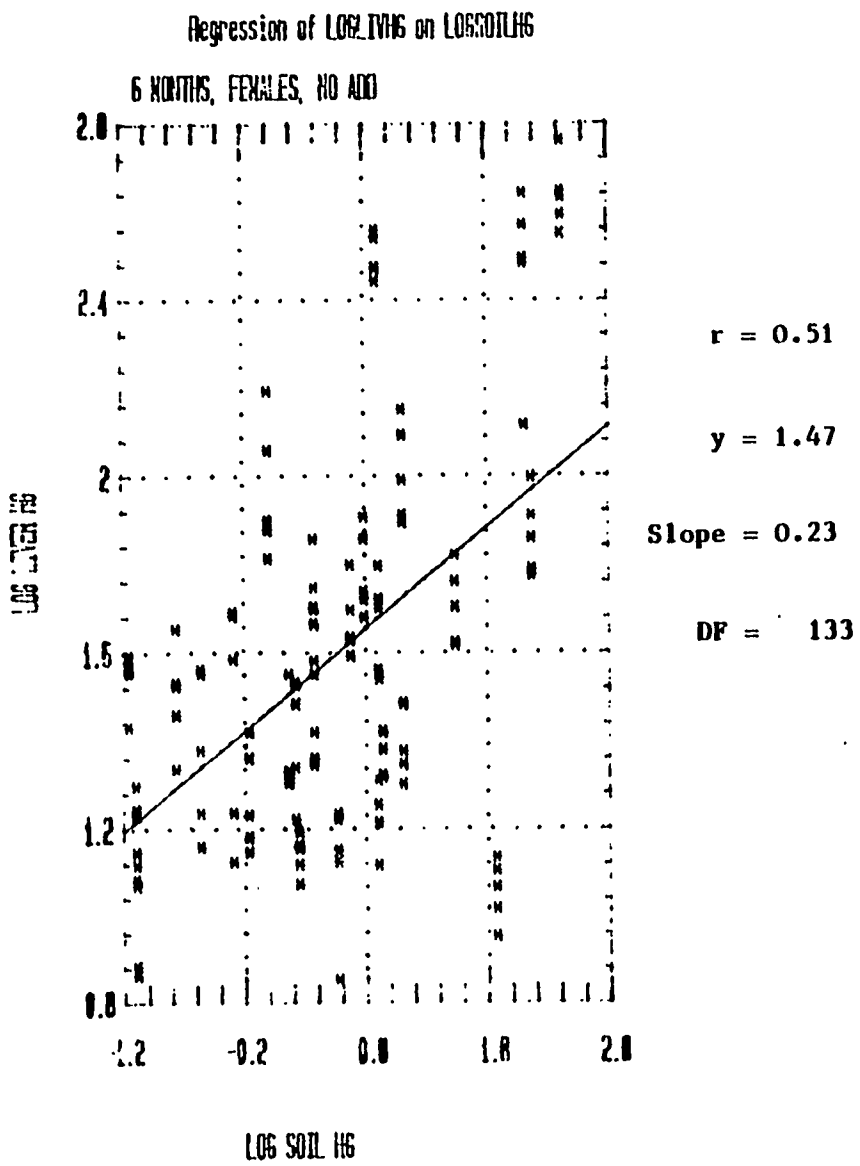

Regression of MKIDHG on MLIVHG
24 MONTHS, MALES
LOG KIDNEY HG
LOG LIVER HG
0
1
2
3
4
.0
.5
1.0
1.5
2.0
2.5
3.0
r = 0.708
y = 0.40
Slope = 1.23
DF = 233

Figure 16



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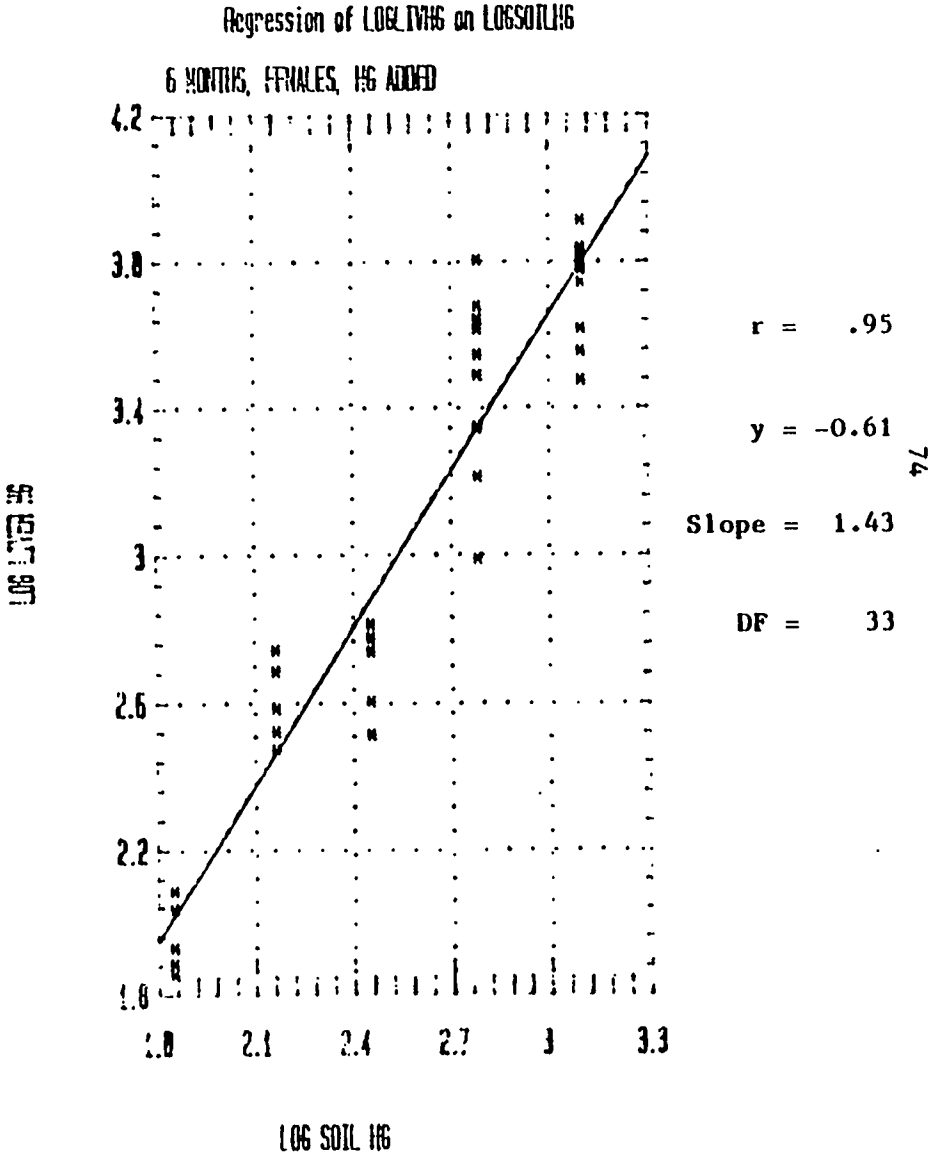

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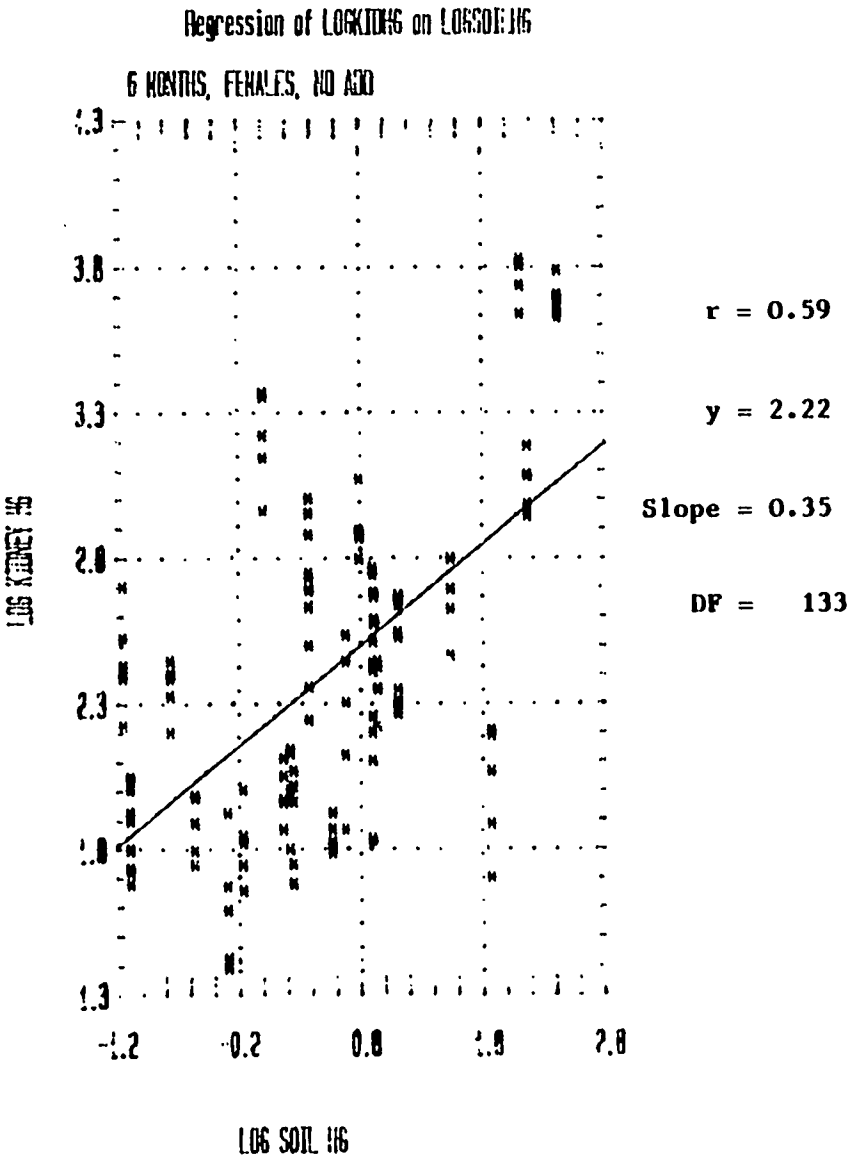

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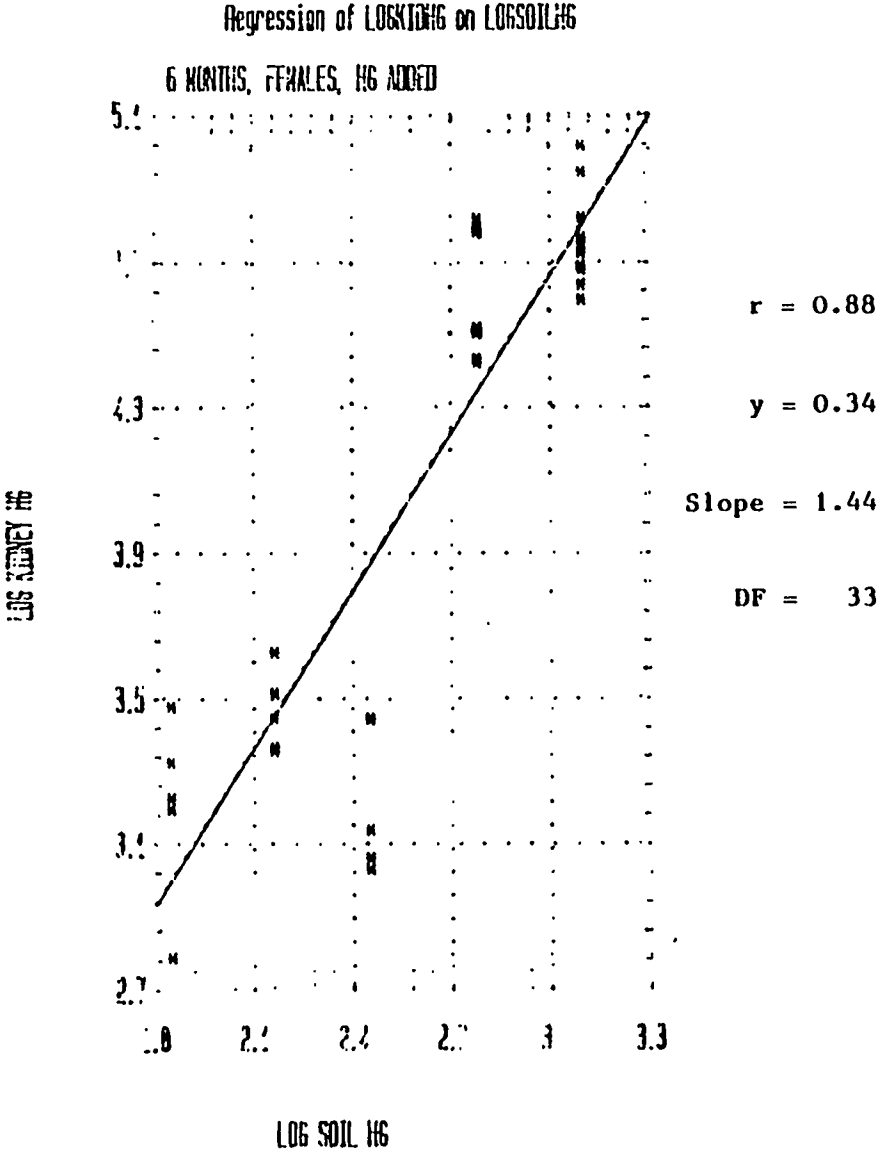

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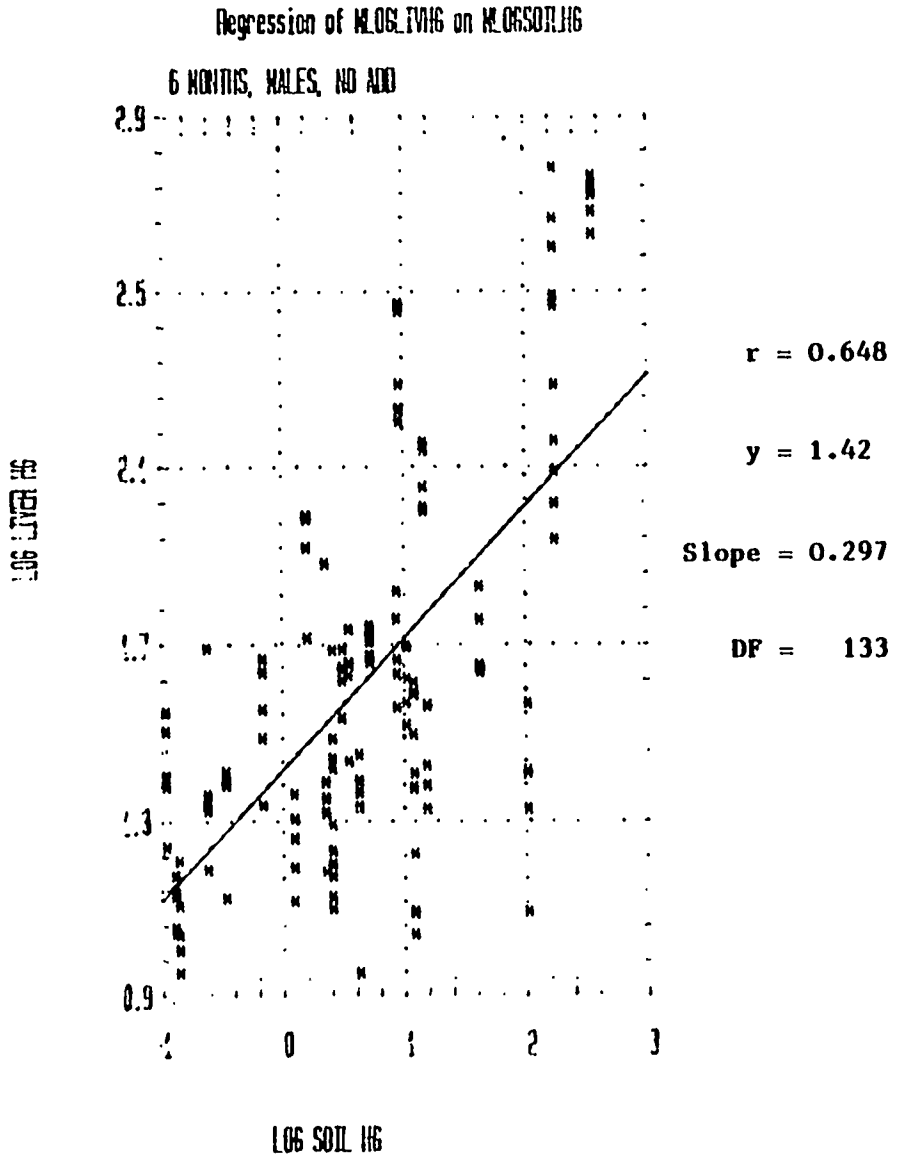

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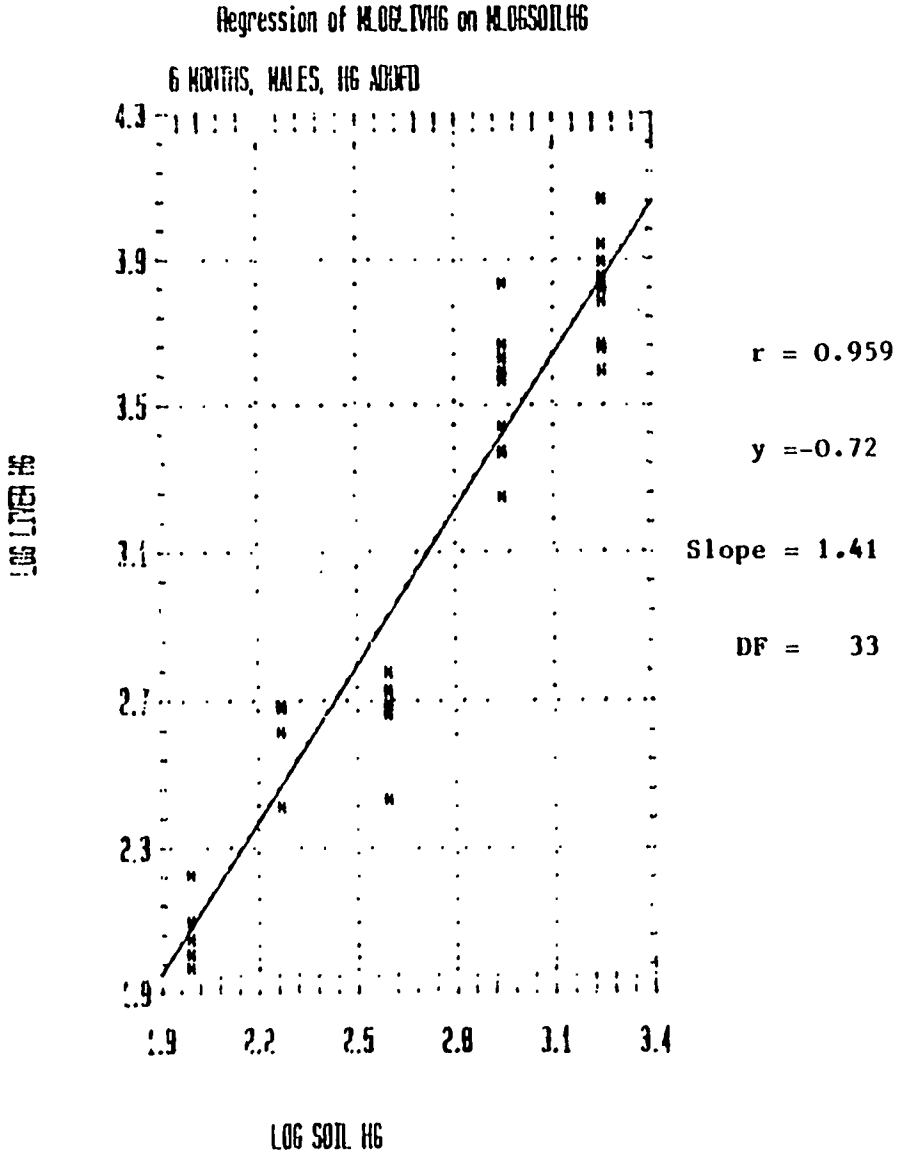

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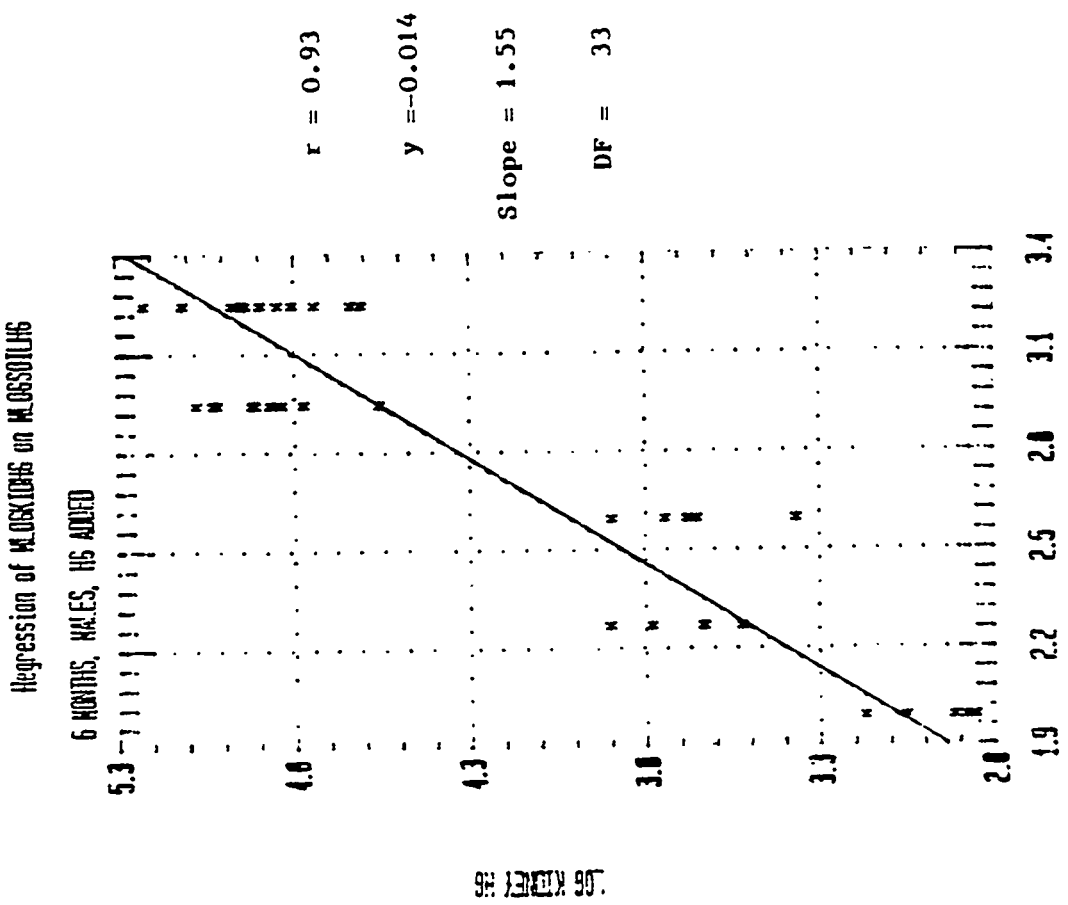

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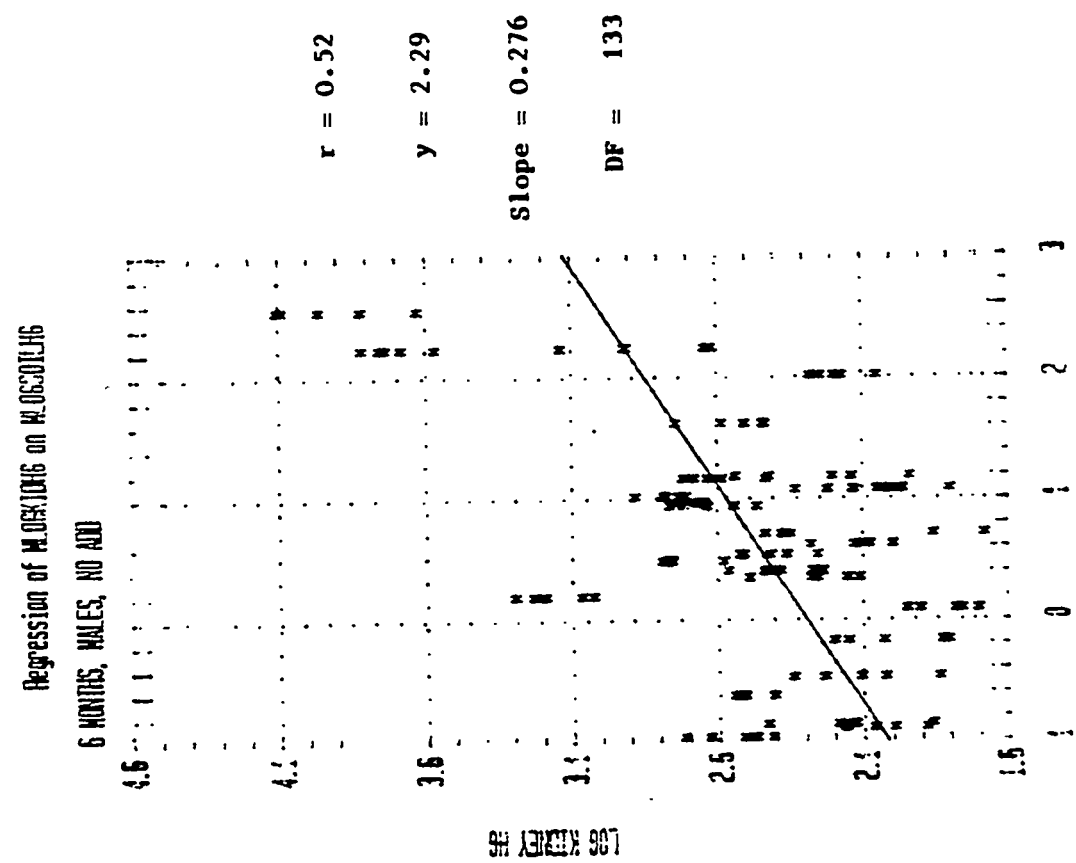

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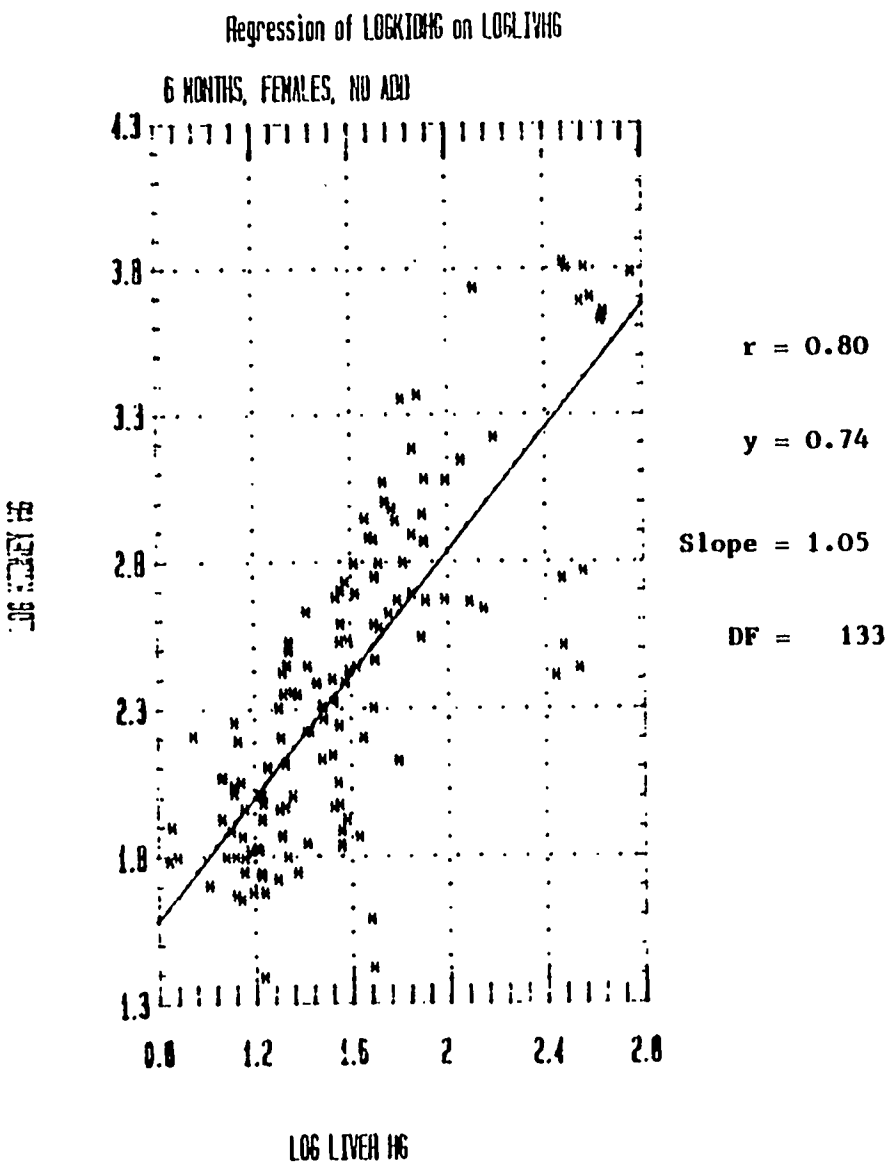

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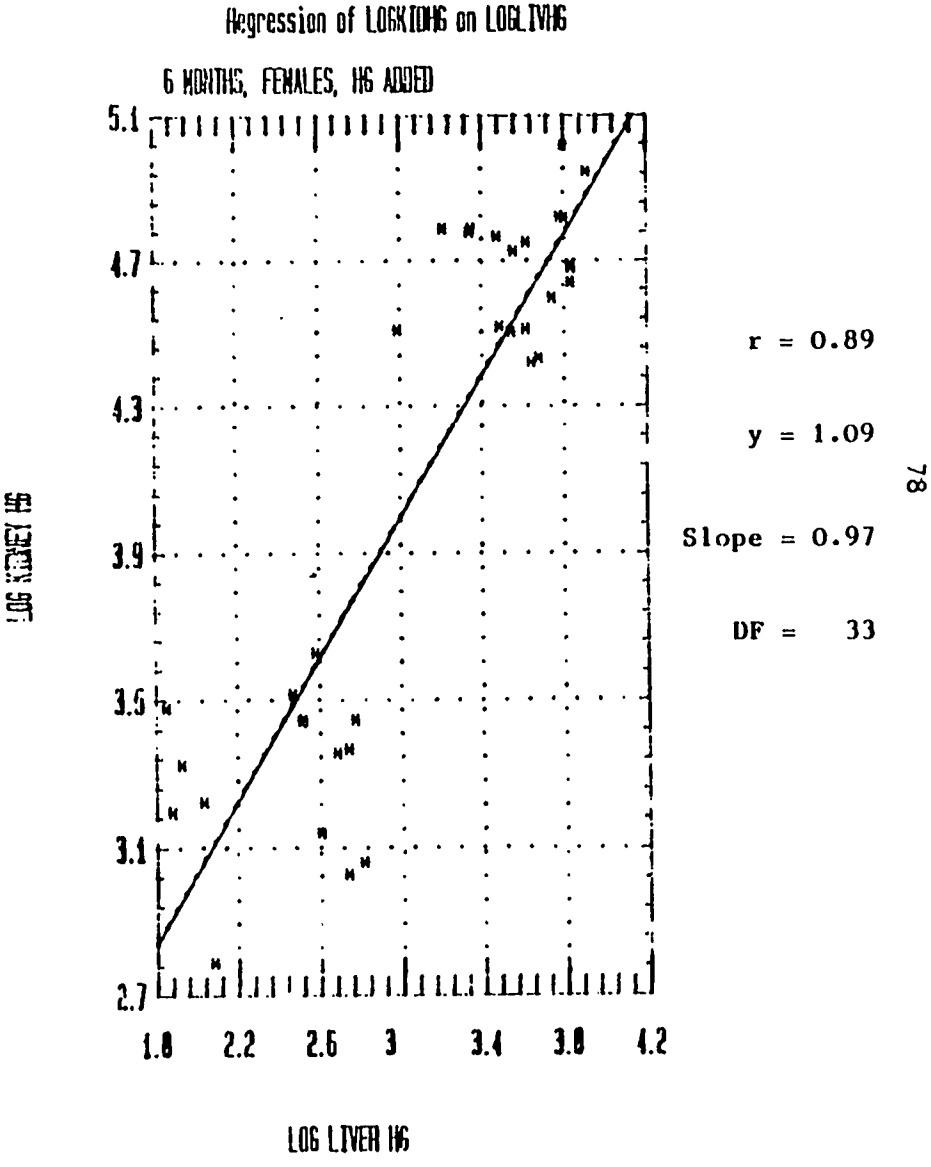

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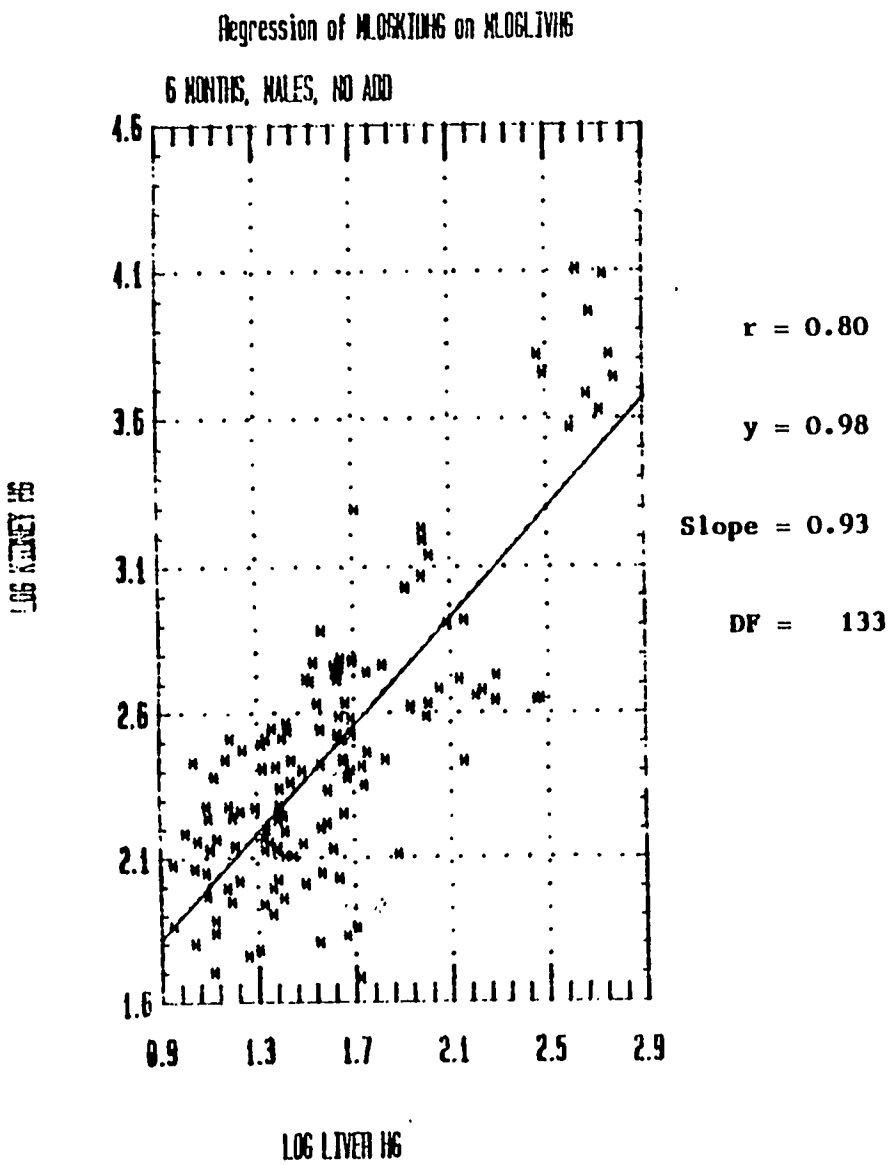

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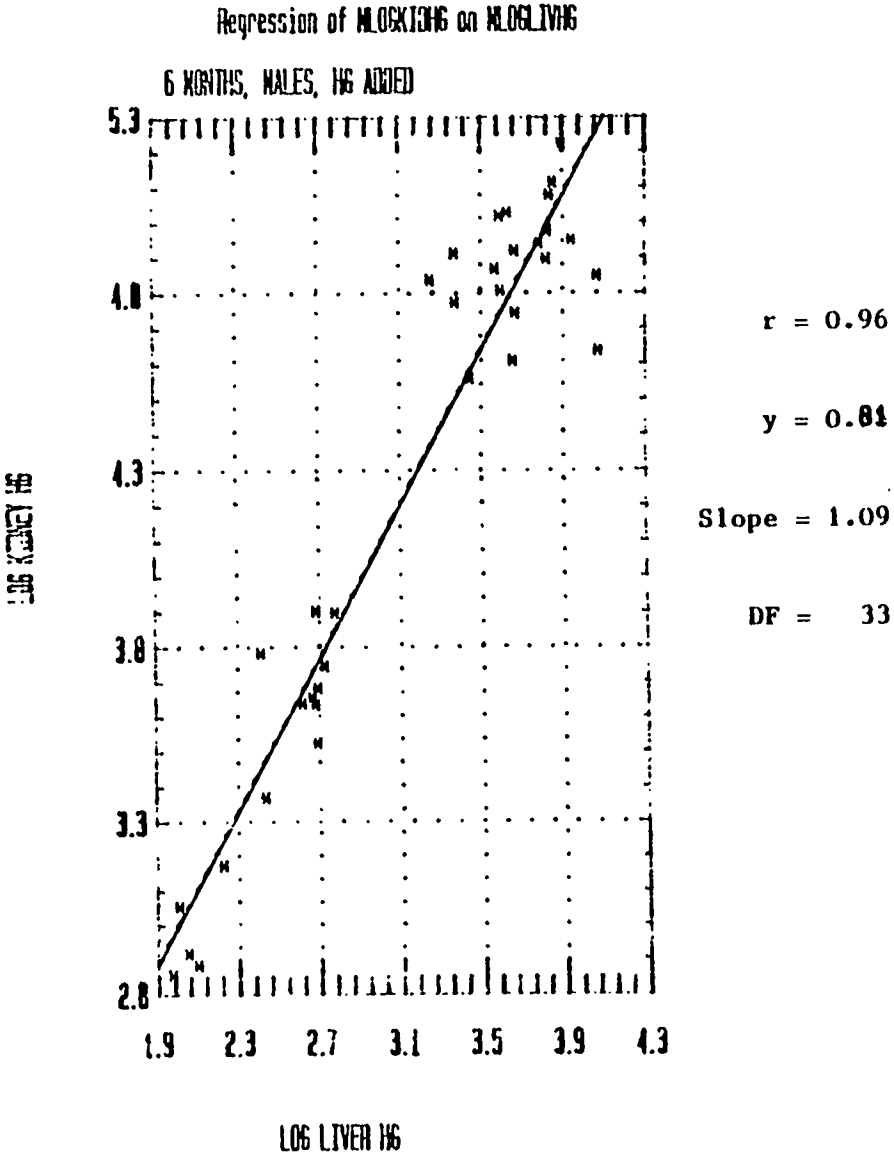

Figure 28

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East Fork Poplar Creek Soils Investigation of Mercury Form and Association

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Y-12 Environmental Restoration Program
Energy Systems Environmental Restoration Program Division

East Fork Poplar Creek Soils Investigation of Mercury Form and Association

Date Issued—October 1993

Prepared by the
Technical Division
Oak Ridge K-25 Site
under an Intercompany Statement of Work and
Work Order PKY 21600-209AG1

Prepared for the
U.S. Department of Energy
Environmental Restoration and Waste Management Program
under Budget and Reporting Code EW 20 10

Oak Ridge Y-12 Plant
Oak Ridge, Tennessee 37831
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MARTIN MARIETTA ENERGY SYSTEMS, INC.
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under contract DE-AC05-84OR21400

CONTENTS

FIGURES v

TABLES vii

ACRONYMS ix

EXECUTIVE SUMMARY xi

1. INTRODUCTION 1-1
   1.1 SITE DESCRIPTION 1-1
   1.2 SITE HISTORY AND CONTAMINATION 1-1
   1.3 REMEDIAL INVESTIGATION 1-2

2. OBJECTIVES 2-1

3. SAMPLE COLLECTION AND PREPARATION 3-1
   3.1 SAMPLE COLLECTION 3-1
   3.2 SAMPLE PREPARATION 3-1

4. OPTICAL MICROSCOPY 4-1
   4.1 METHODS OF INVESTIGATION 4-1
   4.2 OBSERVATIONS 4-4

5. SCANNING ELECTRON MICROSCOPY 5-1
   5.1 METHODS OF INVESTIGATION 5-1
   5.2 OBSERVATIONS AND DISCUSSIONS 5-2

6. SUMMARY 6-1

7. FUTURE WORK RECOMMENDATIONS 7-1

8. REFERENCES 8-1

FIGURES

4.1 An optical micrograph of ash particles: (a) Coke, cenospheres, and mineral matter; (b) ferrospheres; (c) cross-section of a root; and (d) uncombusted coal fragment 4-2

4.2 Ultraviolet light fluoresced hydrogen-rich constituent (extinite, which is a coal entity) in uncombusted coal 4-3

5.1 SEI of mercury-rich particles (shown by arrows) with quartz (Q) and clay (C) 5-3

5.2 BEI of same area shown in Fig. 5.1 where the bright spots are the mercury-rich particles 5-4

5.3 Elemental X-ray maps for (a) mercury, (b) sulfur, and (c) calcium 5-5

5.4 SEI of a mercury-rich particle atop clay 5-6

5.5 Elemental X-ray maps for (a) mercury and (b) sulfur from the feature presented in Fig. 5.4 5-7

5.6 BEI of mercury-rich particles in center of the field 5-8

5.7 BEI of a wing-shaped mercury-rich particle (see arrows) 5-11

5.8 Elemental X-ray maps for (a) mercury and (b) sulfur for the region shown in Fig. 5.7 5-12

5.9 SEI showing an elongated structure (enclosed in the box) containing mercury and sulfur 5-12

5.10 Elemental X-ray maps of (a) mercury and (b) sulfur for the elongated structure shown in Fig. 5.9 5-13

5.11 EDS spectra (a) for as-received material, (b) after an increase in probe current (heating), and (c) after an additional increase in probe current 5-14

5.12 BEI of an elliptically shaped feature (P), probably plant matter, surrounded by quartz and clay 5-16

5.13 Elemental X-ray maps for (a) aluminum, (b) calcium, (c) iron, (d) mercury, (e) silicon, and (f) sulfur 5-17

5.14 BEI of plant matter with a cluster of pyrite crystals 5-18

5.15 Elemental X-ray maps for (a) aluminum, (b) calcium, (c) iron, (d) mercury, and (e) sulfur 5-19

5.16 A BEI of (a) a uranium particle and (b) its X-ray map 5-20

5.17 A BEI of (a) a thorium particle and (b) its X-ray image map 5-21

TABLES

1.1 Toxicity scores based on average of contaminant concentrations in EFPC soil, ranked by toxicity 1-3

3.1 Location and mercury concentrations of samples for mercury soils study 3-2

5.1 Atomic concentrations for a mercury-rich particle and matrix (shown in Fig. 5.6) 5-9

5.2 Atomic concentrations for a mercury-rich particle and matrix (shown in Fig. 5.7) 5-10

ACRONYMS

BEI	backscattered electron image
Colex	column exchange
CRT	cathode ray tube
DOE	U.S. Department of Energy
EDS	energy dispersive spectrometer
EFPC	East Fork Poplar Creek
FS	feasibility study
ORR	Oak Ridge Reservation
RI	remedial investigation
SAIC	Science Applications International Corporation
SEI	secondary electron image
WDS	wavelength dispersive spectrometer

EXECUTIVE SUMMARY

Science Applications International Corporation is performing a Remedial Investigation (RI) of the lower East Fork Poplar Creek (EFPC) under Radian Corporation's Technical Support Contract to the U.S. Department of Energy (DOE). A supportive investigation has been initiated that attempts to determine the form and association of mercury in the floodplain soils of EFPC within the DOE Oak Ridge Reservation and the city of Oak Ridge. The research was supported by funding from the Y-12 Environmental Restoration Program through an intercompany statement of work. This report summarizes the findings of the investigation and will be incorporated into the Administrative Record in compliance with the Comprehensive Environmental Response, Compensation, and Liability Act of 1980 (Public Law 96-510).

The major effort of the first phase of the RI was directed toward identifying the various affected media (i.e., groundwater, surface water, creek and tributary sediments, and floodplain soils) and defining the contaminants of concern for each medium. The medium with the highest concentrations of contaminants and the most widespread extent was determined to be the floodplain soil. Mercury constitutes 85% of the proportional toxicity for the various metals detected. As the primary contaminant, determining the occurrence, form, and association of mercury in the floodplain environment became crucial to the investigation. The form of mercury in the soils directly impacts the remedial treatment selection and the results of the human health baseline risk assessment.

The following objectives were established for this supportive investigation:

- To understand in what form the mercury presently exists (i.e., elemental, sulfide, oxide, or organo-mercury compound).
- To determine the size of the discrete particles containing or being composed primarily of mercury.
- To observe any strong association or paragenetic relationship between mercury and fly ash, organic material, or soil structure.
- To determine if the form and associations of mercury vary over the course of the floodplain.
- To note observable differences in the soil matrices or associations between areas of high and average mercury concentrations.

Twenty samples were collected March 2–4, 1992, with soil sampling equipment that extracted undisturbed cores with the dimensions of approximately 1 in. by 18 in. The locations selected for the samples were identified throughout the EFPC floodplain in varying segments of the creek, but all were from areas of high mercury to maximize the success of the study. Common to all these soil samples are fine-grained micrometer to submicrometer-sized particles that consist of quartz, clays, and plant debris and constitute a matrix in most of the samples. In addition, optical microscopy revealed varying amounts of ash and coal particles

dispersed throughout these core samples. The ash consists of coke, cenospheres, and ferrospheres.

Optical microscopy of the soil cores revealed variations in their content with respect to ash and coal concentrations such that these constituents were found to be more abundant in the mercury-rich cores and to be correspondingly less abundant in those cores that were poorer in mercury. There also appear to be greater amounts of ferrospheres in these mercury-rich soils. In contrast, those soil samples containing less than 300 ppm of mercury appear to be almost devoid of ash and ferrospheres (under 5 surface area percent). Additionally, within some cores we observed significant variations in texture and content, such as extreme variations between the upper sections, which contained significantly less ash and coal and fit the appearance of the mercury-poor samples, and the bottom sections, which were rich in ash and coal and appeared similar to the soils richer in mercury.

Mercury was found to be present in several forms, such as discrete particles or globules, possible coatings on mineral matter, and fillings in pores and/or fissures in plant debris. To date, mercury and sulfur were found to coexist in all the mercury-rich entities. These analyses further revealed that the mode of occurrence of mercury in the mercury-rich and mercury-poor cores is identical; that is, the form and chemistry of the mercury matter is independent of its concentration in the soil.

The coexistence of mercury with sulfur does not imply the presence of a mercury sulfide compound (HgS) because conditions for its formation do not appear to be present in the soils. However, in the absence of conclusive evidence (e.g., X-ray diffraction analyses of a mercury concentrate), one must exercise caution. It is well known that mercury has a strong affinity for sulfur and that sulfur is available in these soils from several sources, including sulfates in the water, organic sulfur in coal, iron sulfides, and plant matter.

One may hypothesize a possible succession of events taking place in the soil with respect to mercury. A first step in these events could be the emplacement of mercury on either mineral or plant substrate. This mode might be governed by the ability of the mercury ion to substitute for the calcium ion and may indeed be one of the reasons for the association of calcium with mercury that has been observed. In addition, sulfur may also be associated with these same substrates and act as an additional attraction for mercury attachment. Finally, the sulfate-rich waters that move through the soil could act to transport sulfur ions to coat and diffuse the mercury entities.

The most important question that still remains unanswered is whether the mercury/sulfur mixtures are present as the crystalline compound HgS.

1. INTRODUCTION

Science Applications International Corporation (SAIC) is performing a Remedial Investigation (RI) of the lower East Fork Poplar Creek (EFPC) under Radian Corporation's Technical Support Contract to the U.S. Department of Energy (DOE). A supportive investigation has been initiated that attempts to determine the form and association of mercury in the floodplain soils of EFPC within the DOE Oak Ridge Reservation (ORR) and the city of Oak Ridge. SAIC solicited the assistance of Mr. Larry Harris, who conducted earlier work on samples from the EFPC floodplain. Mr. Harris, in his capacity as a consultant to the Material Science Section of the Technical Division at the Oak Ridge K-25 Site, agreed to expand on his earlier work of the petrographic and electron microscopy of mercury-contaminated soils. The research was supported by funding from the Y-12 Environmental Restoration Program through an intercompany statement of work. This report summarizes the findings of the investigation and will be incorporated into the Administrative Record in compliance with the Comprehensive Environmental Response, Compensation, and Liability Act of 1980 (Public Law 96-510).

1.1 SITE DESCRIPTION

EFPC is a tributary to the Clinch River system. The perennial stream originates within the DOE Oak Ridge Y-12 Plant boundary and flows 23 km before meeting its confluence with the main branch of Poplar Creek. Creek flow is channelized within the plant boundary but spreads out as it enters the city of Oak Ridge. EFPC collects a significant portion of city drainage through a number of small tributaries. An associated floodplain ranges from 5 m to more than 500 m in width and covers more than 670 acres of adjoining land along its length. Various land uses are encountered along the course of EFPC. After flowing 19 km, EFPC exits the city limits and reenters the DOE reservation.

A second watershed, Bear Creek, also begins within the Y-12 Plant boundary and flows westward. Bear Creek then turns north and enters EFPC before the confluence of EFPC and Poplar Creek.

1.2 SITE HISTORY AND CONTAMINATION

Activities at the Y-12 Plant are associated with weapons component manufacturing, which includes metal finishing, plating, uranium recovery, and facility cleaning operations. Machining and milling of uranium and other metals have been common throughout the plant's history. During the 1950s and early 1960s, millions of kilograms of mercury were used in a column exchange process (Colex) for the production of lithium enriched in the ^{6}Li isotope. The Colex process began in 1950, was expanded in 1953, and ceased in 1963. During that time, large-scale releases of mercury were attributed to spills, maintenance operations, and the discharge of a dilute waste stream into the creek. Of the 109,000 kg of mercury estimated to have been lost to the environment, ~91,000 kg were from the dilute waste stream. Retention basins that have been on line at the plant boundary since 1963 contain a

portion of the mercury. An unknown amount still resides in the soil and bedrock underlying the plant and within the plant-wide drainage system.

1.3 REMEDIAL INVESTIGATION

In 1989 the entire DOE ORR was placed on the U.S. Environmental Protection Agency's National Priorities List of Superfund sites, and in the autumn of 1990, an RI/ feasibility study (FS) was initiated. Earlier studies analyzed areas of the floodplain for mercury concentration and performed miscellaneous multi-element soils analyses and biota contaminant concentrations. The earlier work provided the RI with a conceptual model of transport and distribution of metals within the floodplain. However, a more comprehensive investigation was needed to answer a number of questions in areas as diverse as site characterization, treatability studies, and human health risk assessment.

The major effort of the first phase of the RI was directed toward identifying the various affected media (i.e., groundwater, surface water, creek and tributary sediments, and floodplain soils) and defining the contaminants of concern for each medium. The medium with the highest concentrations of contaminants and the most widespread extent was determined to be the floodplain soil. Table 1.1 lists the contaminants of concern for metals derived from the first phase sampling results. Note that mercury constitutes 85% of the proportional toxicity for the various metals detected. As the primary contaminant, determining the occurrence, form, and association of mercury in the floodplain environment became crucial to the investigation. The form of mercury in the soils directly impacts the remedial treatment selection and the results of the human health baseline risk assessment.

Table 1.1. Toxicity scores based on average of contaminant concentrations in EFPC soil, ranked by toxicity[a]

Parameter	EFPC proportion detected	Average EFPC result	Measurement units	EFPC toxicity score	EFPC percent of total toxicity	EPA toxicity measure	Background proportion detected	Average background result	Background toxicity score
				Noncarcinogenic hazard scores					
Mercury	152/184	105.00	mg/kg	3.51E+05	85.04	3.00E−04	2/41	0.07	2.28E+02
Lead	145/145	37.80	mg/kg	2.70E+04	6.54	1.40E−03	41/41	12.80	9.14E+03
Chromium	146/147	30.70	mg/kg	6.14E+03	1.49	5.00E−03	41/41	24.50	4.90E+03
Cadmium	54/147	2.96	mg/kg	5.93E+03	1.43	5.00E−04	0/41	0.37	7.37E+02
Manganese	146/147	835.00	mg/kg	4.17E+03	1.01	2.00E−01	41/41	574.00	2.87E+03
Copper	141/147	72.20	mg/kg	1.95E+03	0.47	3.70E−02	41/41	10.20	2.76E+02
Nickel	136/147	27.90	mg/kg	1.39E+03	0.34	2.00E−02	41/41	19.10	9.54E+02
Silver	31/147	2.51	mg/kg	8.38E+02	0.20	3.00E−03	0/41	0.87	2.88E+02
Zinc	132/147	63.50	mg/kg	3.18E+02	0.08	2.00E−01	35/41	37.30	1.87E+02

[a]EPA toxicity measures were taken from the IRIS and HEAST tables as updated through January 1991 (EPA 1991a and 1991b). Results less than detection were set to 1/2 DL (except for radionuclides).

Source: EFPC Phase 1A Validated Data from File EFPC Soil on 17JAN92 at 10:37.

2. OBJECTIVES

Several objectives have been established for this supportive investigation. They are to

- understand in what form the mercury presently exists (i.e., elemental, sulfide, oxide, or organo-mercury compound);
- determine the size of the discrete particles containing or being composed primarily of mercury;
- observe any strong association or paragenetic relationship between mercury and fly ash, organic material, or soil structure;
- determine if the form and associations of mercury vary over the course of the floodplain; and
- note observable differences in the soil matrices or associations between areas of high and average mercury concentrations.

3. SAMPLE COLLECTION AND PREPARATION

3.1 SAMPLE COLLECTION

Twenty samples were collected March 2–4, 1992, with soil sampling equipment that extracted undisturbed cores with the dimensions of approximately 1 in. by 18 in. The sampling was conducted under the Quality Assurance Project Plan (LWA 1990a) and in accordance with Technical Procedure-303-8, "Soil Sampling with a JMC Backsaver Soil Sampler System" (LWA 1990b). The soil cores were enclosed within an acetate liner that fits inside the JMC Zero Contamination Tube. Upon extraction, vinyl caps were placed on the ends of the acetate tubes, and the cores were delivered to the investigators at the Oak Ridge K-25 Site with full chain-of-custody documentation. Sections of the core of particular interest were marked, and all samples prepared for the investigation were taken from these sections. Areas of interest were stratigraphic horizons displaying unusual color and known to contain high levels of mercury or sections containing roots, ash, or high levels of organic materials.

The locations selected for the samples were identified throughout the EFPC floodplain in varying segments of the creek, but all were from areas of high levels of mercury to maximize the success of the study. It was recognized that this would bias the selection and not represent a random sampling of the floodplain soils. The specific locations were sampled earlier during the second phase of the RI, and the soils were analyzed by neutron activation analysis for eight analytes including mercury. At the time of the site selection, only preliminary analytical data existed; these data have since been revised. Some samples were found to contain less mercury than originally anticipated. Table 3.1 lists the sample number with the concentration of mercury and a geographic description of the location of the sample. The northing and easting coordinates are referenced to the Oak Ridge Administrative Coordinate Grid.

3.2 SAMPLE PREPARATION

Samples were prepared from the soil cores by cutting them into 2-in. long segments while they were retained in the acetate tubing. In this manner sixty 2-in. segments were generated for analyses. The choice of 2-in.-long segments for these samples was dictated by the maximum available sample size that could be used in the scanning electron microscope. The tubes were split lengthwise to allow moisture to escape while the segments were being vacuum dried. These 2-in.-long core segments were next mounted in araldite epoxy and after curing were split down the middle so that the center of the cores was exposed for analyses. Subsequently, these samples were ground and polished.

Table 3.1. Location and mercury concentrations of samples for mercury soils study

Sample number	Sample ID	Coordinates		Mercury (ppm)	Location
		Northing	Easting		
1	FE 3210016	32155.8	63747.4	1170	Immediately downstream of Lake Reality at Y-12
2	FW 3310812	33140.2	63077.0	135	Immediately upstream of the NO study area
3	FE 3340213	33468.1	63100.0	1320	Within the braided stream of the NO study area
4	FW 3370616	33806.2	62369.5	20	Same as previous sample
5	FE 3671214	36749.0	61097.0	443	In the backwash areas where EFPC crosses Tulsa Road
6	FN 5470016	39835.6	54788.2	121	Within the BR study area of high mercury concentrations
7	FS 5410010	40008.3	54132.1	387	Within BR study area of high mercury concentrations
8	FS 5380015	40201.8	53804.0	470	Within BR study area, south side of stream
9	FN 5380015	40230.3	53804.0	589	Same as previous sample but on north side of stream
10	FN 5340011	40249.5	53475.9	898	Within BR study area

Table 3.1 (continued)

Sample number	Sample ID	Coordinates		Mercury (ppm)	Location
		Northing	Easting		
11	FS 5240019	40180.9	52491.7	293	Within WA study area
12	FN 5210016	40187.3	52163.7	352	Within WA study area
13	FN 5150018	40202.6	51507.4	298	Within WA study area immediately behind convenience store
14	FN 4420010	41497.0	44289.6	125	At sewage treatment plant
15	FS 3870012	40418.5	38712.2	95	At golf course across from Grand Cove development
16	FN 3770010	40221.1	37727.9	345	West edge of Grand Cove development
17	FS 3740017	40391.6	37400.1	191	Golf course across from west edge of Grand Cove development
18	FN 2820016	40576.0	28213.0	99	On DOE reservation near LB sampling station
19	FS 2230015	38878.9	22308.0	186	Immediately downstream of the confluence of Bear Creek with EFPC
20	FS 1960011	40701.4	19682.7	216	Slightly upstream of the confluence of EFPC with Poplar Creek

4. OPTICAL MICROSCOPY

4.1 METHODS OF INVESTIGATION

Characterization of all the polished core samples by optical microscopy was performed with a Zeiss Ultraphot microscope. Reflected light was used exclusively in these analyses, [i.e., the light is directed incident to the sample surface and is subsequently reflected back through the viewing assembly (objective lens and eye piece) to form an image of the surface]. The microscope was configured to do polarized and ultraviolet light (fluorescence) microscopy. The former technique employs a polarizing prism or polaroid (termed polarizer) for the incident light to pass through and a second polarizing prism or polaroid mounted in the viewing assembly (termed an analyzer) for the reflected light to pass through. For normal use, the vibration directions (i.e., the direction that light is constrained by the polaroid) in the polarizer and analyzer are 90° to each other. Conversely, ultraviolet light (fluorescence) microscopy makes use of a mercury lamp using exciter filters for selecting a specific wavelength from the mercury emission spectrum. Barrier filters are placed in the viewing assembly and serve to isolate the visible wavelength that is excited from the fluorescence of a specific material.

Polarized light microscopy is an important technique for identifying minerals and pyrolized organic material such as coke, which is a product of coal combustion. Typical soils in this region of the country consist of alumino-silicate minerals (various clays), quartz (SiO_2), and plant matter. A consequence of the present study has been the recognition of considerable quantities of ash and coal in these soil samples. The amounts of these materials in these soil samples vary from an estimated maximum of between 20 and 30 surface area percent to a low of perhaps a few surface area percent. These observations will be discussed more fully as each core sample is described.

Ash is formed during rapid heating and combustion of coal and included mineral matter. The resulting product can be divided into classes depending upon chemistry and shape. Hollow spheres of alumino-silicate glasses are termed "cenospheres" and are sometimes found packed with spheres or particles. Similarly, more irregular spheres formed from primarily carbonaceous material consist of coke. The third important ash constituent is the ferrosphere composed of iron oxides and formed primarily from the oxidation of pyrite clusters called "framboids" (French for raspberry, alluding to the fact that these clusters have a raspberry-like shape). Finally, there are the fragments of combusted coal that are present in the ash; these fragments are usually coke and retain the remnant structure of the coal.

Figure 4.1 serves as an aid to future discussions in this section by illustrating the appearances of the various constituents described herein. Figure 4.1(a) contains cross-sections of coke, cenospheres, and some associated minerals. The coke appears as an elongated, thin, porous-walled bright material; the grey regions within are epoxy fill. Above the coke is a cenosphere (identified in the micrograph) showing an irregular outline; this material is much darker than the coke and contains some cavities at the surface (black areas). Immediately above and continuous to the cenosphere is a region containing small (<20 μm) SiO_2 grains intermixed with clays. Figure 4.1(b) and (c) are views of ferrospheres and the cross-section of a root, respectively, whereas Fig. 4.1(d) shows a larger (>500 μm) uncombusted coal fragment.

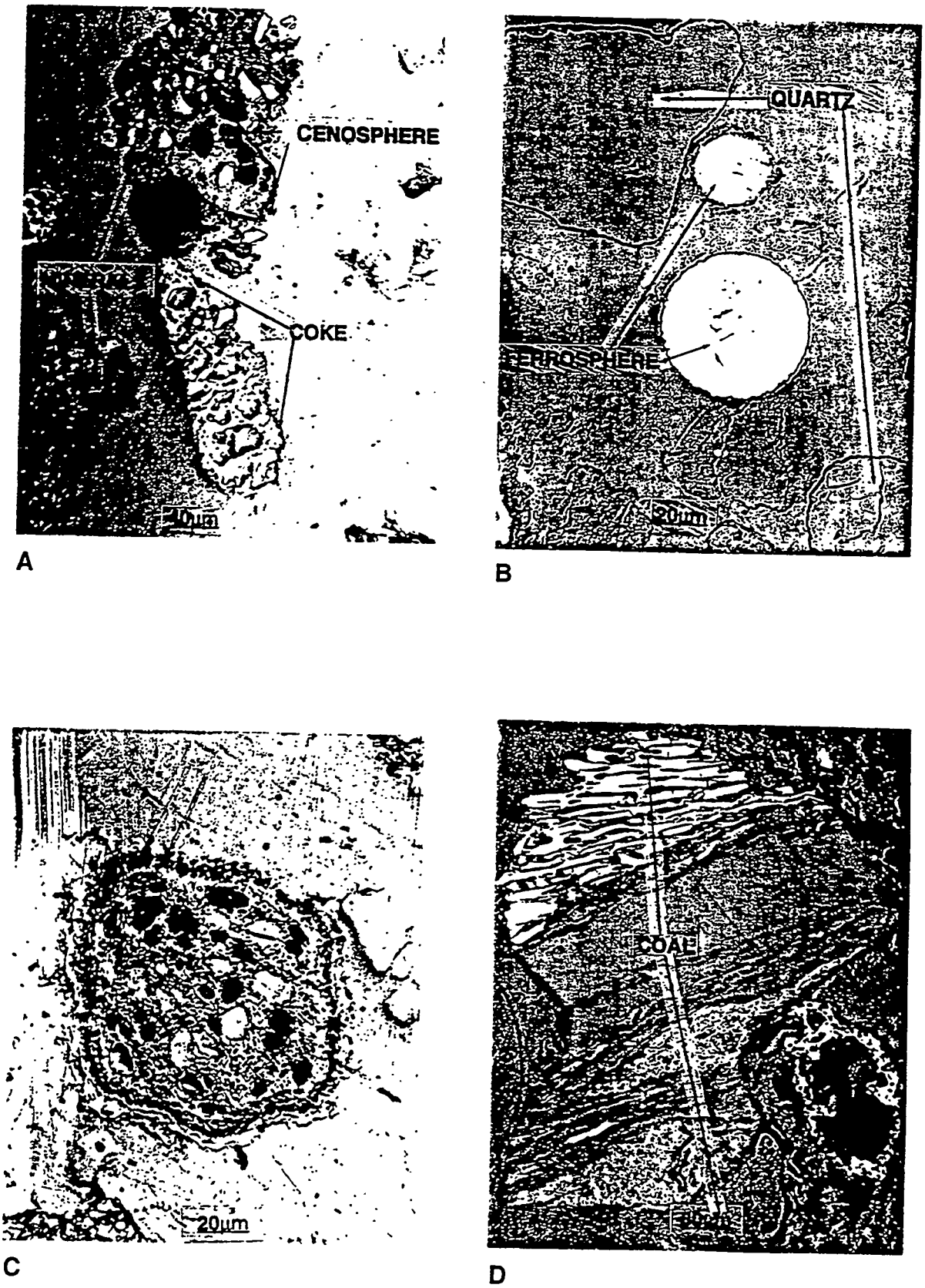


Fig. 4.1. An optical micrograph of ash particles: (a) Coke, cenospheres, and mineral matter; (b) ferrospheres; (c) cross-section of a root; and (d) uncombusted coal fragment.

In addition to the ash, plant matter, and expected mineral assemblages, uncombusted coal fragments are also observed in these soil samples. In several instances, ultraviolet light (fluorescent) microscopy was used to confirm the nature of these coal fragments where they contained hydrogen-rich constituents (named exinite and derived in part from ancient spores) that give a distinctive look in ultraviolet-light fluorescence. This effect is illustrated in Fig. 4.2, which shows the bright stringers of hydrogen-rich material going diagonally across the optical micrograph and confirms the presence of uncombusted coal.

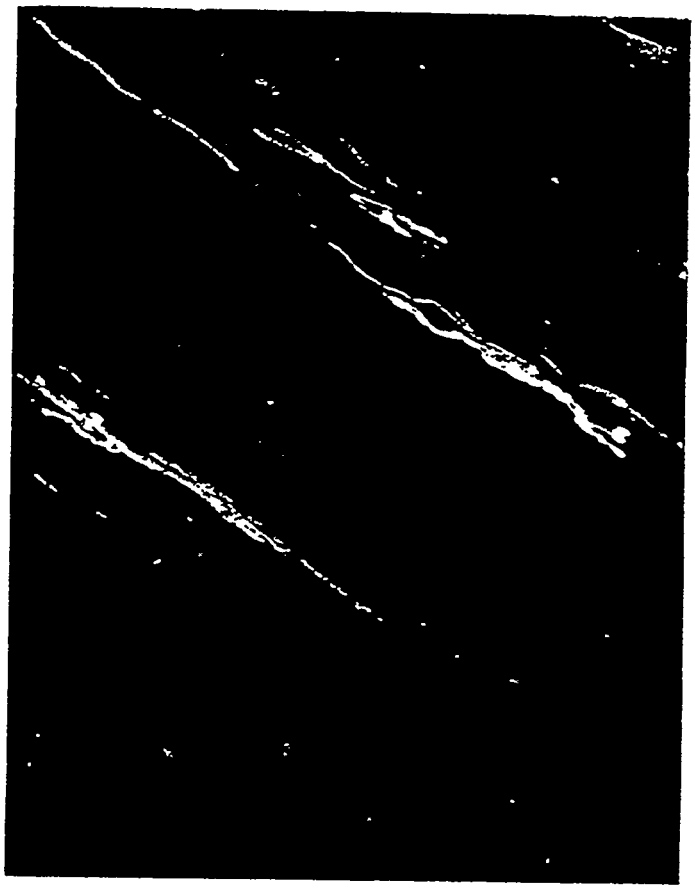


Fig. 4.2. Ultraviolet light fluoresced hydrogen-rich constituent (extinite, which is a coal entity) in uncombusted coal.

Though the optical microscope was not used to identify mercury-bearing matter *per se* (it lacks the capability to do the necessary microchemistry), it nevertheless can help to distinguish textural and content variations between and within soil cores. Because knowledge about the mercury content of the cores was known from prior chemical analyses, it was theorized that optical analyses might correlate mercury content with features identified in the optical microscope.

4.2 OBSERVATIONS

The following is a discussion of the petrographic observations made for each core. The core samples are ordered by their geographic proximity to the Y-12 Plant and in general contain higher concentrations of mercury the closer they were to the plant.

1. **Core Sample FE 32100016 (1170 ppm Hg)**

   Core sample FE 3210016 consists of two sections. In general, there appears to be more ash in the upper section of the core and more coal in the lower portion of the core. This sample contains large amounts of ash and coal fragments. (Note that these are estimates and not quantitative data because no point counts were performed on any of the sample analyses.) Quartz, clays, and organic material can be seen throughout. The ash consists of oxidized pyrite, coke, and cenospheres. The size range of these constituents is nearly identical to that observed in FE 3340213.

2. **Core Sample FW 3310812 (135 ppm Hg)**

   This sample was split into three sections, and all are very similar in appearance. The sections are highly porous, therefore reducing the amount of surface for analyses. The amounts of ash and coal are relatively small (approximately 5 surface area percent) in this sample. Much less ferrospheres, coke, and cenospheres are seen. The major constituent appears to be mineral grains (probably predominantly quartz) that range in size from under 10 μm to greater than 1000 μm.

3. **Core Sample FE 3340213 (1320 ppm Hg)**

   Three specimens were obtained from this core sample. This core differed from the others in that the soil remained soft and had to be mixed with epoxy and then set as powder mounts. All specimens appear to be nearly identical, with no observable differences from the top to the bottom of the core. Typical fields of observation consist of ash, coal, quartz, clays, and plant debris. The ash makes up a considerable quantity of the soil (perhaps up to 20%) and consists of oxidized pyrite (FeS_2), pyrolized coal, and combusted inorganic materials. This sample seems rich in iron oxide and reflects the numerous oxidized pyrite structures that range in size from 10 to 60 μm with some very small crystals (2 to 6 μm). Interspersed with iron oxides are coke and cenospheres. The former is from combusted organic coal matter; the latter is from the mineral matter in coal as discussed earlier.

   Closely associated with the ash are fragments of uncombusted coal ranging in size from 100 to 1000 μm. In many of these coal fragments, the macerals (organic coal constituents) can be seen. In several instances, the exinitic macerals were identified and still fluoresced (see Fig. 4.2) when excited by ultraviolet light. Finally, some quartz grains and fine materials were identified along with plant parts.

4. **Core Sample FW 3370616 (20 ppm Hg)**

   Cut into three sections, this sample consists of a nearly uniform fine matrix. Grains of quartz (55 μm diam) and plant matter are dispersed throughout the sample. Very little ash or coal fragments are observed, although some ferrospheres can be seen.

5. **Core Sample FE 3671214 (443 pm Hg)**

Consisting of four sections, the upper section appears to contain several large root segments while the lower three sections contain large amounts of ash and coal fragments. Coke and converted pyrite are plentiful (approximately 10 surface area percent) as is plant matter. Quartz grains also are present in substantial amounts. It was noted that considerable mineralization occurred in the vesicles (open pores) of the plant tissues.

6. **Core Sample FN 5470016 (121 ppm Hg)**

All three sections of this sample appear to be alike, consisting of fines as defined in our samples (i.e., composed of clays, silt, and plant matter). An increase in ash particles was observed over the similar mercury-poor cores. Plant matter is abundant. The lower section is richer in ash and coal though very few ferrosphere are present. Several large coal fragments greater than 200 μm are seen in this section.

7. **Core Sample FS 5410010 (387 ppm Hg)**

This sample was cut into five sections. The upper two sections appear to differ from the lower in that they contain much less ash and ferrospheres but have many large coal and quartz fragments (>2000 μm). Also found in the upper sections is extensive plant matter with mineralization in the pores. Meanwhile, the lower three sections appear more like some of the mercury-rich samples in that they contain considerable ash (coke and cenospheres) and ferrospheres (perhaps up to ~15%). Typically, the uncombusted coal fragments are smaller and of less quantity than in the upper sections. As in all soil samples, there is a matrix of a fine material.

8. **Core Sample FS 5380015 (470 ppm Hg)**

Cut into two sections, this sample contains a moderate amount of ash and coal particles (~10%) in the upper section. Ferrospheres are also observed along with some large plant sections greater than 2000 μm across. The lower section differs from the upper section in that the amount of ferrospheres seems much less. The ash in both sections is composed of coke and cenospheres. Quartz and uncombusted coal fragments up to 1000 μm are fairly common. The matrix consists of fine material composed of minerals and organic matter (too fine to identify). Much of the plant parts have their vesicles filled with fine mineral matter.

9. **Core Sample FN 5380015 (589 ppm Hg)**

This sample was split into four sections. The upper two sections vary from the lower two in that there is less ash and coal in them; the lower sections are dominated by ash, coal, and oxidized pyrite. Also seen are some larger coal fragments (>500 μm).

10. **Core Sample FN 5340011 (898 ppm Hg)**

This sample was split into three sections. The upper two sections of this sample are alike. They contain ash (~10%) but differ from sample FE 3210016 in that almost no large coal fragments are present. The other constituents are fine quartz particles, clays, and root systems. Minerals fill the open pores of the plant material. There is an abundance of plant matter, much of which is larger than observed in previous core samples, and some of the cross-sections are several millimeters across. The lower section contains

lesser amounts of ash and very few coal fragments. Several rock fragments were identified by their appearance in polarized light.

11. Core Sample FS 5240019 (293 ppm Hg)

Cut into three sections, this primarily fine-grained material is largely composed of clays, silt, and macerated plant material. Coal and ash particles are dispersed throughout this core. All three sections appear to be the same, containing much less ash and coal.

12. Core Sample FN 5210016 (352 ppm Hg)

Cut into three sections, this sample is nearly identical to FN 3770010 with the exception that it contains more ash (such as coke and cenospheres) but very few ferrospheres. Large sections of plant matter (probably roots) as well as quartz grains dominate the sample.

13. Core Sample FN 5150018 (298 ppm Hg)

Cut into three sections, a matrix of fine unidentified material (probably silt, clays, and plant debris) is observed. Large quartz grains (>300 μm) are dominant. Some ash, mostly coke, is seen along with a few ferrospheres. The middle section appears to be much richer in ash and ferrospheres. Also seen throughout this sample are several large coal pieces (>1 mm long).

14. Core Sample FN 4420010 (125 ppm Hg)

The three sections of this sample contain fine-grained matrix material similar to previous samples, but these appear to have more coke (ash) particles. The lower section of the core contains a large cross-section of roots with some pores filled with optically active material, probably minerals. The largest of these cross-sections is 6 mm diam, with many smaller sections throughout. In general, this core seems to contain more plant matter than do the other cores examined.

15. Core Sample FS 3870012 (97 ppm Hg)

Cut into two sections, this sample contains very few ash and ferrosphere particles. The matrix consists of fine material probably similar to previous samples in composition. Quartz grains are abundant but typically less than 100 μm in length. It appears that there is much less plant material in this sample.

16. Core Sample FN 3770010 (345 ppm Hg)

All three sections appear to be nearly identical. Little ash and almost no uncombusted coal fragments can be seen. Plant matter (root sections) and quartz grains (some >2000 μm) are the dominant materials. Several large root cross-sections (some >1 mm across) that contain mineralization in the open pores can be seen. Numerous large, well-rounded quartz grains (>1 mm) (inferring transport) are seen throughout the sample, whereas the matrix consists of smaller, more angular quartz grains mixed with unidentified fines (probably clays) and plant debris.

17. Core Sample FS 3740017 (191 ppm Hg)

Cut into three sections, this sample contains fine material as a matrix surrounding larger quartz grains. Few ash or coal particles can be seen. Plant matter also appears to be scarce.

18. Core Sample FN 2820016 (99 ppm Hg)

Cut into two sections, this sample contains almost no ash or coal. It primarily contains quartz ranging from fines to particles greater than 1000 μm. Several large metallic fragments (highly reflectant opaque material, metallic in appearance) are seen. Mineralization in vesicles of plant matter is observed for root cross-sections.

19. Core Sample FS 2230015 (186 ppm Hg)

This sample, cut into three sections, is identical to FS 1960011. Very small amounts of ash or coal can be seen, and there are not too many recognizable organic fragments.

20. Core Sample FS 1960011 (216 ppm Hg)

The three sections of this predominantly fine-grained material are similar to those of FS5240019 in that they consist of clays, silt, and plant matter. Some large plant cross-sections containing mineralization in their pores can be observed. Several larger quartz grains (>100 μm) can be seen, but very few ash or ferrosphere particles are present.

5. SCANNING ELECTRON MICROSCOPY

5.1 METHODS OF INVESTIGATION

This section pertains to the imaging, detection, and microanalysis of mercury-bearing particles in the soil samples. The sample preparation and the methodology employed in their analyses are described. Although optical microscopy was useful in illuminating certain relationships between mercury and ash in these soils, it nevertheless cannot be used to do elemental analyses nor to resolve very small particles, for example, those less than 2 μm. Electron microscopy, which utilizes the very short wavelengths of electrons, can resolve particles in the submicrometer range down to less than 0.1 μm in a very rapid manner. In addition, with electron microscopy there are methods for performing microanalysis of any observed feature.

Like the optical microscope, the electron microscope generates images of the regions being surveyed that can be directly photographed to yield a photomicrograph. The electron microscope is also equipped with accessory analytical tools that are capable of doing elemental analyses of regions or specific particles down to less than 0.02 μm as well as plotting of elemental (dot) maps that are superimposed over features viewed on the cathode ray tube (CRT). Perhaps one of the most important functions of the instruments is their ability to be utilized for conducting quantitative multielement analyses. Using these analytical and survey methods makes electron microscopy an ideal technique to search for trace elements such as mercury dispersed in a matrix of alumino-silicates (typical soil mineralogy) and plant debris.

A representative sample from each core was selected to undergo microanalysis. Because the soil samples are composed of nonconducting materials, it was necessary to coat their surfaces with a thin film of conducting material to prevent electrical charging of the sample surface and beam instability. The coating used in these analyses was carbon that was deposited on the sample surfaces in a vacuum coater.

For completeness, a brief description of the instruments used in these analyses and their operation is included. The microchemistry of these soil samples was performed almost exclusively with a JEOL-733 superprobe and a JEOL-840 scanning electron microscope. The superprobe may be considered a dedicated microanalytical microscope because it has a special nontilting and nonrotatable stage, thus ensuring accurate registry and constancy between the beam axis and specimen surface. Both systems use high energy, small diameter (focused) electron beams that interact with the specimen surface. The microscopes require a vacuum (10^{-5} TORR) to prevent the beam from interacting with gas molecules and beam electrons, thereby averting drifts in beam current.

The impingement of the electron beam on the sample surface produces secondary and backscattered electrons. The secondary electrons are derived from the electrons that lose energy from the interaction of the incident beam electrons with the electrons in the specimen. Therefore, secondary electrons have relatively low energies that range from a few electron volts to an arbitrary upper limit of 50 eV. The mean free path length in a typical material for secondary electron is about 0.001 μm. Therefore, only those electrons generated at shallow depths escape the specimen surface. These secondary electron images (SEIs) provide surface topological information at the highest resolution and can be directly viewed on a CRT.

In contrast, backscattered electrons contain the original beam electrons that have been elastically (no energy loss) scattered back through the specimen surface. The interaction of those electrons is primarily with the specimen's atomic nuclei where there is little energy transferred from the electrons. Backscattered electrons are classified as having high energies (>50 eV) and an increase in yield with increasing average atomic number of the specimen. Thus backscattered electron images (BEI) show significant differences in the appearance of entities composed of high atomic number elements versus features composed of low atomic numbered elements. Like secondary electrons, backscattered electrons also can be imaged on a CRT to yield BEI.

In addition to the secondary and backscattered electrons, the interaction of the electron beam with the sample generates X-rays. When an electron in an inner atomic shell of an element is ejected by an incident electron, a transition from an outer energy level (shell) to the vacancy may occur with concomitant emission of an X-ray photon, the energy being determined by the initial and final electron energy levels. The letters K, L, M, . . . (where K is the innermost shell followed by L, M, . . .) are used in the notations for listing X-ray lines and refer to the shell initially ionized. These characteristic X-rays emitted under the effect of an electron beam yield data pertaining to the nature and number of elements present in the volume excited by the primary beam.

Analyses of the X-rays can be performed in the following two ways: with an energy dispersive spectrometer (EDS) and/or with a wavelength dispersive spectrometer (WDS). The former method uses a lithium-drifted silicon crystal detector in which the X-ray photon is converted into an electrical pulse. A voltage applied to the crystal collects the charges that are proportional to the energies of the X-rays. This pulse is further amplified and converted and then passed to a multichannel analyzer where the pulses are sorted out according to their energies for presentation on a CRT or for plotting on a chart. Typical resolution of EDS is 150 eV, which means that an overlap of lines exists such as for mercury $M\alpha_1$ = 2.195 keV, $M\alpha_2$ = 2.283 keV, and sulfur $K\alpha$ = 2.308 keV, where the difference is approximately 25 eV.

In wavelength dispersive spectrometry, a crystal of a known atomic spacing separates X-rays according to Bragg's law $n\lambda = 2d \sin \theta$ [where n is an integer, d is an interplanar spacing of the analyzing crystal, λ is the wavelength of the incident X-radiation, and θ (theta) is half the angle between the incident and diffracted rays] so that for a given angle theta (θ) X-rays of a specific wavelength are detected. Several different crystals are required to cover the entire range of wavelengths. In the systems employed in this study, there are three WDSs mounted. X-rays obeying Bragg's law for a specific analyzing crystal are detected by a proportional counter. The X-rays thus being counted are treated in a manner similar to that reported for EDS. The resolution achieved with WDS is 10 eV or less and therefore is the technique of choice for discriminating between mercury and sulfur. In addition, WDS has a higher counting rate, which means greater sensitivity and improved statistics.

5.2 OBSERVATIONS AND DISCUSSIONS

Mercury in core samples was observed to be present in several modes, such as discrete particles or globules, possible coating on mineral surfaces, and fillings in pore openings or fissures in plant matter. Detection of mercury in those core samples containing under 400 ppm mercury was more difficult because searching for mercury at lower concentrations was more time

consuming. However, the only difference in mercury occurrence regarding the soil cores appears to be in the concentrations but not in the mode or microchemistry.

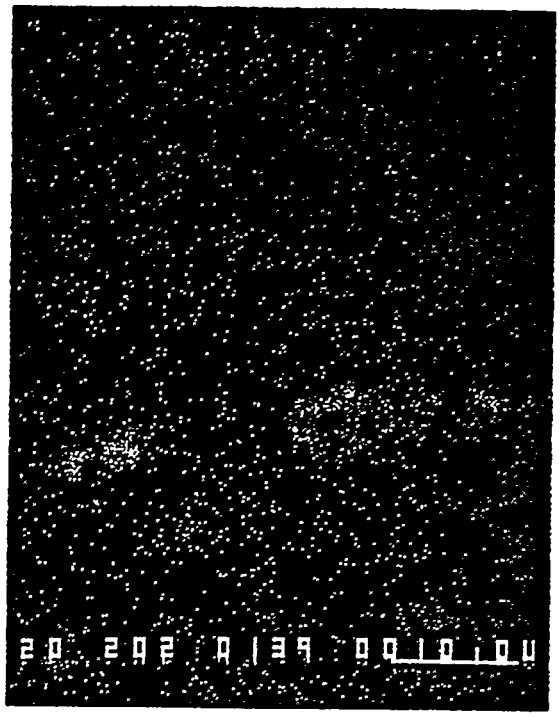
Mercury-rich micrometer-sized particles are a common mode of occurrence for mercury in these soil samples. The particles are usually associated with fragments of plant matter and/or minerals. To date, no mercury-rich particles have been observed in association with ash even though the cenospheres contain an abundance of open pores and cracks. Figure 5.1 is an SEI photomicrograph that contains several mercury-bearing particles (shown by the arrows) along with such minerals as quartz (Q) and clays (C) as well as other unidentified matter. The BEI of this same area is shown in Fig. 5.2, where the signals from the low atomic numbered elements have been suppressed to accent the mercury-bearing particles that range in size from <1 μm to 5 μm.

Fig. 5.1. SEI of mercury-rich particles (shown by arrows) with quartz (Q) and clay (C).

X-ray dot maps for mercury Mα, sulfur Kα, and calcium Kα are shown in Fig. 5.3(a) through (c). The superimposition of mercury and sulfur on the particle sites while calcium appears unrelated can be observed from these maps. Additional inspection of the sulfur map shows that

Fig. 5.2. BEI of same area shown in Fig. 5.1 where the bright spots are the mercury-rich particles.

the adhered-to fragment is rich in sulfur. It should be noted that several observations were made of sulfur-rich matter that did not contain any of the elements detectable with EDS [i.e., elements of atomic number 11 (sodium) or greater]. From these observations, it was assumed that the fragment was either sulfur or an organic sulfur compound; the latter choice appears more likely because it is well known that coal contains organic sulfur compounds and that coal is a constituent of these soils.



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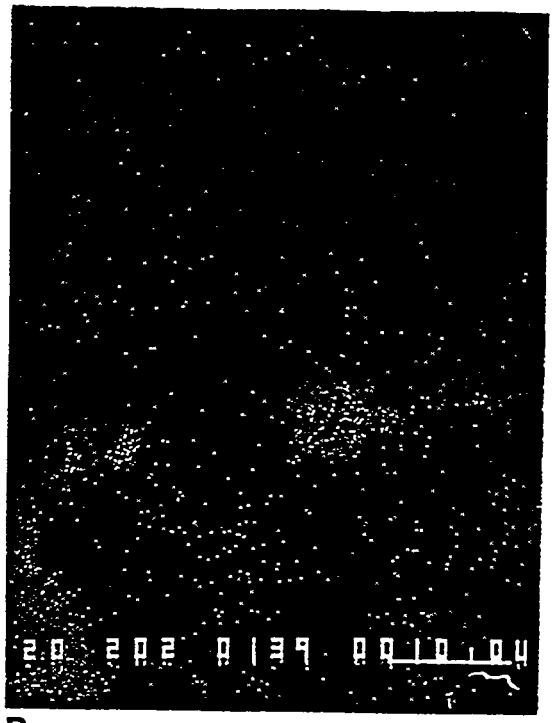

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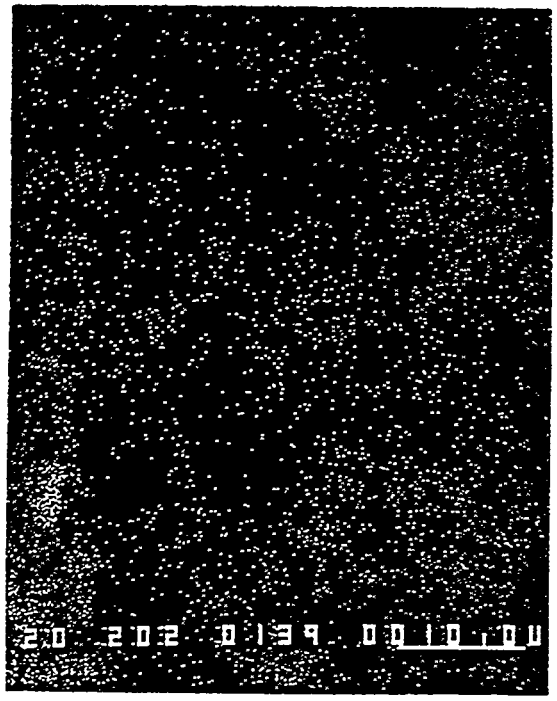


C

Fig. 5.3. Elemental X-ray maps for (a) mercury, (b) sulfur, and (c) calcium.

The relationship of mercury and sulfur in all the mercury-rich particles observed to date is further illustrated in Fig. 5.4, which is an SEI of an approximate 1-μm particle atop a probable clay mineral. Figure 5.5(a) and (b) are X-ray maps for mercury and sulfur that show the superimposition of those elements. The diffusion of the mercury and sulfur X-rays appears to be indicative of a thin coating of those elements on the substrate. The energy dispersion spectrum for the region surrounding the particle clearly shows aluminum and silicon to be the primary elements present, with much lesser amounts of calcium, potassium, and iron supporting the thesis that the substrate is composed of clays.

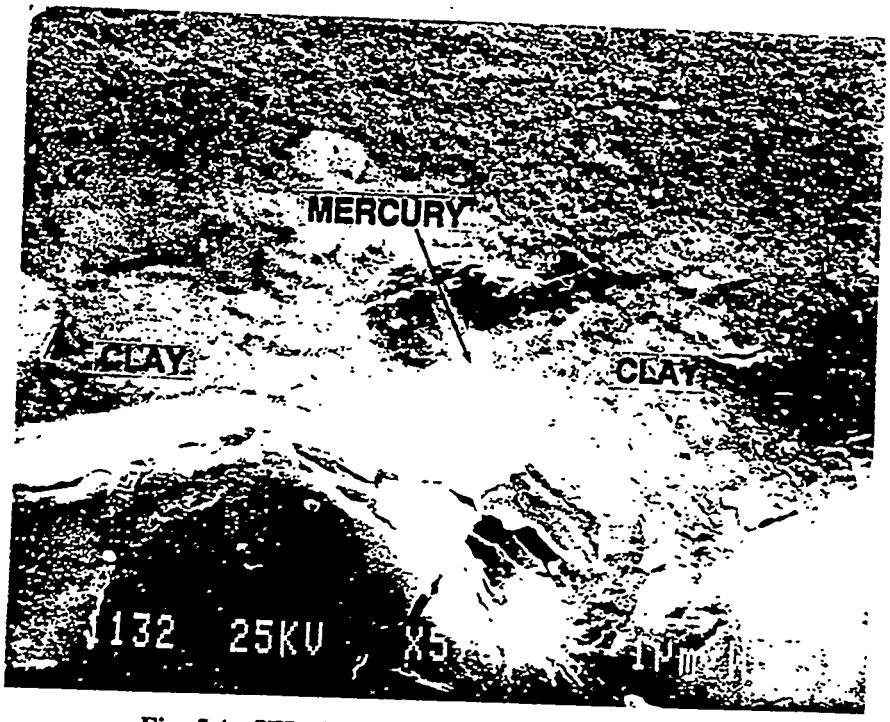
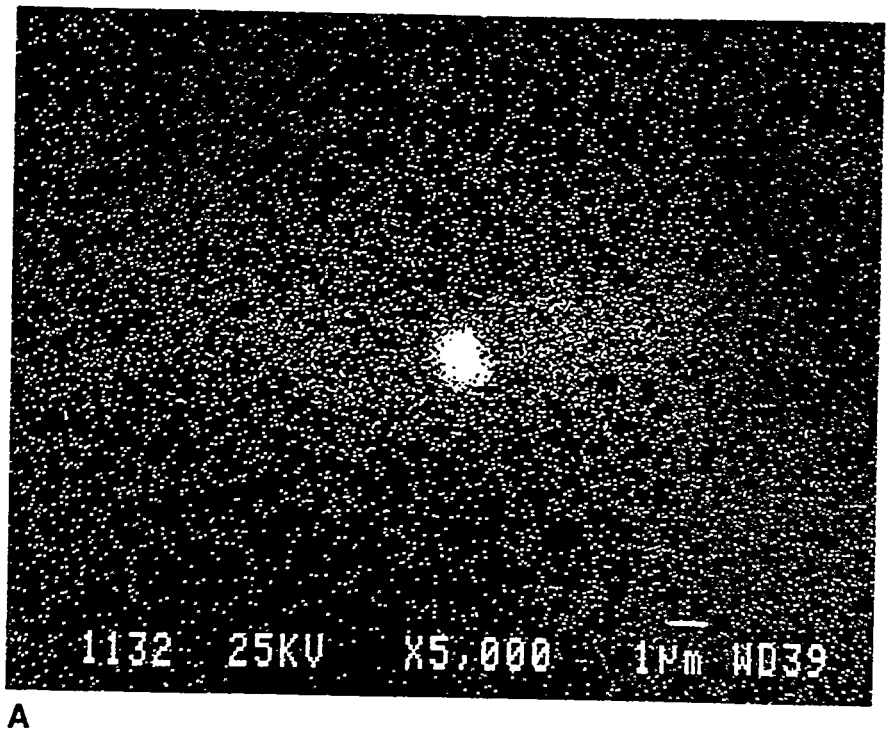


Fig. 5.4. SEI of a mercury-rich particle atop clay.

Numerous additional instances of mercury and sulfur impositions on the particles throughout these core samples were observed and recorded. An attempt to establish whether possible relationships exist between these particles and other associated elements identified either in the substrates or in adjacent matter indicated a need for quantitative multi-element analyses. These analyses were performed on mercury-rich particles and their immediate surroundings using the JEOL electron probe microanalyzer. Data reduction and corrections for atomic number, absorption, and fluorescence (ZAF) were applied to raw data to yield atomic concentrations. Figure 5.6 is a BEI of one such particle which appears as a very bright spot (~1 μm long) located near the center of the micrograph. Microanalyses of the particle and surrounding matter were performed for the following elements: aluminum, calcium, iron, mercury, potassium, silicon, sulfur, and titanium. Table 5.1 lists these elements and their concentrations with respect to the particle (A and B) and its matrix (Nos. 1, 2, 3, 4). Substantial amounts of mercury, sulfur, and calcium were revealed in



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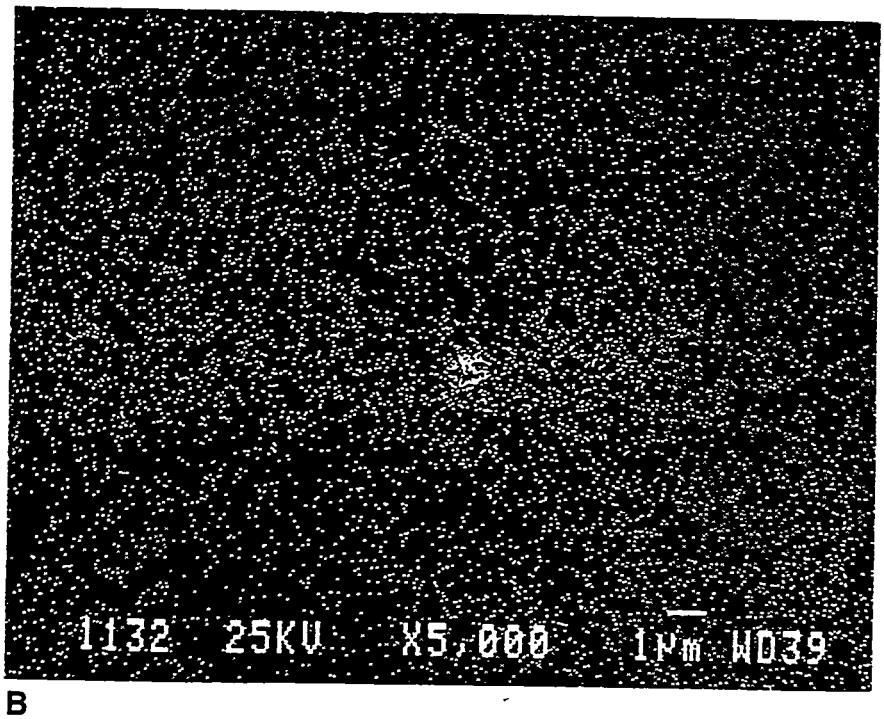


B

Fig. 5.5. Elemental X-ray maps for (a) mercury and (b) sulfur from the feature presented in Fig. 5.4.

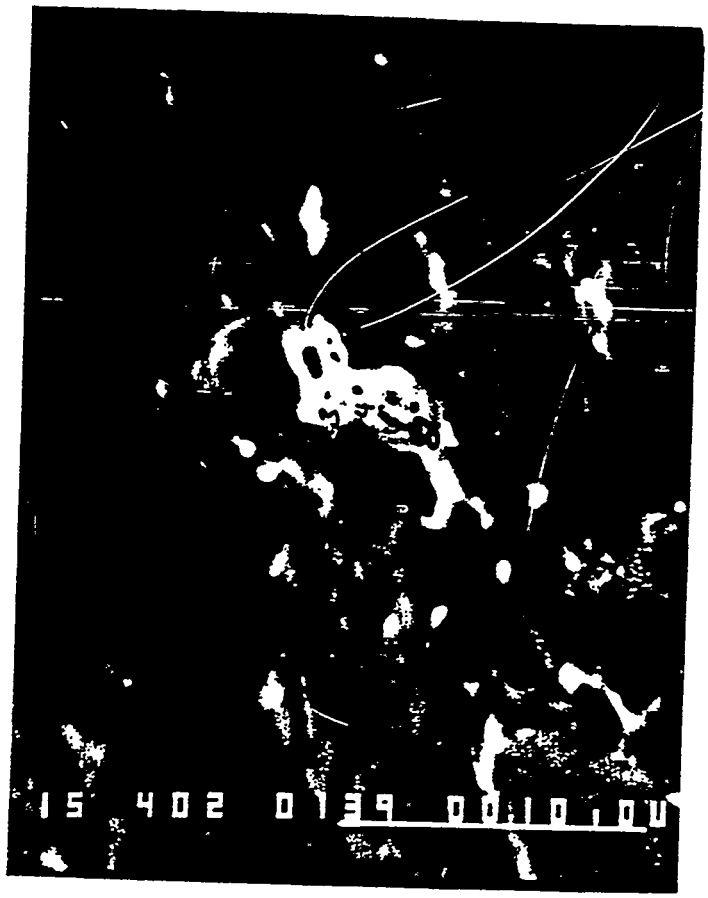


Fig. 5.6. BEI of mercury-rich particles in center of the field.

the particle probe. The presence of calcium where mercury and sulfur superimpose is not unique in these soil samples, but it is relatively common as will be demonstrated by additional data. Present thinking is that some calcium may be due to fragments of plant matter because it is known that calcium is an invariable constituent of all plants, which contain it both as a structural constituent and as a physiological ion. In addition, sulfur is also one of the essential plant nutrients.

Analyses for a region identified by spots 1 and 2 in Table 5.1, located about 5 μm from the particle and lying in the adjacent grey, kidney-shaped feature just below the particle, show that the region contains no mercury, sulfur, calcium, or iron but is rich in aluminum, silicon, and potassium, which suggests a mixed clay mineral. Closer to the particle, data from spots 3 and 4 show some mercury, sulfur, and calcium but indicate that this region is still primarily rich in the clay elements. It may be that the mercury signal is due to some overlap with the mercury-rich particle because the X-ray source volume is larger than the probe spot. This means that small particles (<1 μm) can display X-ray signals that originate from the matrix.

Probe analyses of a second mercury-rich region (A, B, and C in Table 5.2) and its contiguous areas (Nos. 1, 2, and 3 in Table 5.2) shown in Fig. 5.7 resulted in the atomic concentration data listed in Table 5.2. The bright, wing-shaped region consists of a large grain (~5 μm) and several smaller associated particles. X-ray maps of mercury Mα and sulfur Kα for the region are shown in Fig. 5.8(a) and (b), respectively, and further serve to illustrate the superimposition of these elements in the soil samples. The relative ratios of sulfur to mercury in the particles are 0.91, 0.89,

Table 5.1. Atomic concentrations for a mercury-rich particle and matrix (shown in Fig. 5.6)

Elements	A[a]	B[a]	No. 1[b]	No. 2[b]	No. 3[b]	No. 4[b]
Aluminum	9.41	7.26	21.94	20.42	28.34	28.62
Silicon	5.47	5.25	71.29	73.26	36.28	41.17
Sulfur	21.71	25.34	—	—	5.71	4.48
Iron	1.38	1.40	—	—	5.43	6.47
Mercury	23.42	26.61	—	—	2.92	1.67
Calcium	35.27	32.43	—	—	13.58	10.75
Titanium	0.40	0.24	—	—	0.47	0.20
Potassium	2.95	1.47	6.49	6.06	7.27	6.63

[a]A and B are two locations upon the bright spot in the center of Fig. 5.6.
[b]Numbers 1, 2, 3, and 4 are four locations upon the matrix adjacent to the bright spot shown in Fig. 5.6.

Table 5.2. Atomic concentrations for a mercury-rich particle and matrix (shown in Fig. 5.7)

Elements	A[a]	B[a]	C[a]	No. 1[b]	No. 2[b]	No. 3[b]
Aluminum	24.46	24.19	25.95	27.51	28.18	31.48
Silicon	34.94	37.50	35.62	53.93	46.88	50.48
Sulfur	11.76	10.79	8.69	2.18	4.99	1.18
Iron	5.22	4.69	5.25	6.77	6.20	6.14
Mercury	12.82	12.13	14.48	0.66	0.06	0.00
Calcium	4.29	4.34	3.87	1.43	2.38	1.26
Titanium	0.53	0.24	0.29	0.76	2.75	0.63
Potassium	5.98	6.11	5.85	6.76	7.55	8.39

[a]A, B, and C are locations upon the mercury-rich particle shown in Fig. 5.7.
[b]Numbers 1, 2, and 3 are locations in contiguous areas adjacent to the mercury-rich particle shown in Fig. 5.7.

Fig. 5.7. BEI of a wing-shaped mercury-rich particle (see arrows).

and 0.60, which may be compared with a ratio value of 0.98 computed for the mercury sulfur standard (the mineral cinnabar HgS). Although the sulfur-to-mercury ratios of 0.91 and 0.89 approach a value of 0.98 computed from crystalline mercury sulfide (HgS, cinnabar), this does not confirm that mercury sulfide has been formed in the soil. In the absence of X-ray diffraction data, the nature of the mercury sulfur mixture cannot be confirmed. The synthesis of mercury sulfide is normally carried out at mildly elevated temperatures that are not present in soils. The presence of regions with differing sulfur-to-mercury ratios, for example, the 0.60 ratio, may simply be due to the dynamics of the soil system and/or the availability of sulfur. Interestingly, in this specific area, calcium is not significant nor does it correlate with mercury and sulfur.

Figure 5.9 shows an elongated structure (boxed area) that contains mercury and sulfur as shown by their respective X-ray maps in Figs. 5.10(a) and (b). The structure appears to have form, perhaps even crystal faces, and, indeed, was thought to possibly be a crystal. However, it would be unexpected for mercury to react with sulfur at ambient conditions to produce a crystal about 80 μm long. Indeed, it appears more likely that the mercury accumulated on the surface of a crystal as a coating. To explore that thesis, a simple series of experiments was devised to increase the probe current, resulting in a concomitant heat increase. In this way it was thought that the mercury/sulfur mixture would evaporate (especially in a vacuum) and reveal the nature of the substrate, assuming it was a coating. Figure 5.11(a), (b), and (c) show EDS spectra for as-received material, after an increase in probe current, and after an additional increase in probe current,

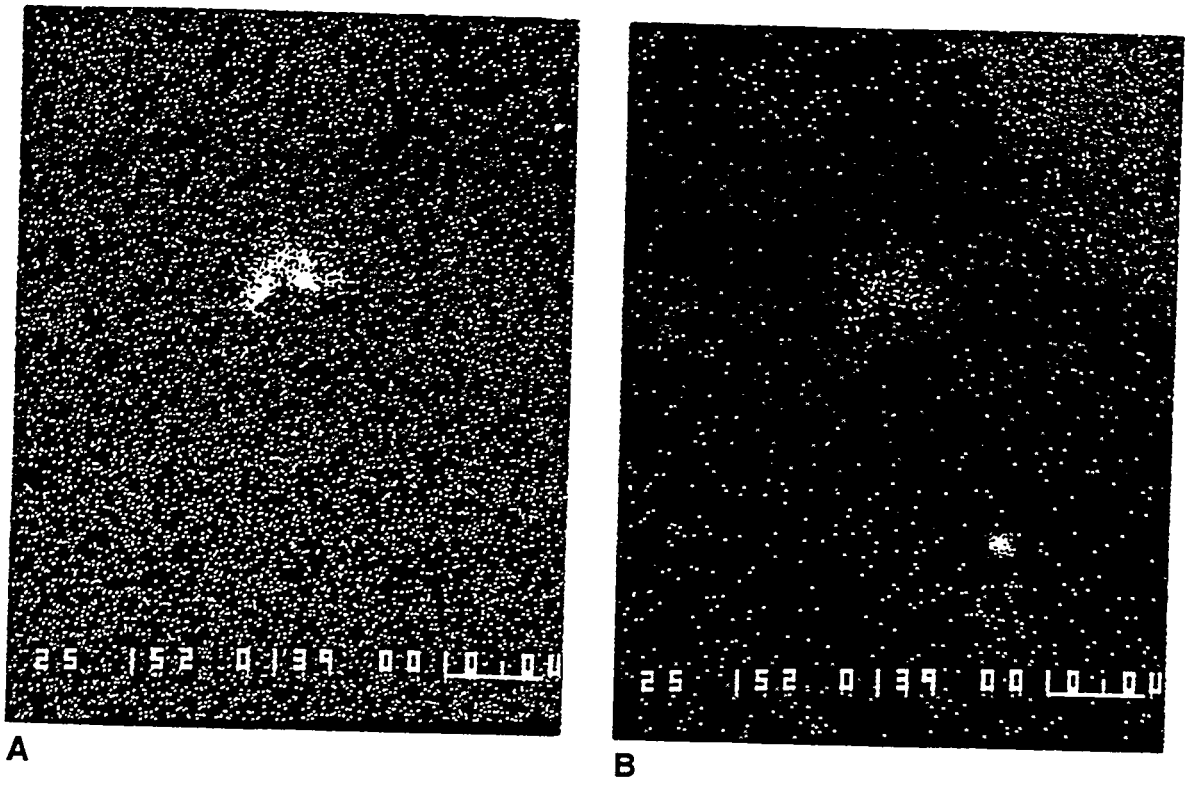


Fig. 5.8. Elemental X-ray maps for (a) mercury and (b) sulfur for the region shown in Fig. 5.7.

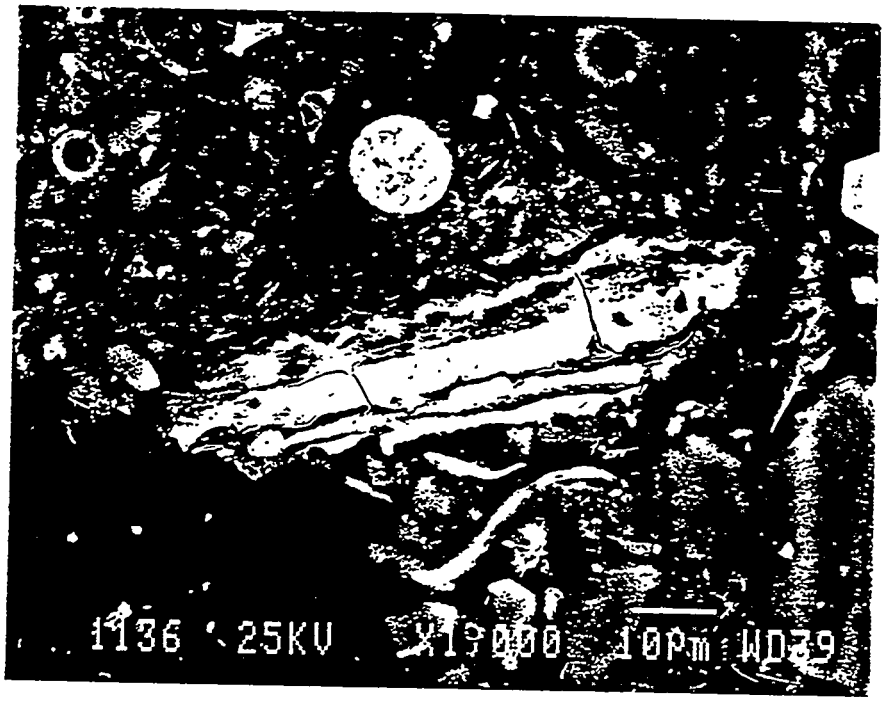


Fig. 5.9. SEI showing an elongated structure (enclosed in the box) containing mercury and sulfur.

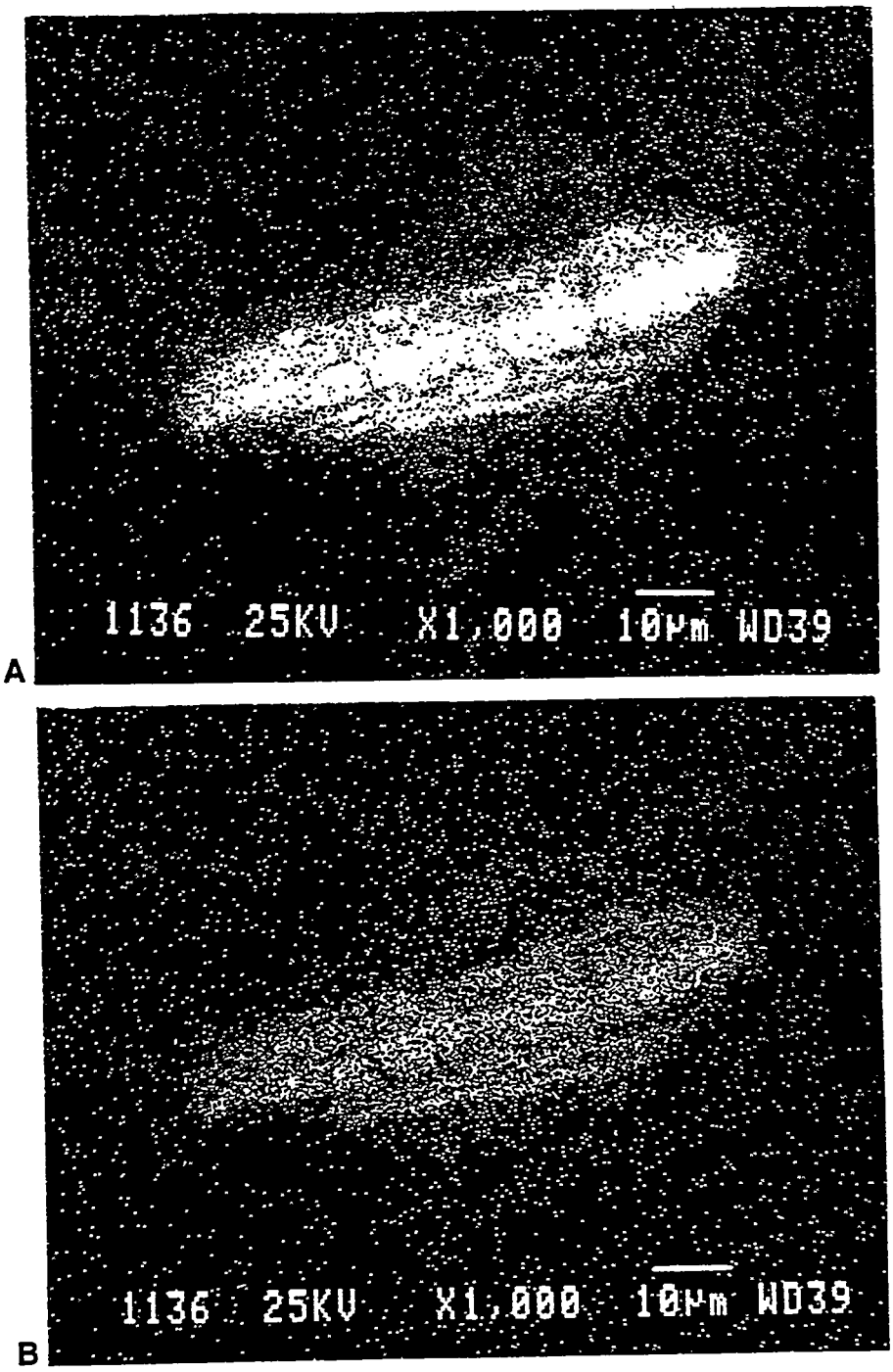
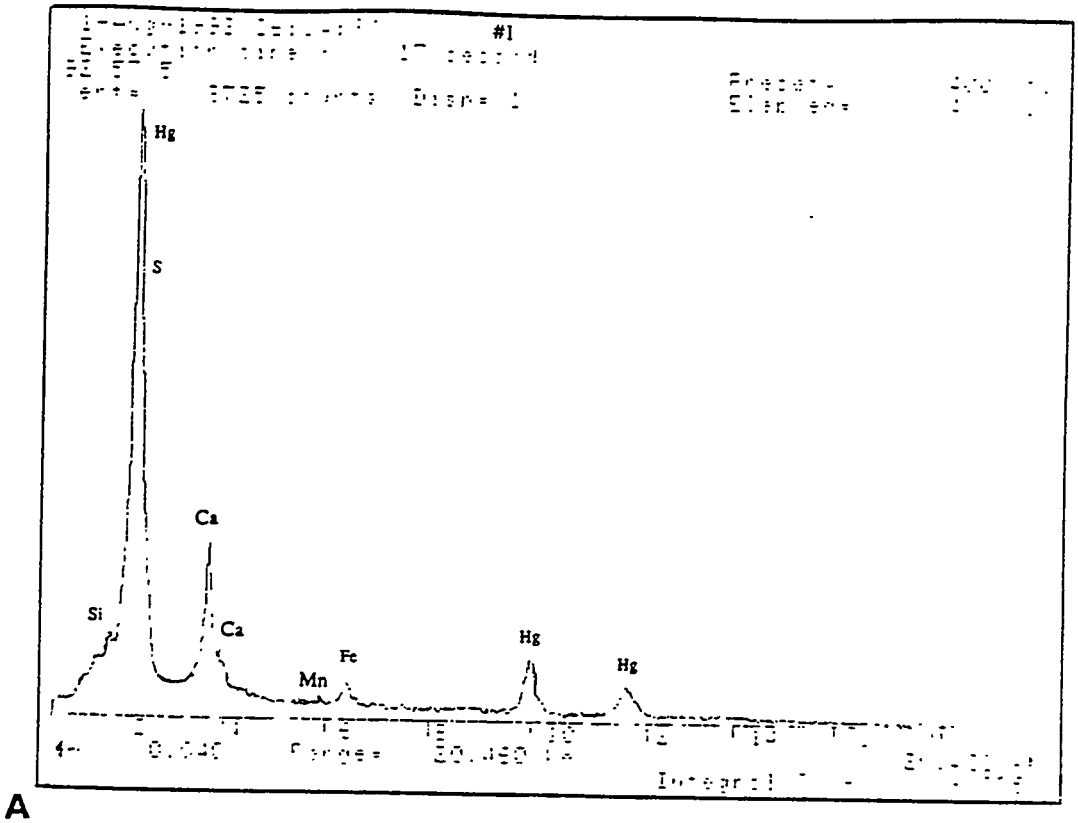


Fig. 5.10. Elemental X-ray maps of (a) mercury and (b) sulfur for the elongated structure shown in Fig. 5.9.

respectively. These spectra show that a change (as observed by the relative intensity of the combined mercury-sulfur peak as compared with the other peaks) occurred after the initial heating, but no subsequent changes occurred on the second heating. The changes included a 12-fold increase in the ratio of iron to mercury and a doubling of the calcium-to-mercury ratio. A possible explanation for the continued presence of mercury and sulfur is one previously discussed, specifically, the greater X-ray volume from which the X-rays originate compared with the probe



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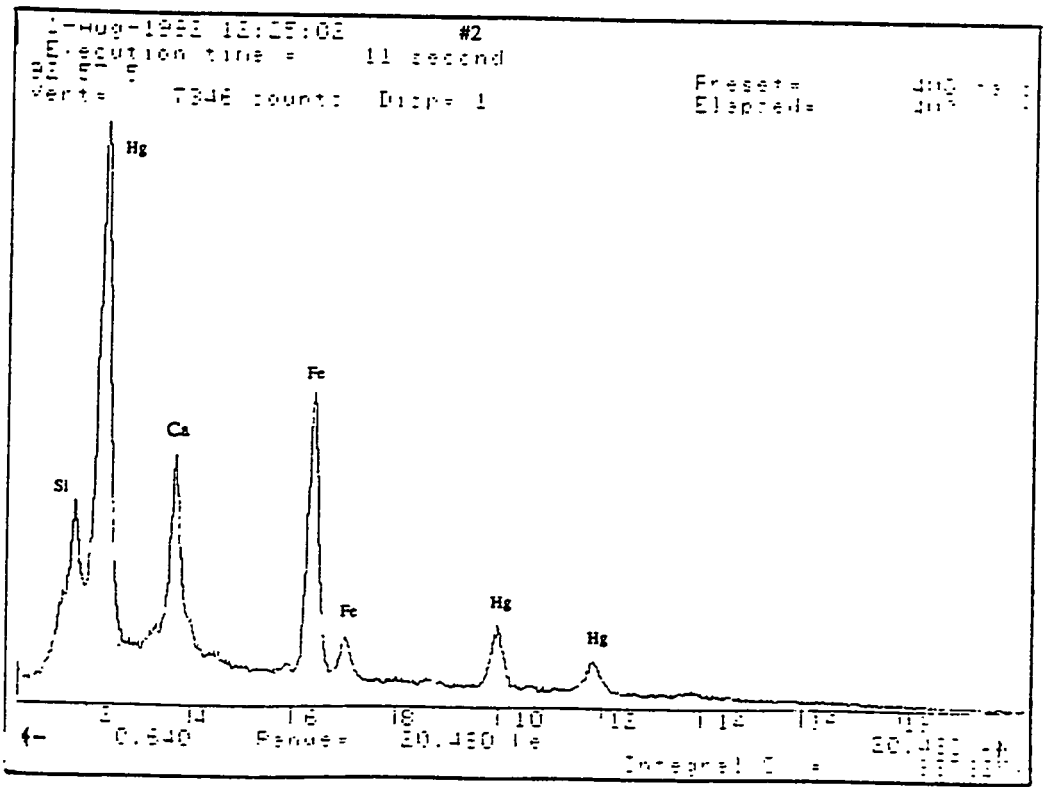


B

Fig. 5.11. EDS spectra (a) for as-received material and (b) after an increase in probe current (heating).

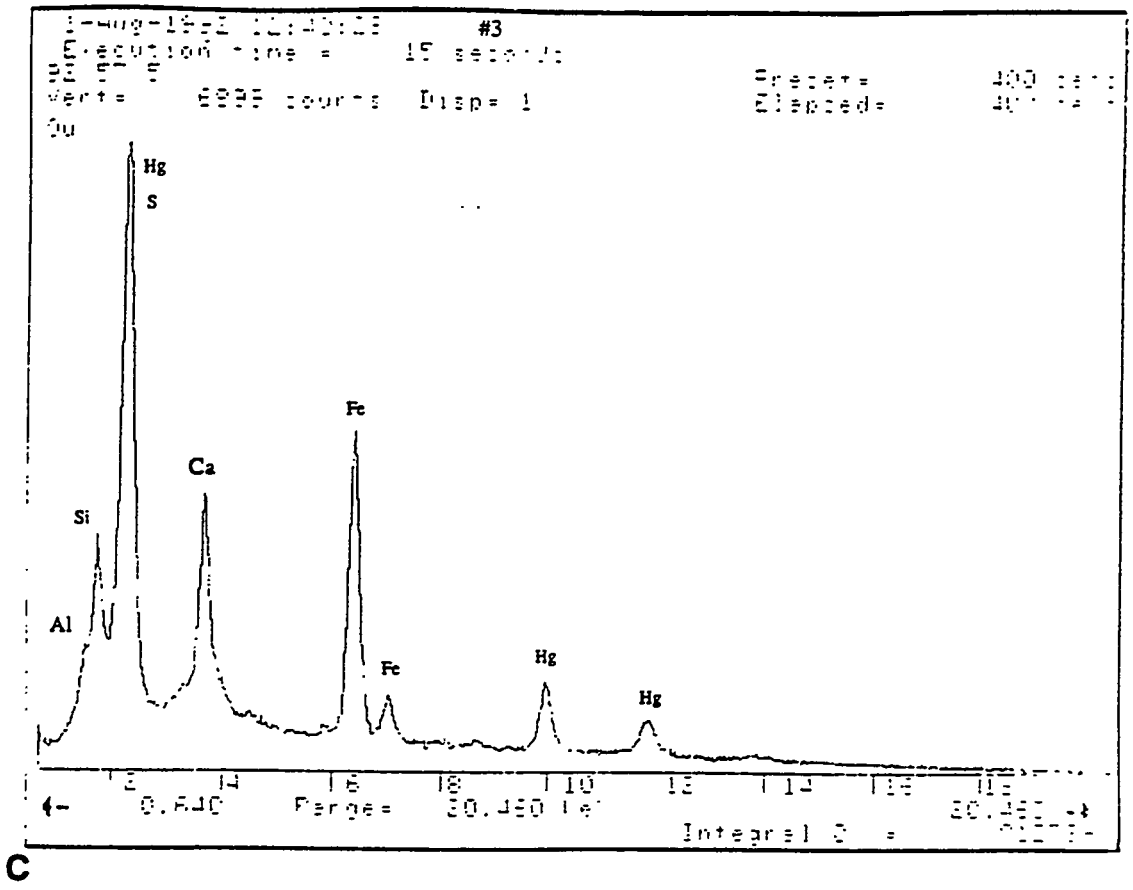


Fig. 5.11 (continued). EDS spectra (c) after an additional increase in probe current.

diameter. This means that many X-rays are originating outside the probe spot so that elemental data from the immediate area around the probe spot are being recorded. Apparently, future work must be done to confirm or deny the coating thesis. However, these data do support that phenomena in a very limited way (e.g., the increase in calcium and iron).

As reported previously, some of the mercury appears to be associated with calcium-rich matter. Figure 5.12 is a BEI of just such a feature (the elliptically shaped structure designated by the letter "P") and is surrounded by a profusion of grains ranging in size from 100 to <1 μm. The identity of many of these grains was established by the following X-ray microanalysis. Figure 5.13(a) through (f) are the X-ray maps for the elements identified by EDS. The superimposition of the mercury and sulfur X-ray maps within the feature is almost in perfect register. Superimposed on these elements is calcium, which appears to occupy additional areas within the feature not covered by mercury and sulfur. Because no calcium, mercury sulfide, or sulfate compound is reported in the literature, it may be that calcium is related to the feature itself. Aluminum, silicon, and iron X-ray maps define the chemistry of the adjacent matter surrounding the feature; for example, the superimposition of these three elements identifies clay minerals, while grains covered by silicon only must be quartz.

Plant matter containing pyrite (FeS_2) (Fig. 5.14) also contains mercury. These elemental relationships are illustrated by a series of X-ray maps for aluminum, calcium, iron, mercury, and

Fig. 5.12. BEI of an elliptically shaped feature (P), probably plant matter, surrounded by quartz and clay.

sulfur [Fig. 5.15(a) through (e)]. The plant cell wall is composed of calcium and aluminum, while within the cell, sulfur and iron are superimposed forming an iron sulfide (probably pyrite). The overall appearance of the iron and sulfur in this figure is reminiscent of a pyrite framboid. Though mercury is present, it appears to be diffuse over the inner cell surface. It may be that the mercury adheres to the pyrite surfaces because it has a strong affinity for sulfur.

Finally, it is worth noting that the floodplain soil is the repository for a host of foreign elements besides mercury that have been deposited over the years. In the course of these microanalyses, many other high atomic number materials were imaged, most notably uranium and thorium fragments that were identified and X-ray mapped. Figure 5.16(a) and (b) present a BEI of uranium and its associated X-ray map. The uranium particle is about 5 μm as compared with the thorium particle, which is 60 μm [see Fig. 5.17(a) and (b), the BEI and thorium X-ray map, respectively]. In addition to those materials, rare earths, zirconium, and stainless steel were relatively common.

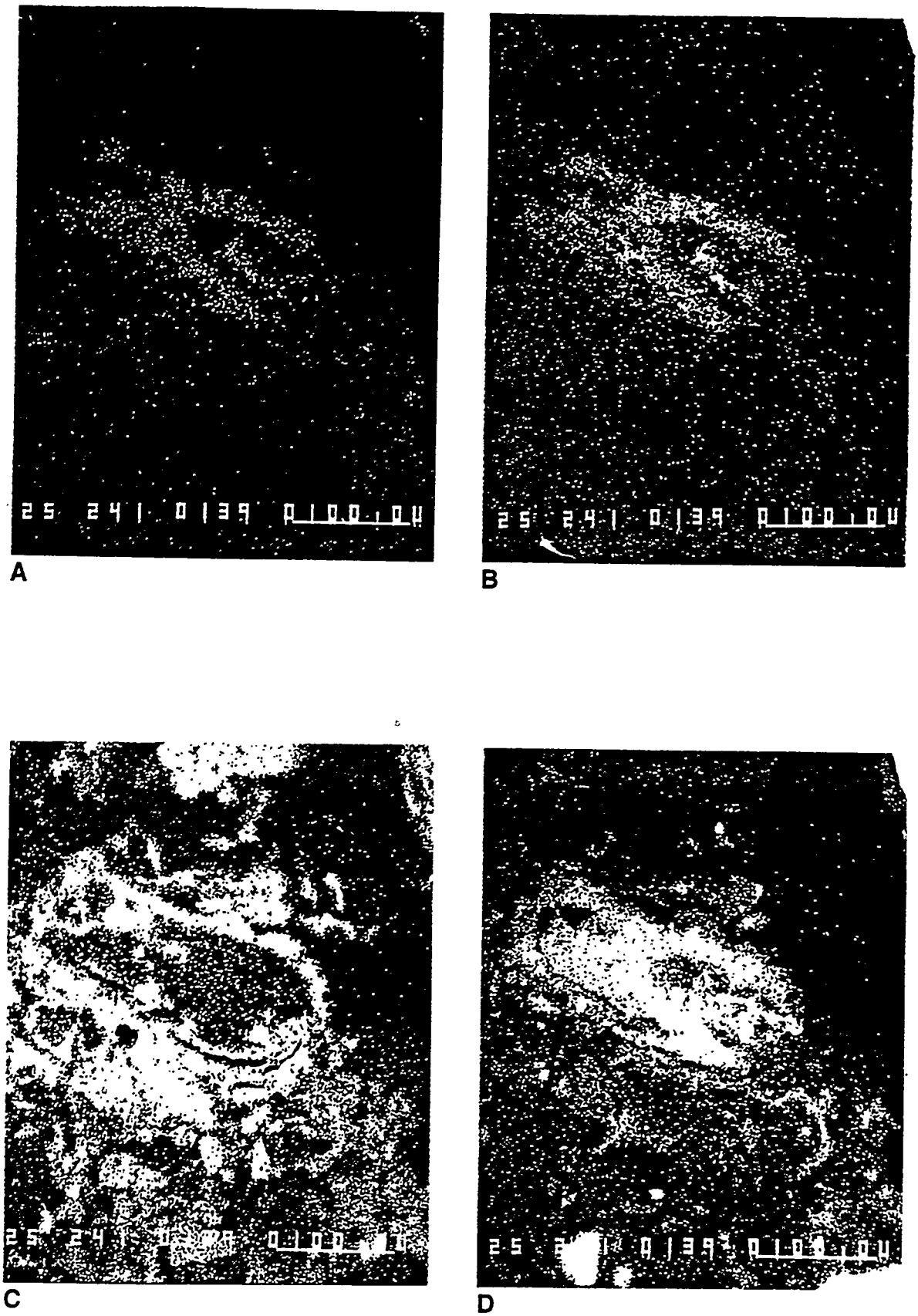
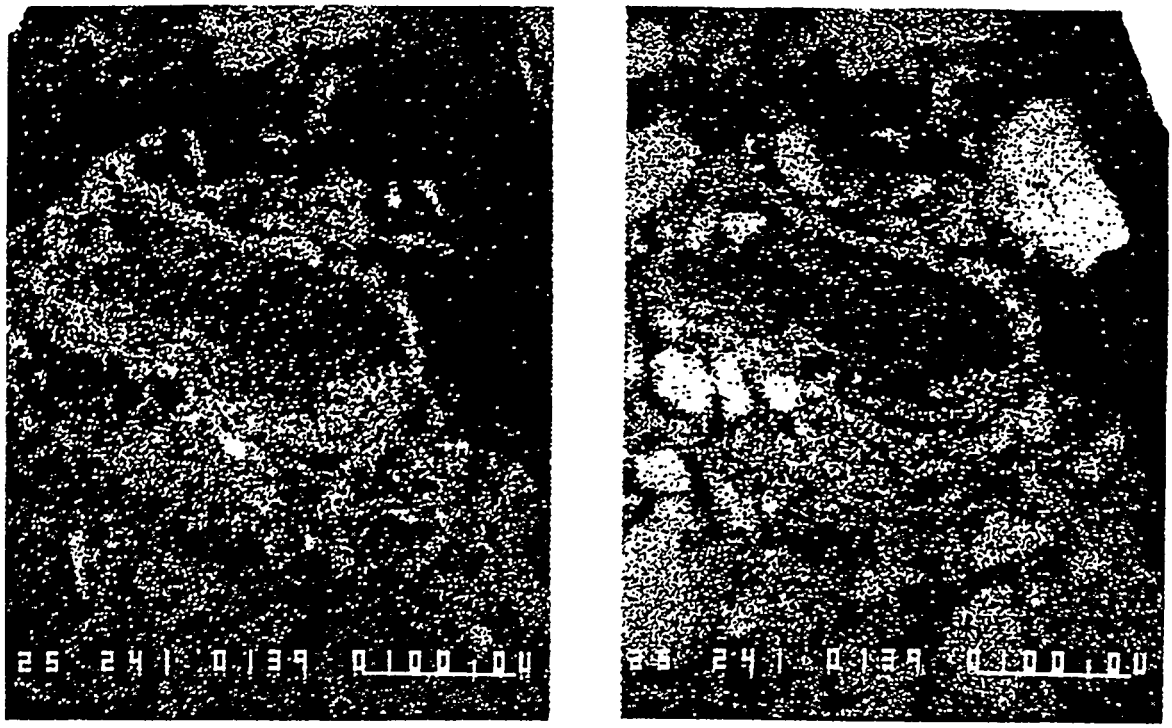


Fig. 5.13. Elemental X-ray maps for (a) aluminum, (b) calcium, (c) iron, and (d) mercury.



E F

Fig. 5.13 (continued). Elemental X-ray maps for (e) silicon and (f) sulfur.

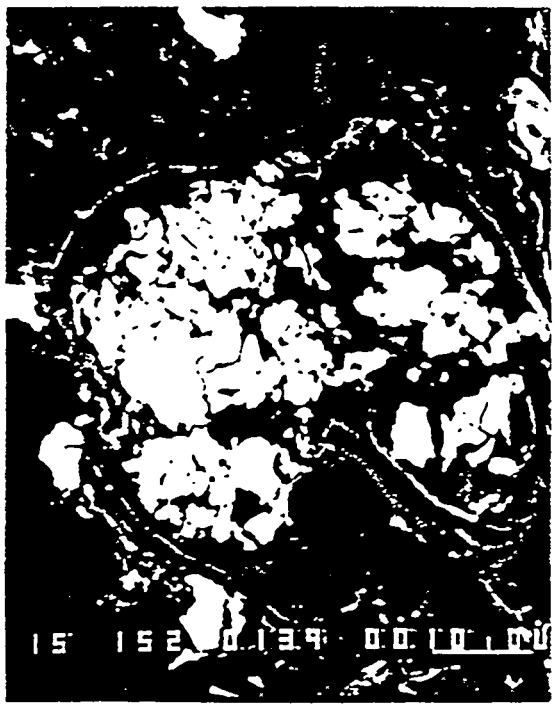


Fig. 5.14. BEI of plant matter with a cluster of pyrite crystals.

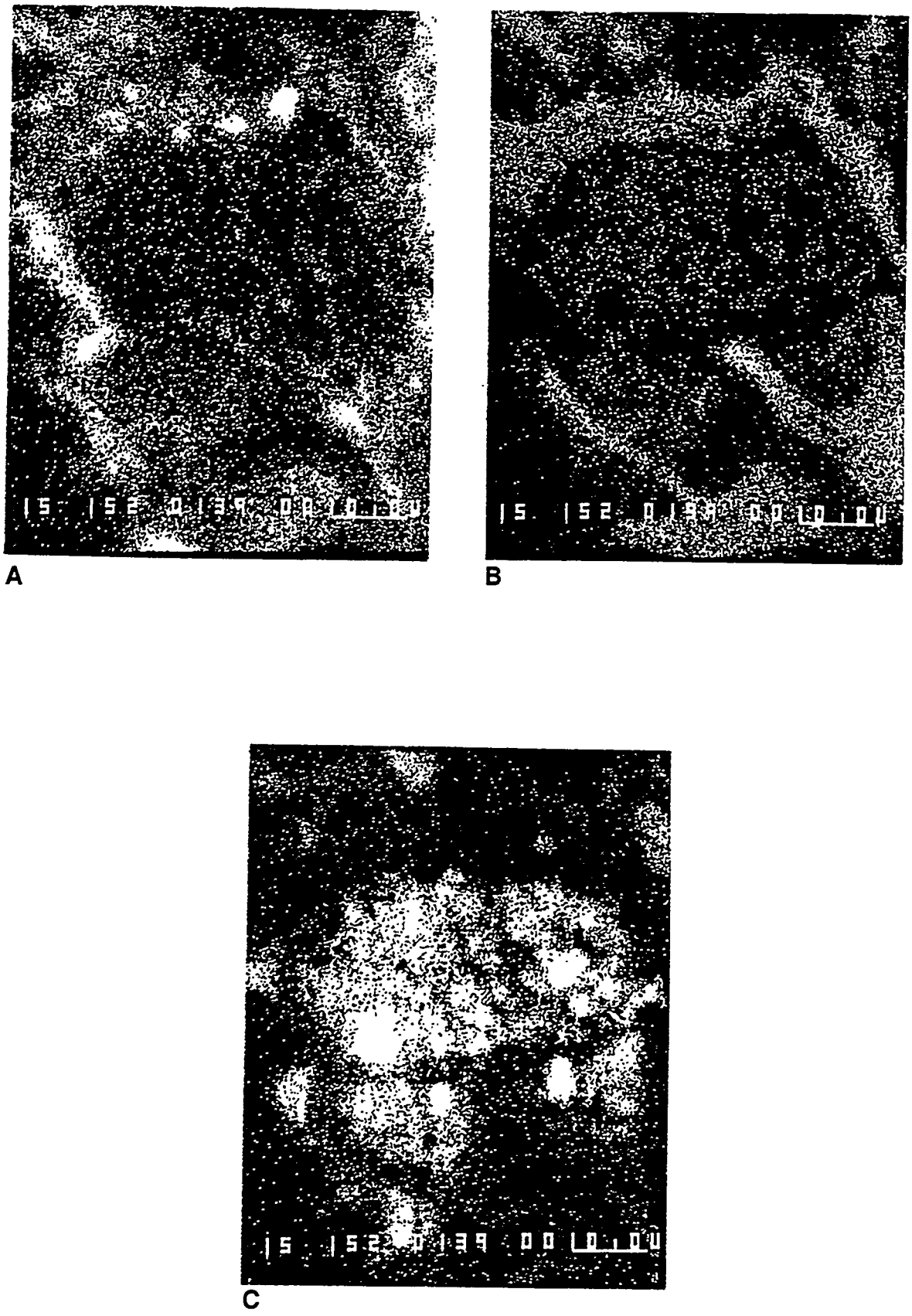
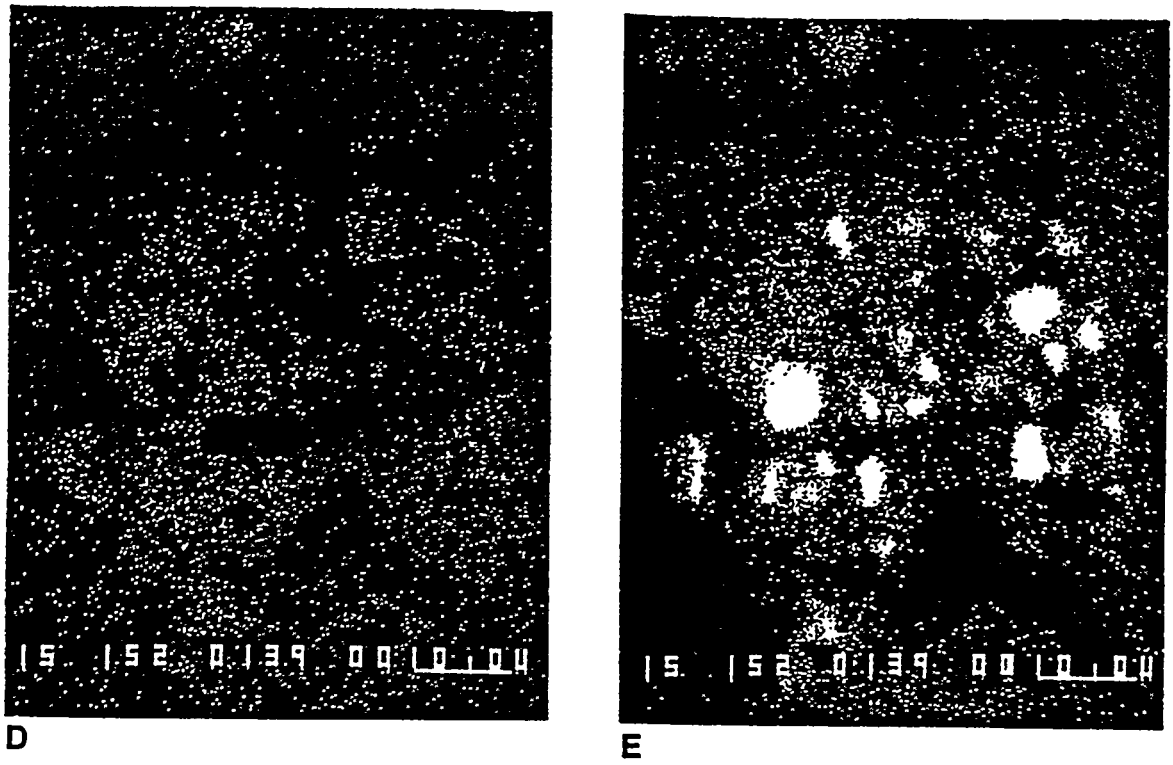
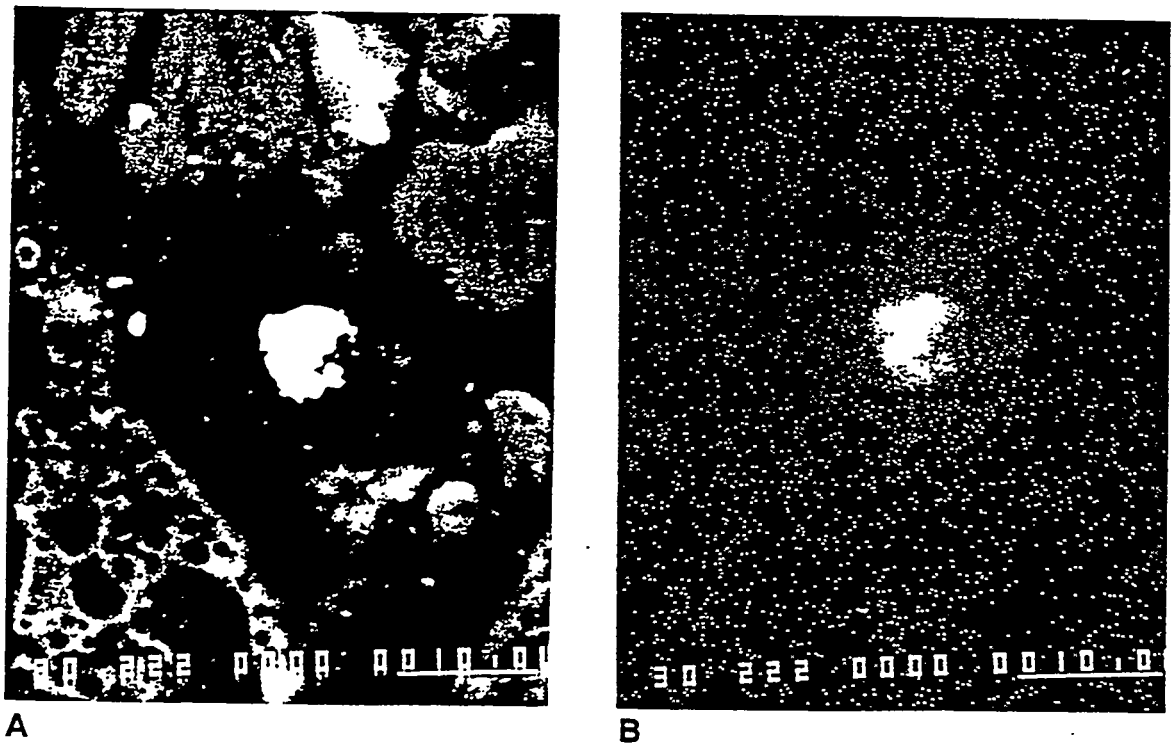


Fig. 5.15. Elemental X-ray maps for (a) aluminum, (b) calcium, and (c) iron.



D E

Fig. 5.15 (continued). Elemental X-ray maps for (d) mercury and (e) sulfur.



A B

Fig. 5.16. A BEI of (a) a uranium particle and (b) its X-ray map.

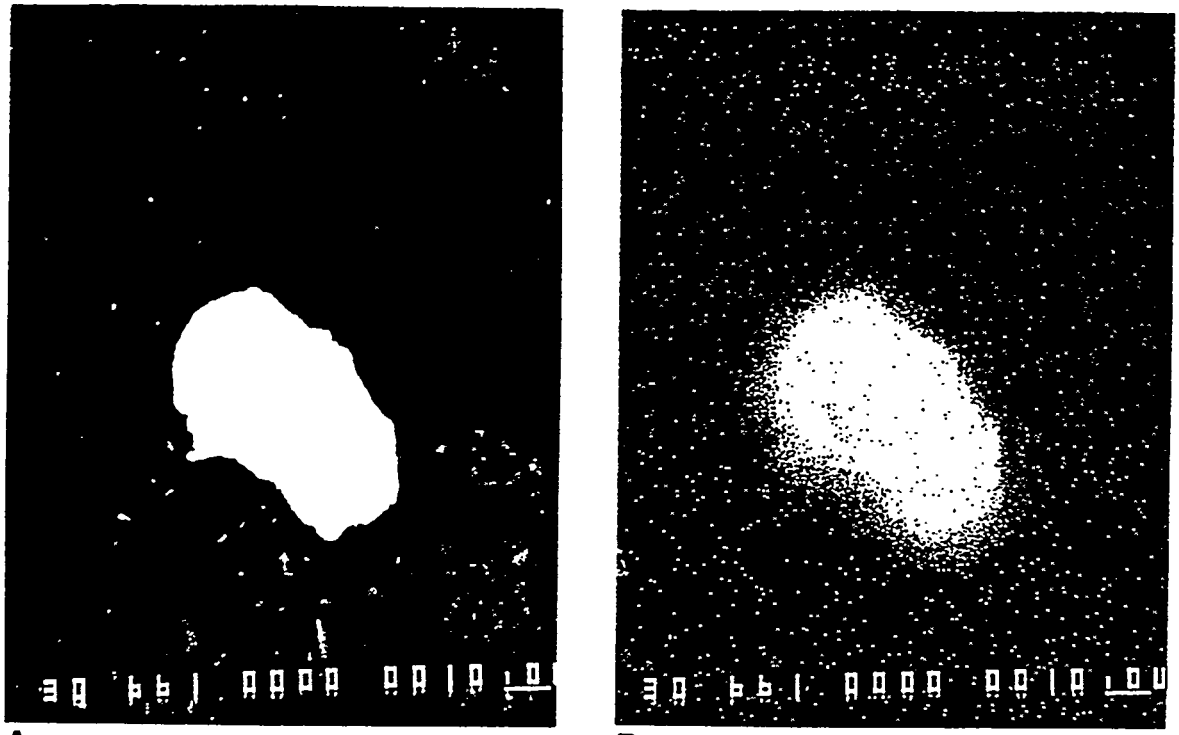


Fig. 5.17. A BEI of (a) a thorium particle and (b) its X-ray image map.

6. SUMMARY

Common to all these soil samples are fine-grained micrometer to submicrometer-sized particles that consist of quartz clays and plant debris and constitute a matrix in most of the samples. In addition, optical microscopy revealed varying amounts of ash and coal particles (ranging from an estimated 20 to 30 surface area percent to less than a few surface area percent) dispersed throughout these core samples. The ash consists of coke (pyrolized organic material, probably coal that can be distinguished by its appearance in polarized light), cenospheres (hollow spheres composed of an alumino-silicate glass), and ferrospheres (iron oxides formed from oxidized pyrite).

Optical microscopy of the soil cores revealed variations in their content with respect to mercury concentrations. Ash and coal fragments were found to be more abundant in the mercury-rich cores and to be correspondingly less abundant in those cores that are poorer in mercury. There also appear to be greater amounts of ferrospheres in these mercury-rich soils. In contrast, those soil samples containing less than 300 ppm of mercury appear to be almost devoid of ash and ferrospheres (under 5 surface area percent). Additionally, within some cores we observed significant variations in texture and content. For example, when core sample FS 5410010 (388 ppm Hg) was cut into five sections, it showed extreme variations between the two upper sections and the three bottom sections. The bottom sections were rich in ash and coal, appearing similar to the soils that are richer in mercury, whereas the top two sections contained significantly less ash and coal and fit the appearance of the mercury-poor samples.

Electron microscopy was employed to detect, image, and microanalyze the mercury-bearing matter in the soil samples. No physical or chemical relationship could be established between the mercury and ash. However, this observation does not negate the optical finding in the sense that these constituents may have traveled together without interacting so that ash-rich soils may still imply mercury enrichment.

Mercury was found to be present in several forms, such as discrete particles or globules, possible coatings on mineral matter, and fillings in pores and/or fissures in plant debris. To date, mercury and sulfur were found to coexist in all the mercury-rich entities. These analyses further revealed that the mode of occurrence of mercury in the mercury-rich and mercury-poor cores is identical; that is, the form and chemistry of the mercury matter is independent of its concentration in the soil.

The coexistence of mercury with sulfur does not imply the presence of a mercury sulfide compound (HgS) because conditions for its formation do not appear to be present in the soils. However, in the absence of conclusive evidence (e.g., X-ray diffraction analyses of a mercury concentrate), one must exercise caution. It is well known that mercury has a strong affinity for sulfur and that sulfur is available in these soils from several sources, including sulfates in the water, organic sulfur in coal, iron sulfides, and plant matter.

One may hypothesize a possible succession of events taking place in the soil with respect to mercury. A first step in these events could be the emplacement of mercury on either mineral or plant substrate. This mode might be governed by the ability of the mercury ion to

substitute for the calcium ion and may indeed be one of the reasons for the association of calcium with mercury that has been observed. In addition, sulfur may also be associated with these same substrates and act as an additional attraction for mercury attachment. Finally, the sulfate-rich waters that move through the soil could act to transport sulfur ions to coat and diffuse the mercury entities.

7. FUTURE WORK RECOMMENDATIONS

Several fresh approaches to help clarify the data described and discussed in the previous chapters are suggested. The most important question that still remains unanswered is whether the mercury/sulfur mixtures are present as the crystalline compound HgS. X-ray diffraction analyses of concentrates of mercury-rich particles should unequivocally answer this question. Of lesser importance, but still not truly understood, is whether mercury/sulfur mixtures are truly coatings on mineral surfaces. To address this problem, an auger spectrometer might be ideal because the surfaces can be sputtered in a controlled manner while that surface is being analyzed.

The optical microscopy of several of the soil cores revealed major intracore differences with respect to ash and coal. It may be that mercury-rich layers are interspersed with mercury-poor soil such that the true nature of the mercury distribution is unclear. Careful electron microanalyses of these cores by segments would do much to clarify this situation.

8. REFERENCES

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LWA. 1990b. *Environmental Restoration Program, Technical Support Contractor, Quality Assurance Technical Procedures Manual*, DOE/OR-933 and LWA/90-008.

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FIGURES

Fig. 4.1 An optical micrograph of ash particles; (a) coke, cenospheres, and mineral matter,(b) ferrospheres, (c) cross-section of a root, and (d) uncombusted coal fragment.

Fig. 4.2 Ultraviolet light fluoresced hydrogen-rich constituent (extinite which is a coal entity) in uncombusted coal.

Fig. 5.1 SEI of mercury-rich particles (shown by arrows) with quartz (Q) and clay (C).

Fig. 5.2 BEI of same area shown in Fig. 5.1 where the bright spots are the mercury-rich particles.

Fig. 5.3 Elemental x-ray maps for (5.3a) mercury, (5.3b) sulfur, and (5.3c) calcium.

Fig. 5.4 SEI of a mercury-rich particle atop clay.

Fig. 5.5 Elemental x-ray maps for (5.5a) mercury and (5.5b) sulfur.

Fig. 5.6 BEI of mercury-rich particles in center of the field.

Fig. 5.7 BEI of a wing-shaped mercury-rich particle (see arrows).

Fig. 5.8 Elemental x-ray maps for (5.8a) mercury and (5.8b) sulfur.

Fig. 5.9 SEI showing an elongated structure (enclosed in the box) containing mercury and sulfur.

Fig. 5.10 Elemental x-ray maps of (5.10a) mercury and (5.10b) sulfur.

Fig. 5.11 EDS spectra for (5.11a) as-received material, (5.11b) after an increase in probe current (heating), and (5.11c) after an additional increase in probe current.

Fig. 5.12 BEI of an elliptically shaped feature (P), probably plant matter, surrounded by quartz and clay.

Fig. 5.13 Elemental x-ray maps for (5.13a) aluminum, (5.13b) calcium, (5.13c) iron, (5.13d) mercury, (5.13e) silicon, and (5.13f) sulfur.

Fig. 5.14 BEI of plant matter with a cluster of pyrite crystals.

Fig. 5.15 Elemental x-ray maps for (5.15a) aluminum, (5.15b) calcium, (5.15c) iron, (5.15d) mercury, and (5.15e) sulfur.

Fig. 5.16 A BEI of a uranium particle (5.16a) and its x-ray map (5.16b).

Fig. 5.17 A BEI of a thorium particle (5.17a) and its x-ray image map (5.17b).

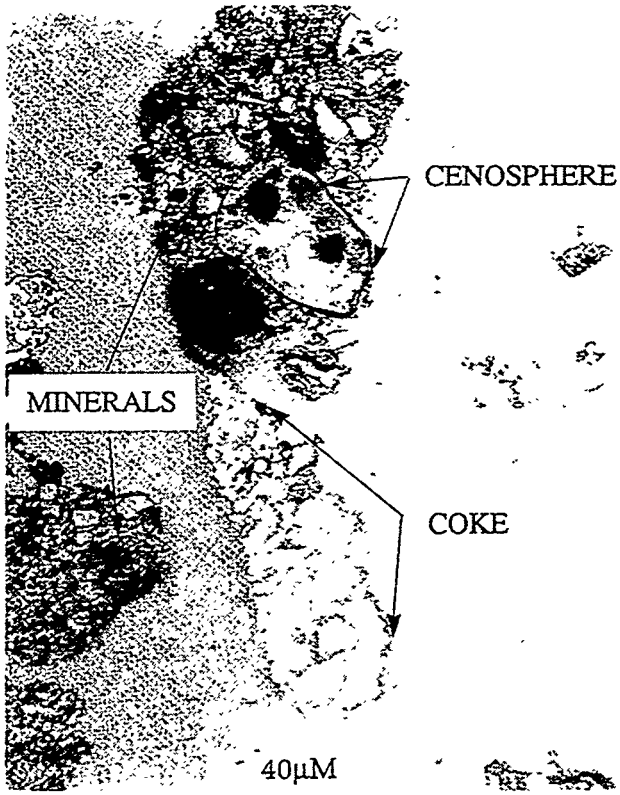
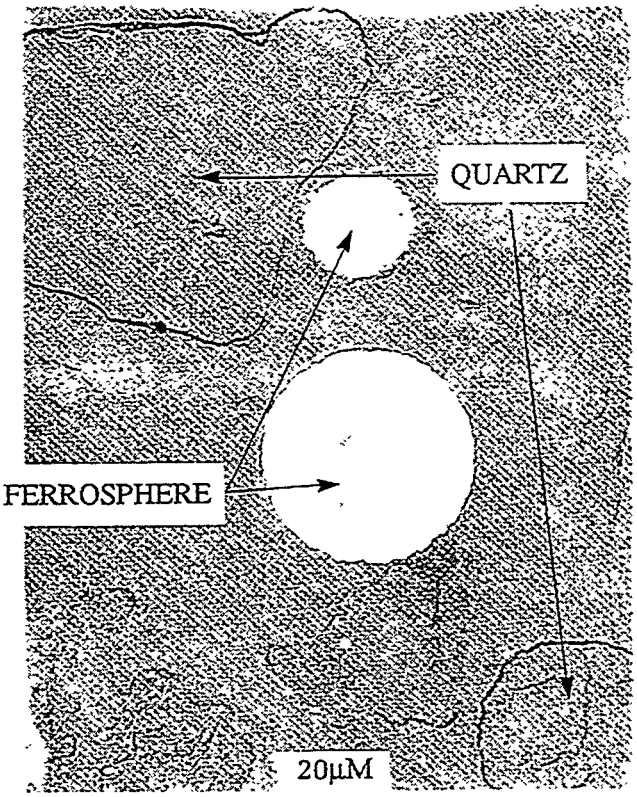


Fig. 4.1a



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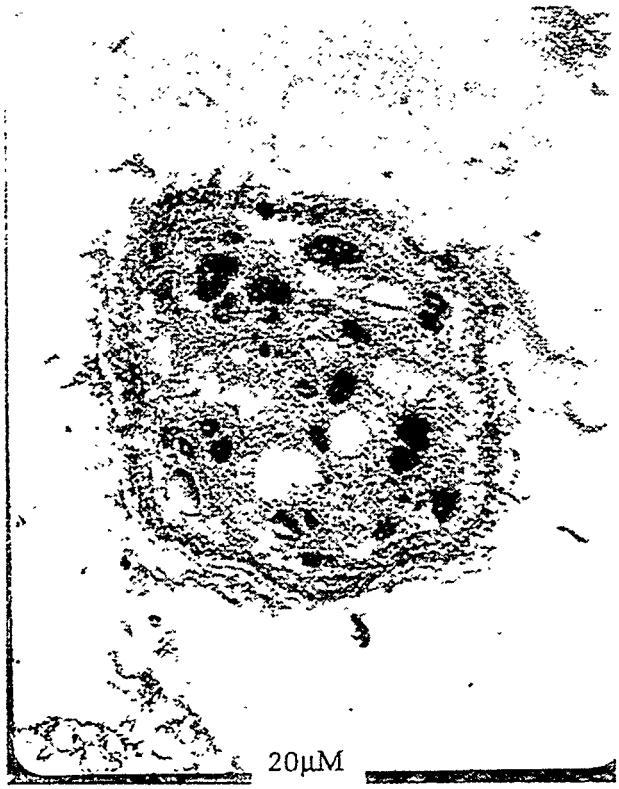

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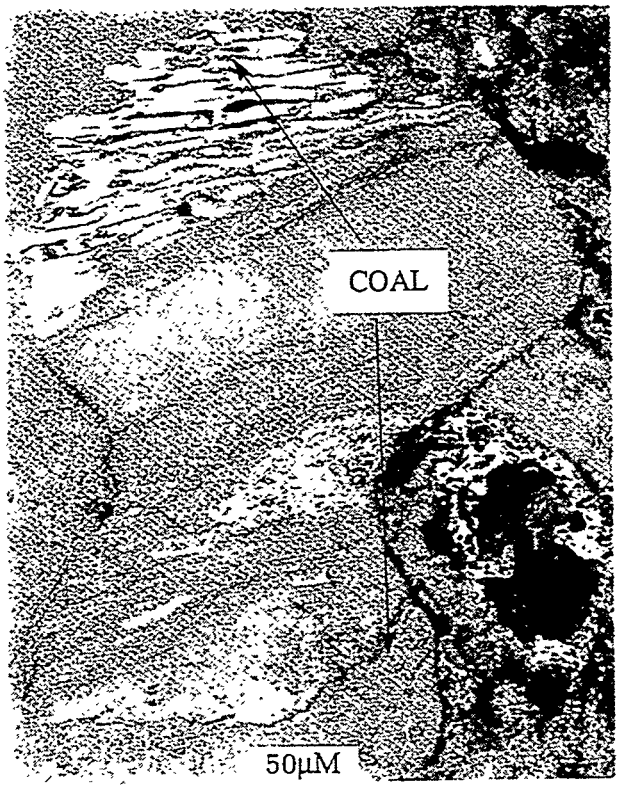

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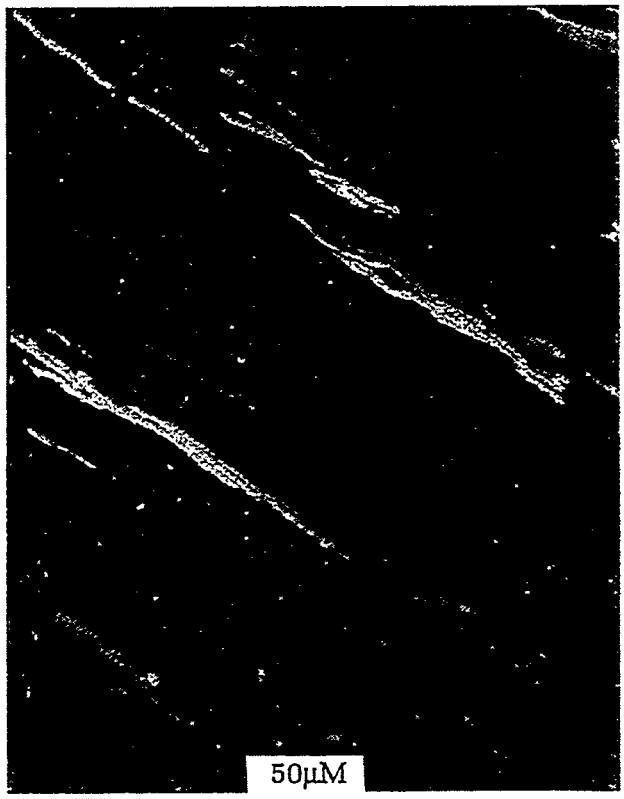
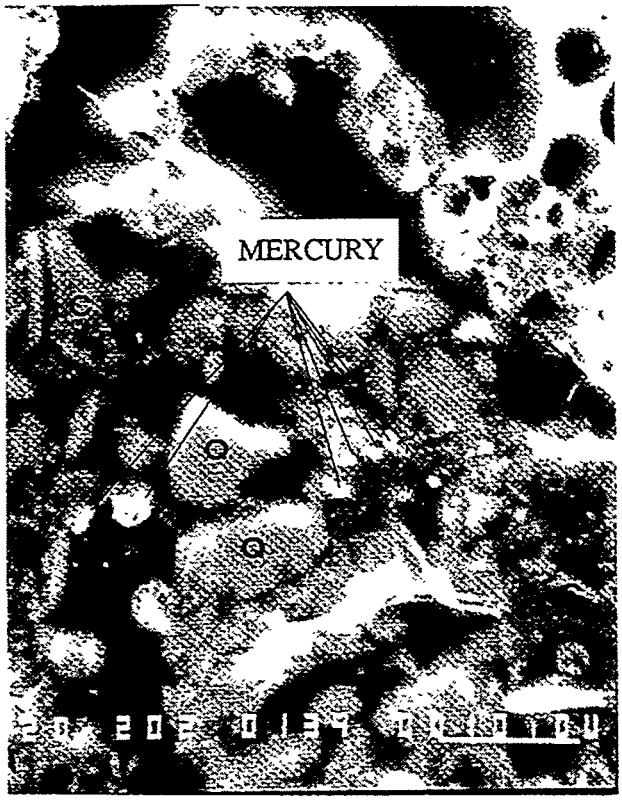


Fig. 4.2



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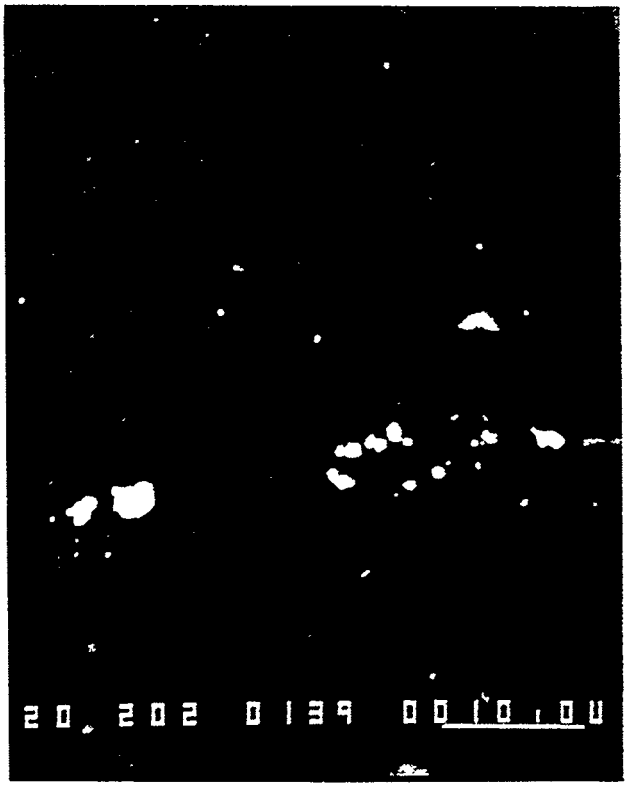

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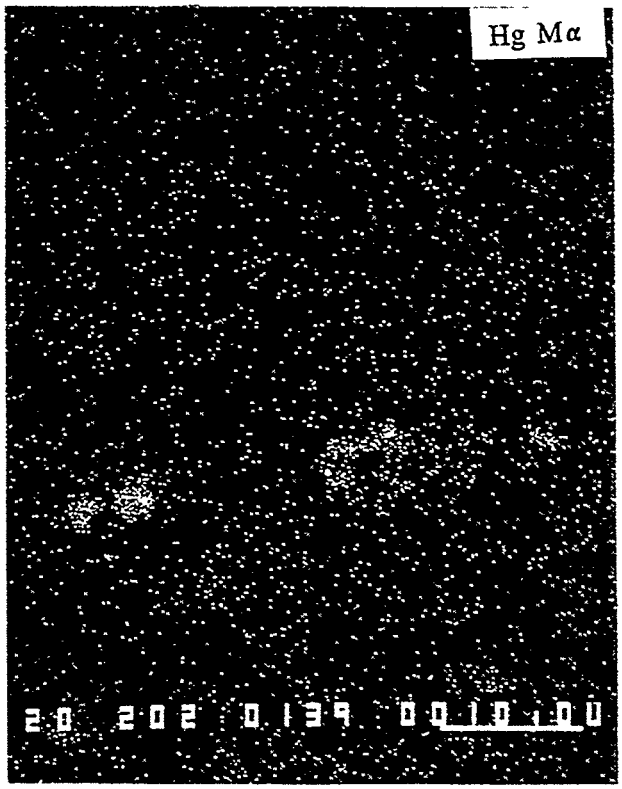

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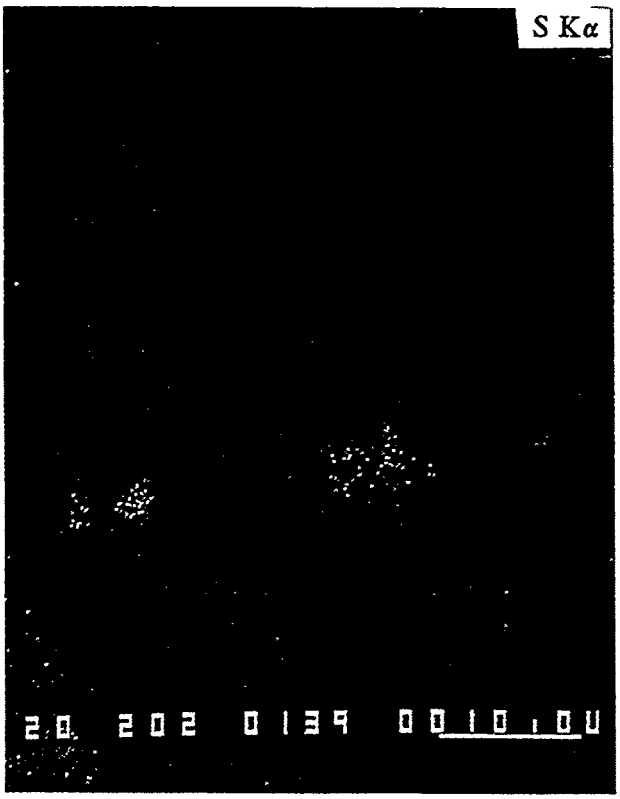

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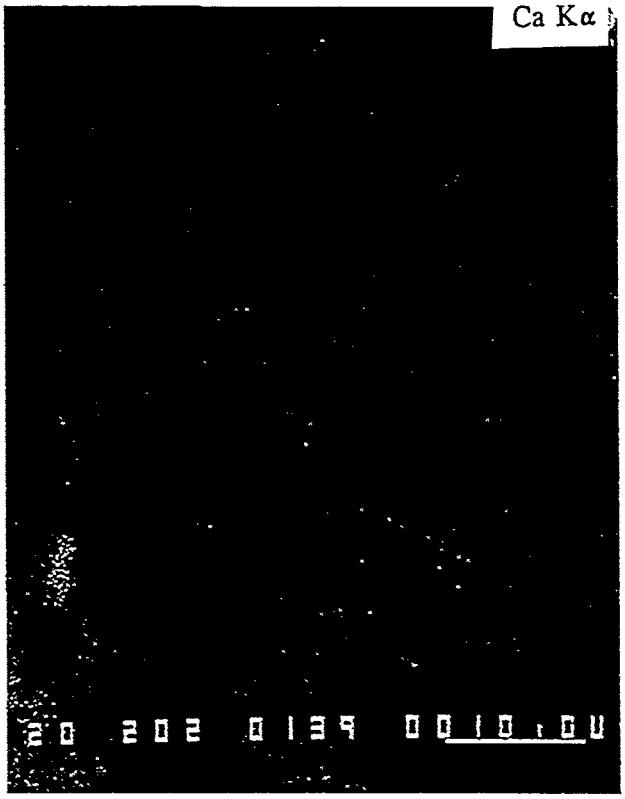

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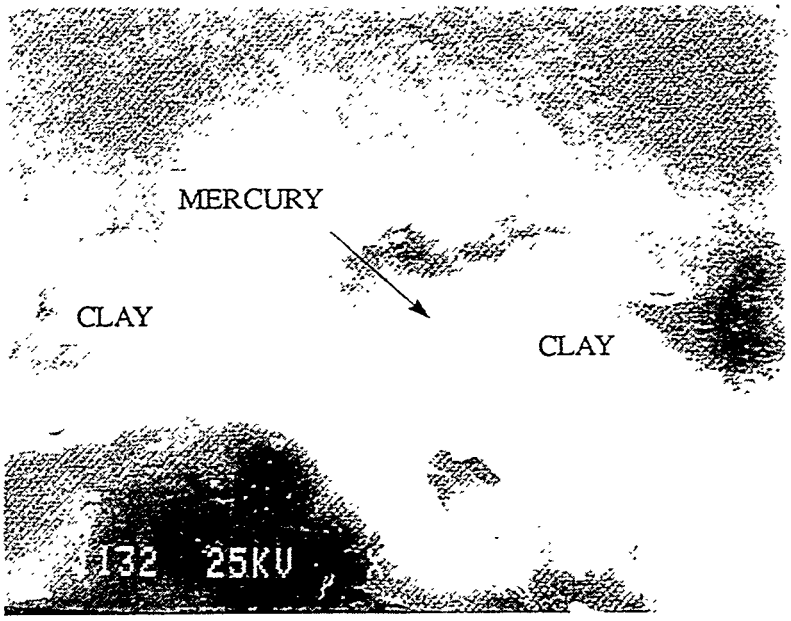

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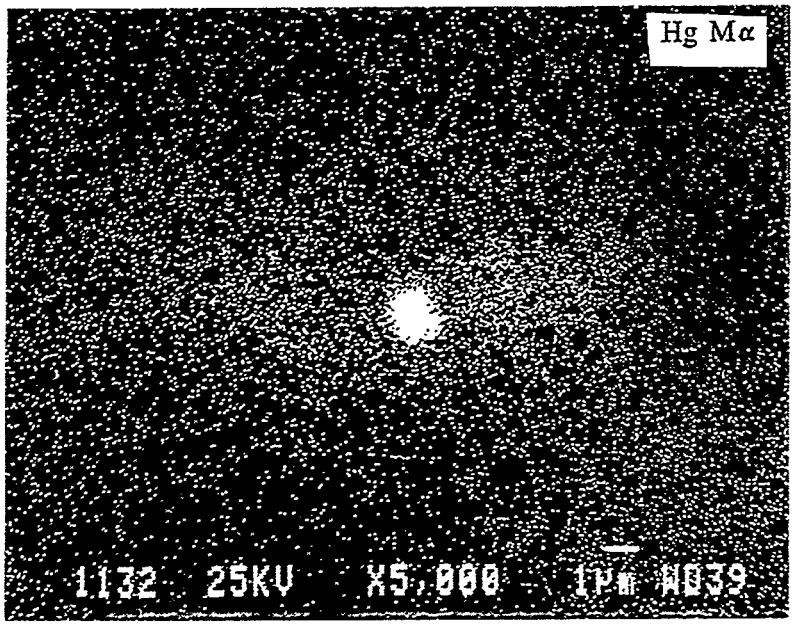

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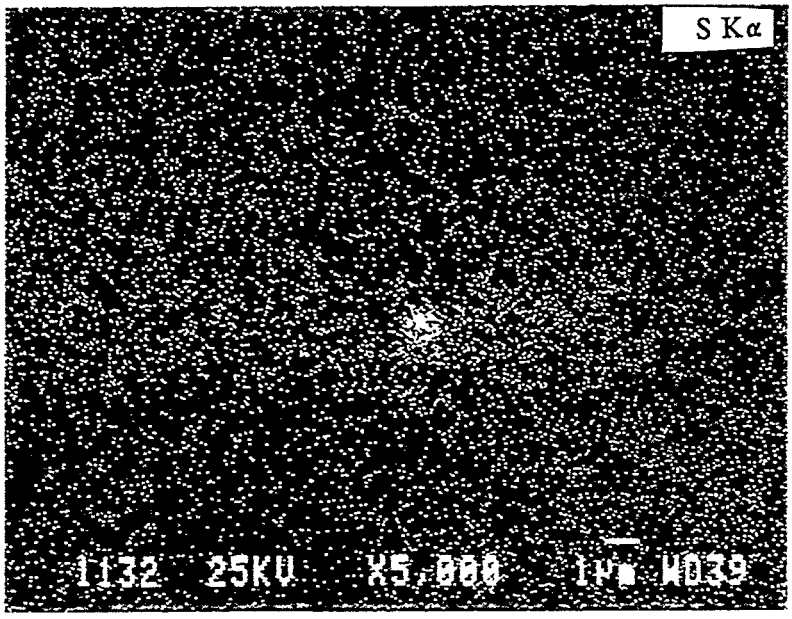

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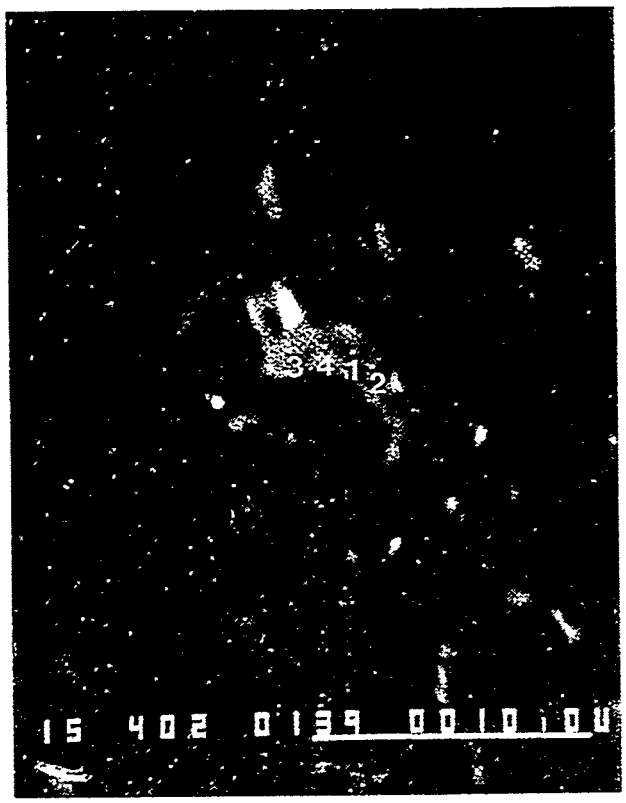

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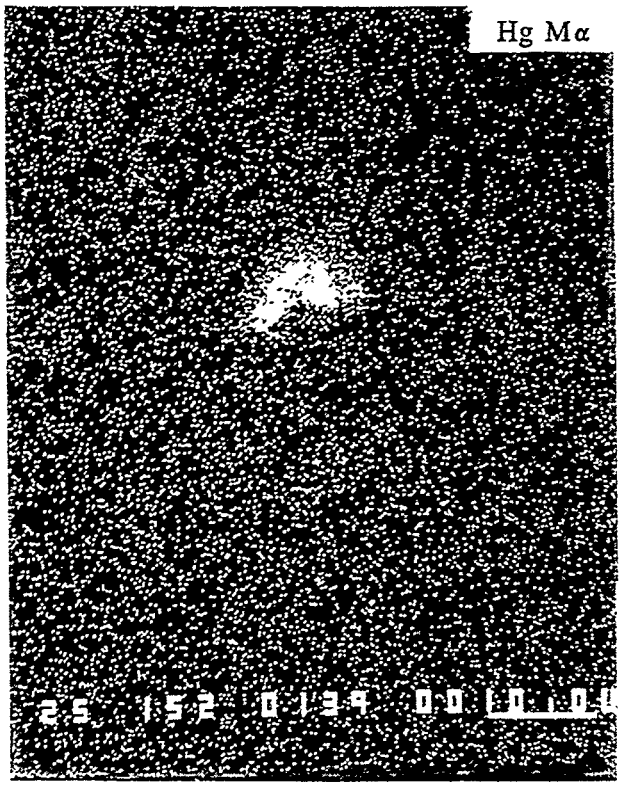

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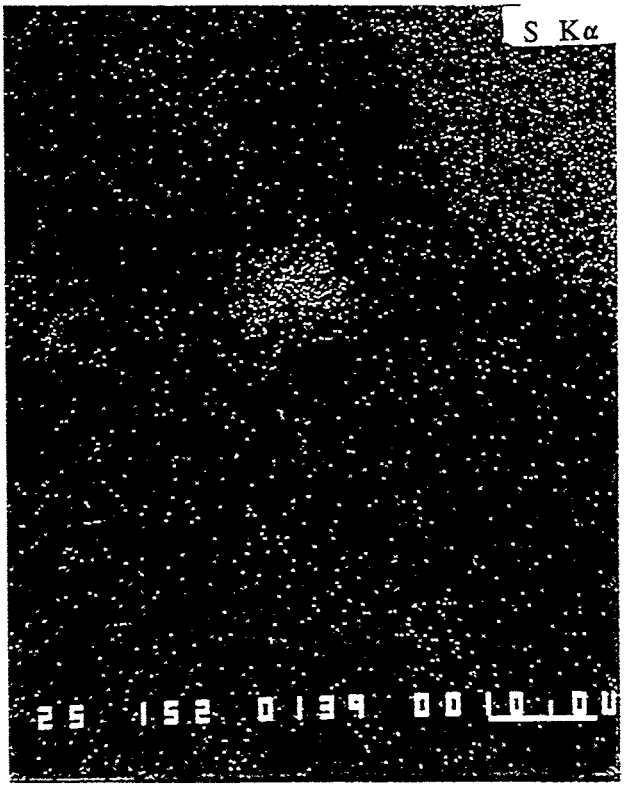

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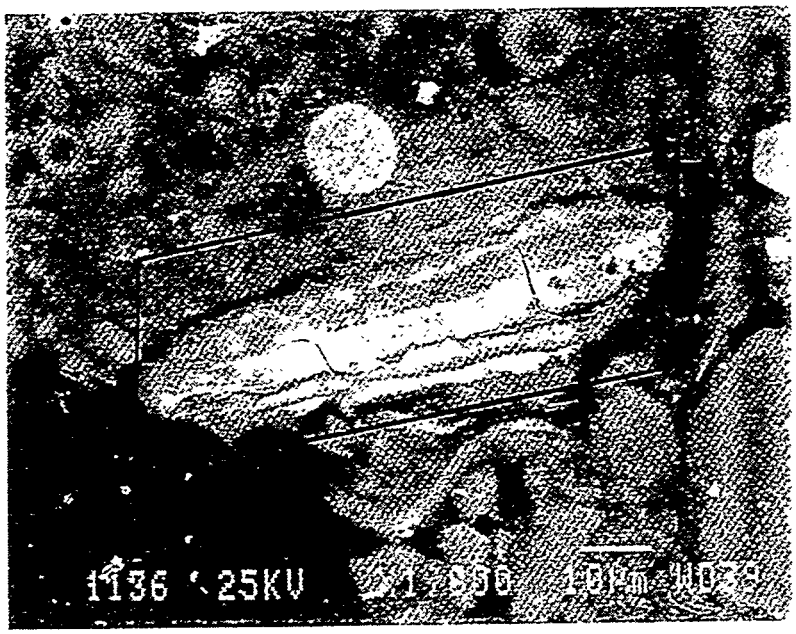

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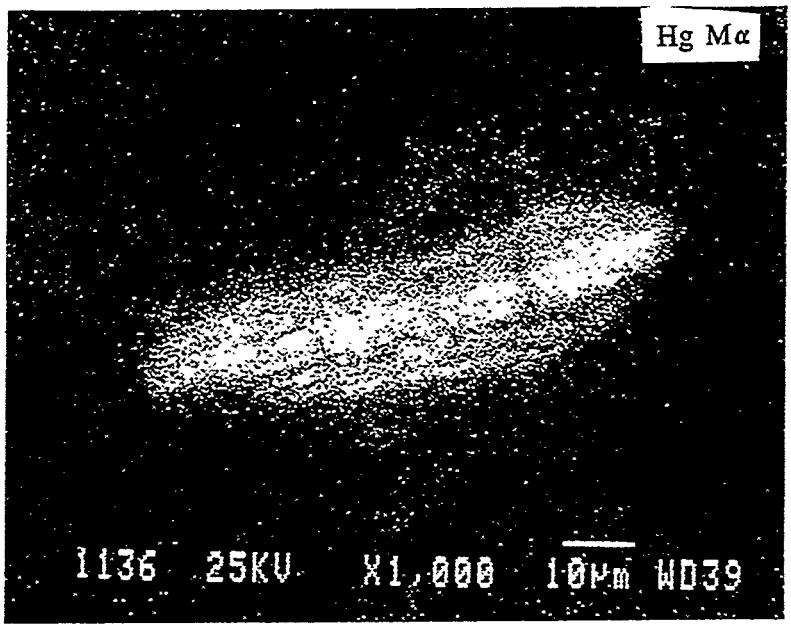

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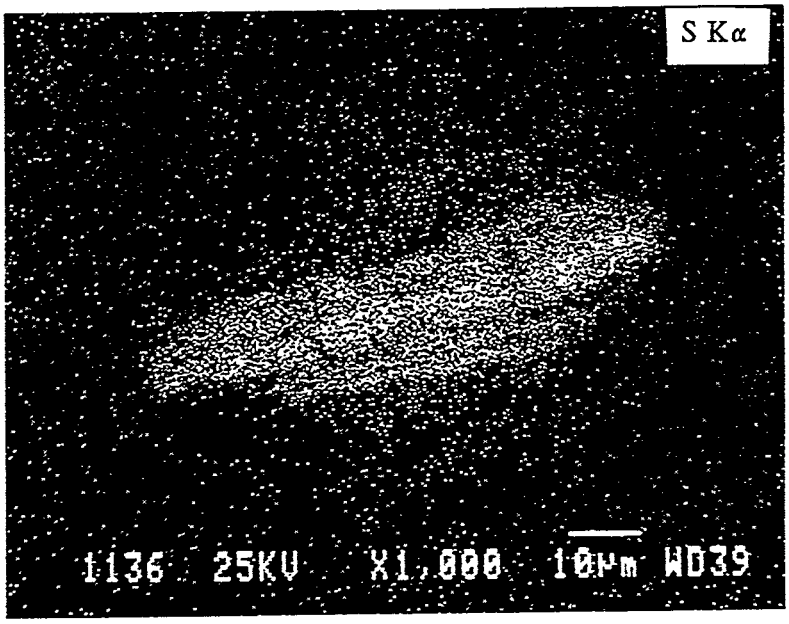

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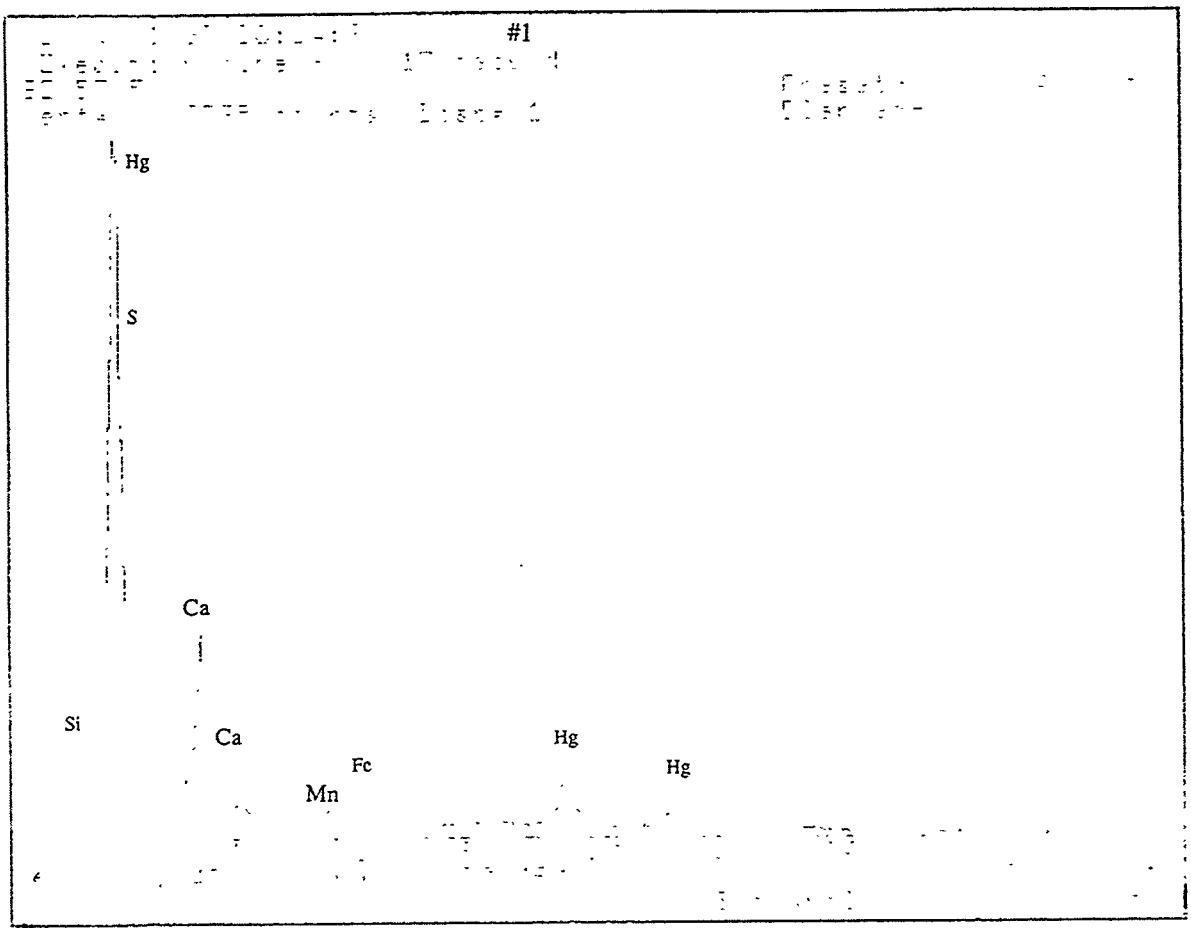

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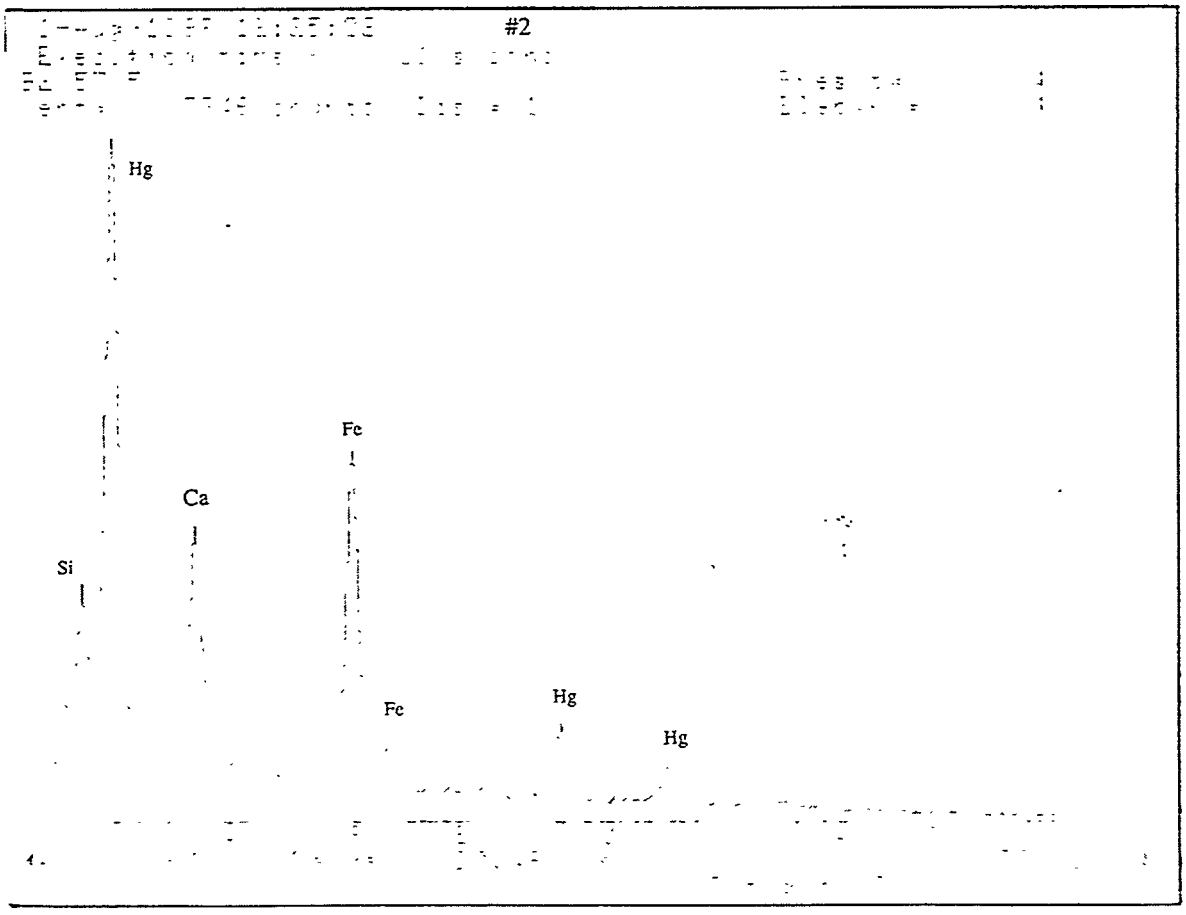

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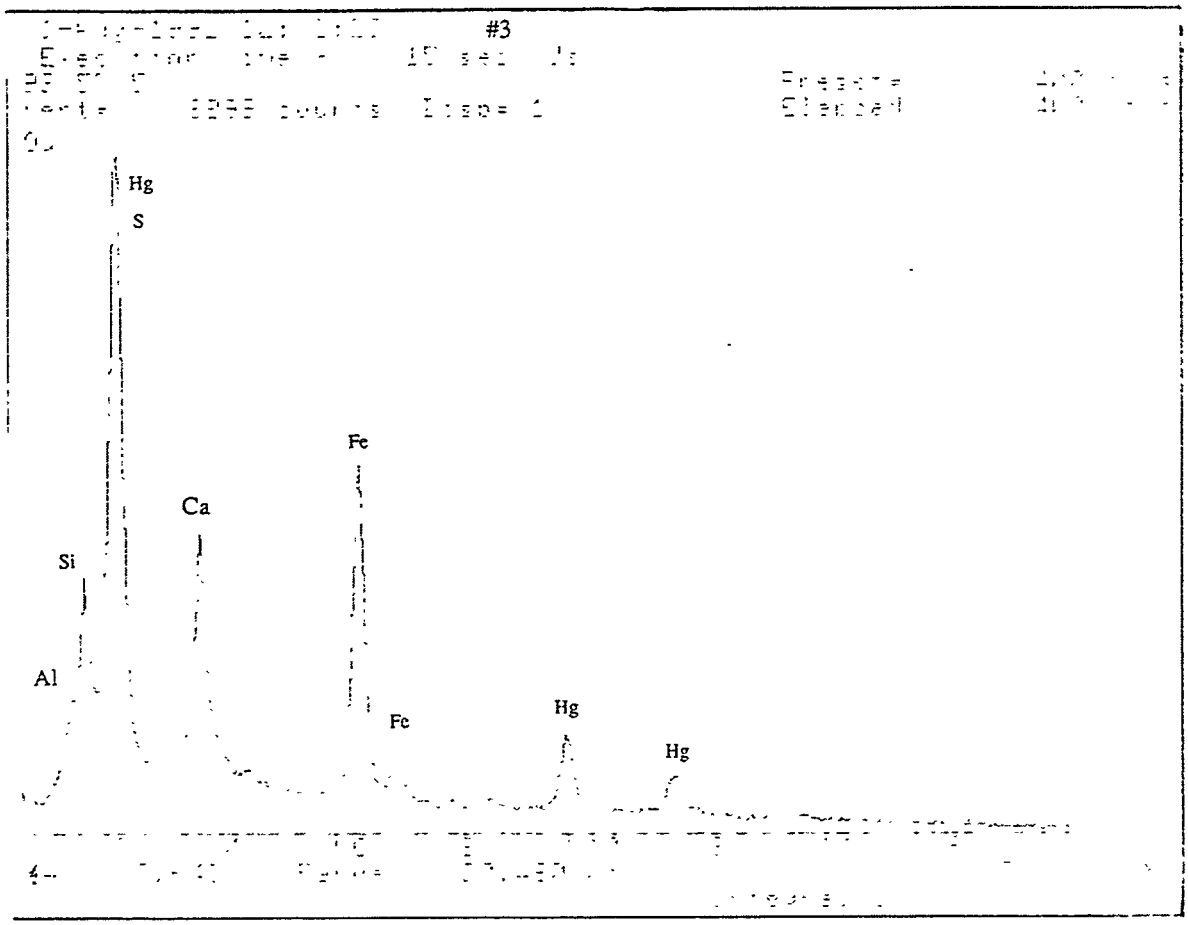


Fig. 5.11c

Fig. 5.12

Fig. 5.13a

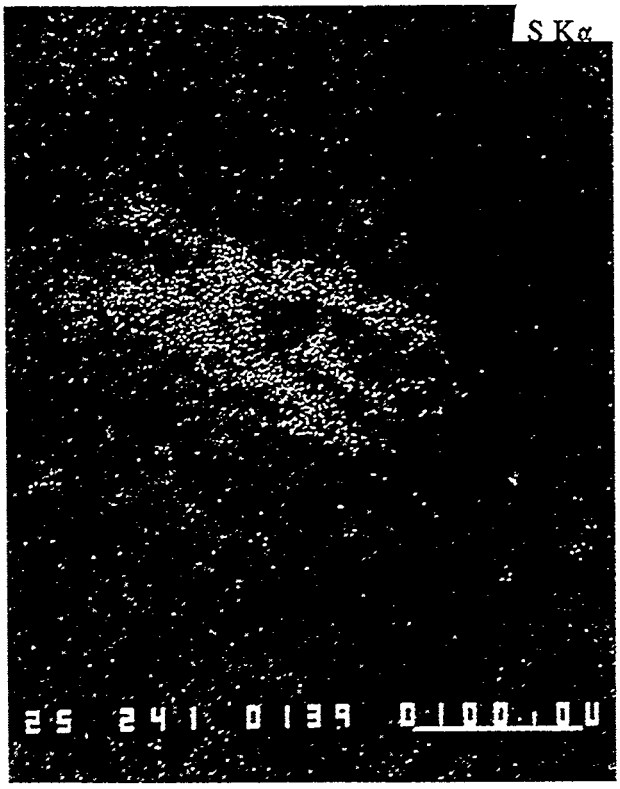


Fig. 5.13b

Fig. 5.13c

Fig. 5.13d

Fig.5.13e

Fig. 5.13f

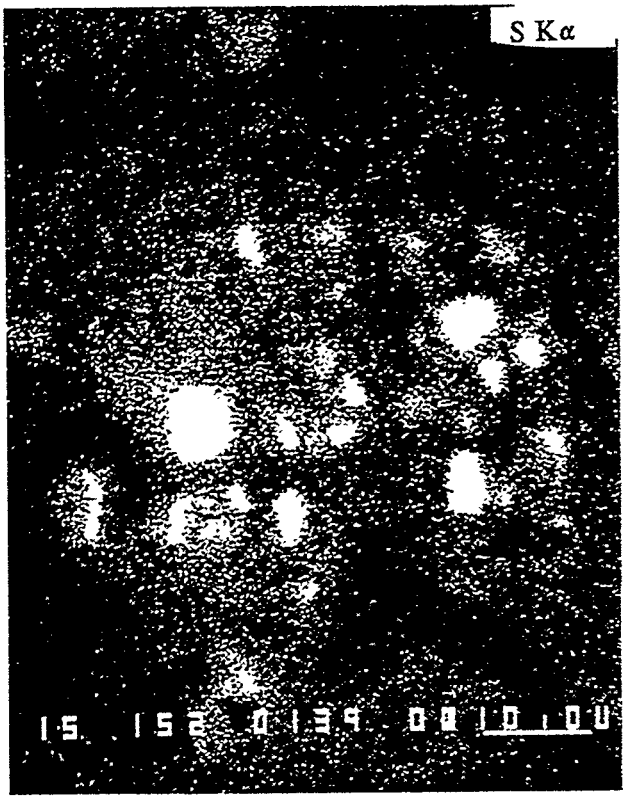
Fig. 5.14

Fig. 5.15a

Fig. 5.15b

Fig. 5.15c

Fig. 5.15d



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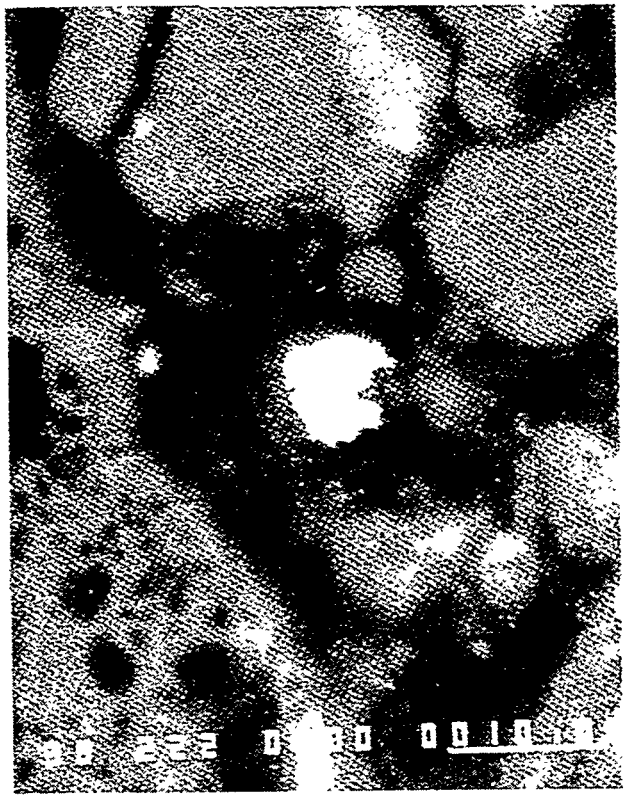

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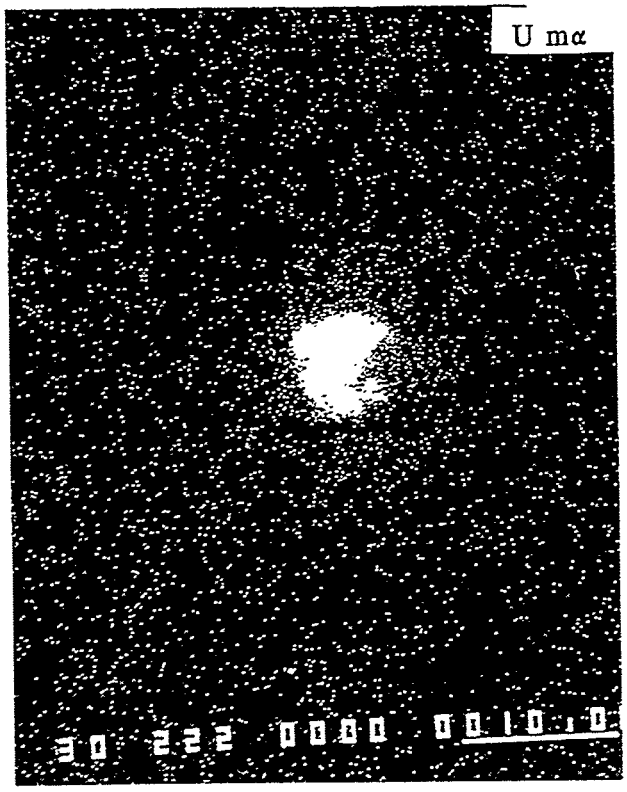

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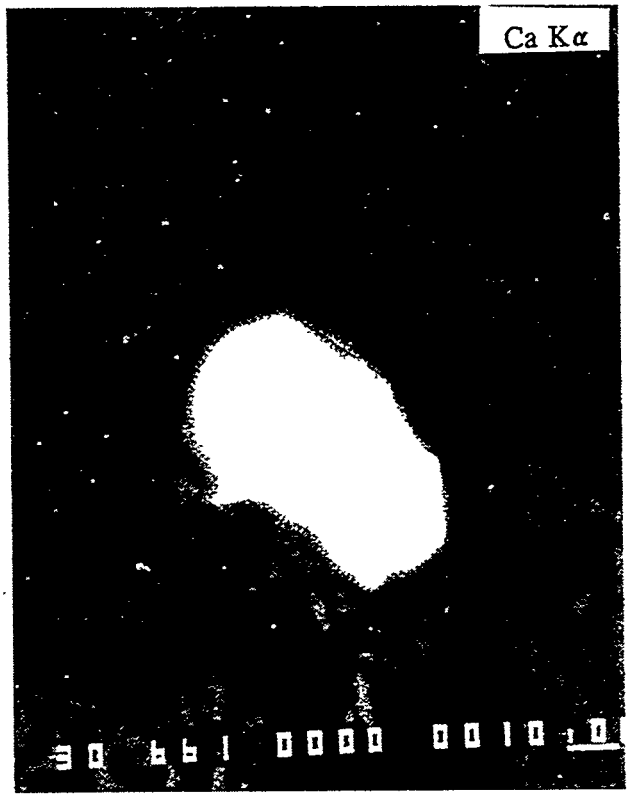

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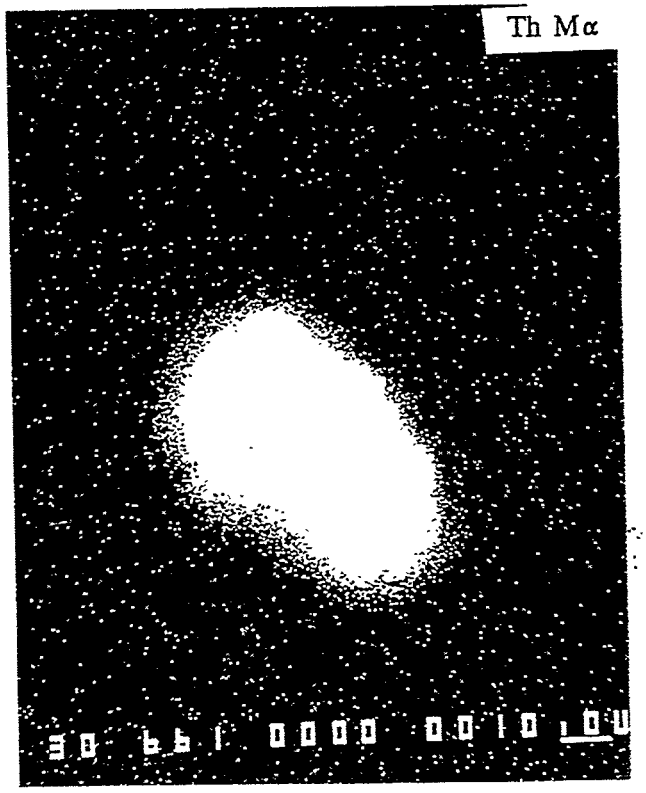

Fig. 5.17b

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BROOKS RAND, LTD.
MARTIN MARIETTA ENERGY SYSTEMS
RADIAN SOIL REPORT
SOW 266

The soil samples were received, stored and analyzed according to their respective Brooks Rand, Ltd., Standard Operating Procedures.

Shipping and Receiving

Eighteen soil samples were received 10/19/93, intact and cooled to 4°C with *blue ice*. Upon receipt, the samples were assigned the batch # OR03, numbers -01 through -20 (excluding -12) and refrigerated to 4°C until sample preparation. We noticed that the 28-day holding time had expired for samples taken 9/21/93. I contacted Teena Rhoden of Radian Corporation and had a conference call with Teena, myself and Walt Oldendorf on October 20, 1993. I was advised to proceed with the analysis as requested for all samples received 10/19/93. Total Mercury sample preparation began the morning of October 22, 1993, including dry weight determinations. Later October 22, 1993, we received instruction from Walt Oldendorf to discard the samples associated with the collection date 9/21/93 and 9/24/93.

A second group of twelve Radian soils were received 10/26/93, intact and cooled to 4°C with *blue ice*. Upon receipt, the samples were assigned the batch # OR03, numbers -21 through -32 and refrigerated to 4°C until sample preparation.

Analytical

Approximately 2.0 grams of soil sample were dried at 85°C for at least 24 hours for dry weight determination.

For Methyl Mercury, approximately 1.0 gram of soil sample was distilled 10/22/93 and 10/29/93. The Methyl Mercury analysis was troublesome because the concentrations were very high and initial attempts to analyze the distillate swamped the instrument. The samples had to be screened for Total Mercury to determine proper aliquot amounts that would be within the linearity of the instrument. All samples were analyzed as one batch 11/16/93 by aqueous phase ethylation, room temperature Tenax trap precollection, isothermal GC separation and CVAFS detection.

For Total mercury determination, approximately 1.0 gram of soil sample was digested in 10 ml of 7:3 HNO_3:H_2SO_4 concentrated acid moisture. The samples were refluxed for 3 hours. After digestion, Mercury free water and 1 ml BrCl were added to volume (25.6 ml). Sample aliquots are analyzed using $SnCl_2$ reduction, single-stage gold amalgamation, and cold vapor atomic fluorescence spectrometry.

Sample ZN3341326 was spilled and needed to be redistilled for MeHg analysis. The sample was lost.

Results

All results are reported in ug/g, on a dry weight basis and without blank subtraction.

111693M1.XLS
110293T2.XLS

All QC is in control.

102793T1.XLS

Sample ZE5381425, OR03-17, was run in triplicate at runs 2.12, 2.13, 2.14. The % RSD for the triplicate is 30%. The % RSD for runs 2.12 and 2.13 is 7%. The average value of 2.12, 2.13 and 2.14 is reported in the summary of results although the average of 2.12 and 2.13 may be more representative of the sample.

All other QC is in control.

Miscellaneous

Because we already had the 9/21/93 collection date samples in process before being notified to discard we are billing out for the dry weight and sample prep time. The amount charged will be seven dry weights and technician prep time of $50.00. The samples were not pulled from the work plan so we are offering the Total Mercury data for the samples at no charge to SOW 266.

I certify this data package is in compliance with the terms and conditions agreed upon by Radian Corporation/Martin Marietta Energy Systems and BRL both technically and for completeness, for other than the conditions detailed above. In addition, I certify, that to the best of my knowledge and belief, the data as reported are true and has been authorized by the QA Director, as verified by the following signature.

Dorthy Spurling

Dorthy Spurling
QA Director

BROOKS RAND, LTD.

Martin Marietta Energy Systems
Radian Soil Report
SOW 266

CASE NARRATIVE ..... i-ii

SUMMARY OF RECEIVING AND ANALYSIS DATES 1

SUMMARY OF Methyl Hg AND TOTAL Hg RESULTS 2

111693M1.XLS 3-4
Associated Data - Methyl Hg 5-12

DISTILLATION LOGS - Methyl Hg Sample Prep 13-14

110293T2.XLS 15-16
Associated Data - Total Hg 17-31

102793T1.XLS 32-34
Associated Data - Total Hg 35-55

102293R1.XLS 56
Dry Weight Determination 57

110393R1.XLS 58
Dry Weight Determination 59

110693T2.XLS 60
Screening Run 61-73

STANDARD REFERENCE MATERIALS
NRCC BEST-1 74-75
IAEA-356 76-95

BRL RECEIVING - 10/19/93 96
CHAIN OF CUSTODY
No.S02602 97
No.S02607 98
No.S02613 99
No.S02629 100
No.So2635 101
AIRBILL - 10/12/93 102

BRL RECEIVING - 10/26/93 103
CHAIN OF CUSTODY
No.S01037 104
No.S01036 105
AIRBILL - 10/25/93 106

BROOKS RAND, LMT.

SUMMARY OF RECEIVING AND ANALYSIS DATES

RADIAN SOILS

Martin Marietta Energy Systems, SOW 266

12/13/93

Sample ID	Batch #	Lab ID	Date Received	MeHg Prep Date	Me Hg Date Analyzed	Total Hg Prep Date	T-Hg Date Analyzed
ZN3341124	OR-3	01	10/19/93	**	**	10/22/93	10/27/93
ZN3341225	OR-3	02	10/19/93	**	**	10/22/93	10/27/93
ZN3341326	OR-3	03	10/19/93	**	**	10/22/93	10/27/93
ZN3341439	OR-3	05	10/19/93	**	**	10/22/93	10/27/93
ZN3371228	OR-3	06	10/19/93	**	**	10/22/93	10/27/93
ZN3371329	OR-3	07	10/19/93	**	**	10/22/93	10/27/93
ZE5441221	OR-3	08	10/19/93	**	**	10/22/93	10/27/93
ZE5441423	OR-3	09	10/19/93	**	**	10/22/93	10/27/93
ZE5442122	OR-3	10	10/19/93	**	**	10/22/93	10/27/93
ZE5442324	OR-3	11	10/19/93	**	**	10/22/93	10/27/93
ZN3372129	OR-3	13	10/19/93	**	**	10/22/93	10/27/93
ZN3773121	OR-3	14	10/19/93	**	**	10/22/93	10/27/93
ZE5381223	OR-3	15	10/19/93	10/22/93	11/16/93	10/22/93	10/27/93
ZE5381324	OR-3	16	10/19/93	10/22/93	11/16/93	10/22/93	10/27/93
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ZN0001145	OR-3	19	10/19/93	10/22/93	11/16/93	10/22/93	10/27/93
ZN3371331	OR-3	20	10/19/93	10/22/93	11/16/93	10/22/93	10/27/93
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ZN3373121	OR-3	26	10/26/93	10/29/93	11/16/93	10/26/93	11/2/93
ZN3341124	OR-3	27	10/26/93	10/29/93	11/16/93	10/26/93	11/2/93
ZN3341225	OR-3	28	10/26/93	10/29/93	11/16/93	10/26/93	11/2/93
ZN3341326	OR-3	29	10/26/93	10/29/93	***	10/26/93	11/2/93
ZN3341439	OR-3	30	10/26/93	10/29/93	11/16/93	10/26/93	11/2/93
ZN3371329	OR-3	31	10/26/93	10/29/93	11/16/93	10/26/93	11/2/93
ZN3371228	OR-3	32	10/26/93	10/29/93	11/16/93	10/26/93	11/2/93

**Walt Oldendorf phoned 10/22/93 to advise us to discard these samples. They were already in the work plan so we do have T-Hg results for those samples.

***Sample lost

Dorthy Spurling

Dorthy Spurling
QA Director

12/03/93
Page 1 of 2

Hg Analysis Results
111693M1.XLS

			Analyst:	L.Liang	Project:	Oak Ridge		Matrix:	Soil	Anal. Req.:	MeHg	
	Sample Calculations			Sample Weight	Dilution Volume	Analyzed Volume			Result	Dry wt	Result	Result
Sample ID	Run	Batch #	Lab ID	(g)	(mL)	(mL)	PH	pg	(ng/g,wet)	%	(ng/g, dry)	(ug/g, dry)
ZE5441423	1.10	OR-03	22	0.982	25.6	0.5	281	324.3	16.9	74%	22.9	0.023
ZE5441221	1.12	OR-03	21	1.056	25.6	0.5	227	262.0	12.7	75%	16.9	0.017
ZE5381324	1.13	OR-03	16	1.118	25.6	1.0	83.0	95.8	2.19	81%	2.71	0.003
ZE5381223	1.14	OR-03	15	0.981	25.6	0.5	433	499.8	26.1	74%	35.1	0.035
ZN3371331	1.15	OR-03	20	0.983	25.6	1.0	154	177.8	4.63	98%	4.70	0.005
ZE5442324	1.19	OR-03	24	1.071	25.6	0.5	193	222.8	10.6	74%	14.4	0.014
ZE5442122	1.20	OR-03	23	1.081	25.6	0.5	137	158.1	7.49	77%	9.74	0.010
ZN3341124	1.21	OR-03	27	1.043	25.6	0.5	184	212.4	10.4	61%	17.1	0.017
ZN3341439	1.22	OR-03	30	1.155	25.6	0.5	36.0	41.6	1.84	75%	2.47	0.002
ZN3371228	1.23	OR-03	32	1.141	25.6	0.5	99.9	115.3	5.17	77%	6.75	0.007
ZE0001145	1.24	OR-03	19	1.159	25.6	0.5	12.5	14.4	0.64	85%	0.75	0.001
ZN3341225	1.26	OR-03	28	0.952	25.6	0.5	307	354.4	19.1	59%	32.1	0.032
ZN3373121	1.27	OR-03	26	1.045	25.6	0.5	175	202.0	9.90	73%	13.6	0.014
ZN3371228	1.28	OR-03	31	1.048	25.6	0.5	30.5	35.2	1.72	77%	2.24	0.002
ZE5382124	2.01	OR-03	18	1.220	25.6	0.5	275	317.4	13.3	74%	18.1	0.018
ZE53821241	1.09	OR-03	17 B	1.666	25.6	0.5	400	461.7	14.2	73%	19.4	0.019
ZE53821241	1.11	OR-03	17 C	1.014	25.6	0.5	333	384.4	19.4	73%	26.6	0.027
								Average:	16.8		23.0	
								RSD:	22%		22%	
ZN3372129	1.25	OR-03	25 A	1.008	25.6	1.0	235	271.3	6.89	72%	9.59	0.010
ZN3372129	1.16	OR-03	25 B	1.041	25.6	1.0	251	289.7	7.12	72%	9.92	0.010
								Average:	7.01		9.76	
								RSD:	2%		2%	

Dorthy Spurling

Dorthy Spurling, QA Director

BROOKS RAND, LMT.					
SUMMARY OF RECEIVING AND ANALYSIS DATES					
RADIAN SOILS					
Martin Marietta SOW 266					
12/13/93					
Sample ID	Batch #	Lab ID	Me Hg ug/g, dry	T-Hg ug/g, dry	Comments
ZN3341124	OR-3	01	**	443*	
ZN3341225	OR-3	02	**	177	
ZN3341326	OR-3	03	**	46.8	
ZN3341439	OR-3	05	**	3.17	
ZN3371228	OR-3	06	**	3.42	
ZN3371329	OR-3	07	**	0.200	
ZE5441221	OR-3	08	**	122	
ZE5441423	OR-3	09	**	191	
ZE5442122	OR-3	10	**	209	
ZE5442324	OR-3	11	**	182	
ZN3372129	OR-3	13	**	56.2*	
ZN3773121	OR-3	14	**	351	
ZE5381223	OR-3	15	0.035	823	
ZE5381324	OR-3	16	0.003	47.5	
ZE5881425	OR-3	17	0.023*	552*	T-Hg %RSD Out of Control, see case narrative
ZE5382124	OR-3	18	0.018	226	
ZN0001145	OR-3	19	0.005	0.220	
ZN3371331	OR-3	20	0.005	65.1	
ZE5441221	OR-3	21	0.017	214	
ZE5441423	OR-3	22	0.023	191	
ZE5442122	OR-3	23	0.010	128	
ZE5442324	OR-3	24	0.144	356	
ZN3372129	OR-3	25	0.010	18.6*	
ZN3373121	OR-3	26	0.014	299	
ZN3341124	OR-3	27	0.017	353	
ZN3341225	OR-3	28	0.032	171	
ZN3341326	OR-3	29	**	30.4	***sample lost, see case narrative
ZN3341439	OR-3	30	0.002	1.21	
ZN3371329	OR-3	31	0.002	0.271*	
ZN3371228	OR-3	32	0.007	4.41	
*average of replicate analyses					
**not analyzed for Me-Hg					

Dorthy Spurling

Dorthy Spurling
QA Director

Hg Analysis Results
111693M1.XLS

Standards

Run	pg	PH/PA	Corr. fac.
1.06	500	433.0	1.155
1.03	300	280.0	1.071
1.07	300	238.0	1.261
1.17	300	245.0	1.224
2.02	300	285.0	1.053
2.12	300	261.0	1.149
1.01	100	83.2	1.202
1.08	100	81.2	1.232
2.11	100	96.0	1.042
1.04	5	5.0	1.000
2.03	5	6.1	0.820
		Average:	1.154
		St.dev.:	0.0824
		Slope:	1.1319
		Intercept:	3.7241
		RSQ:	0.9830

Bubbler:

Reagent:

Blanks

Run	PH/PA
1.02	0.3
1.18	0.5
Average:	0.4
Stdev:	0.1
2.04	1.7

Method Detection Limit (pg)

Blanks:	0.49
Low pg standards:	2.69

Standard Reference Materials

Run	Sample ID	Certified Value (ng/g)	Sample Weight (g)	Dilution Volume (mL)	Anal. Vol. (mL)	PH	pg	Recovery (ng/g)	% Rec.
1.05	IAEA-356	5.256	1.066	25.6	1	198	226.6	5.441	104%

Spike Recoveries on another customer's samples

Run	Sample ID	Spike amt. (pg)	Sample Volume (mL)	PH	Unspiked pg	Spiked pg	Rec. (pg)	% Recovery
2.05	PTI 069	100	44.2	110	8.2	127.3	119.1	119%
2.07	PTI 094	100	44.9	107	2.7	123.8	121.1	121%
2.09	PTI 070	100	44.1	103	4.0	119.2	115.2	115%

Dorthy Spurling

Dorthy Spurling, QA Director

7-5

Hg Analysis Sheet

☐Total Hg ☒MeHg ☐Hg(II) ☐Other:_____ Project/Matrix: OR. Soil

Analyst: Liang
Date: 11/16-93
QA: High☒ Research☐
OR Batch

Blank $\bar{X}$: 0.4 ± 0.1
N= 2
RB: 1.7

Calibration Coefficient $\bar{X}$: 1.153 (7.7%)
SD: 0.088
N=: 9

Run	Trap	Sample ID	Sample vol/wt.	Dilution Volume	Analyzed volume	BrCl (pg)	PH/PA	pg (x cor.fact.)	Result ng/g
1		100 pg		~~100 ml~~ 83.2-0.4			83.2	1.2077	
2		BB					0.3		
3		300 pg					280	1.0359	
4		5 pg					5.0		
5		CRM IAEA-356	1.066 g	25.6 ml	1 ml		198		5.435 103.4
6		500 pg					433	1.1558	
7		300 pg					238	1.2626	
8		100 pg					81.2	1.2376	
9		#17B	1.166 g	25.6 ml	0.5 ml		400	459.2	20.17 19.732
10		#22	0.982 g	"	"		281	322.0	16.79
11		#17C	1.014 g	"	"		333	382.0	19.29
12		#21	1.056 g	"	"		227	259.8	12.59
13		#16	1.118 g	"	1 ml		83.0	93.74	2.146
14		#15	0.981 g	"	0.5 ml		433	497.3	25.95
15		#20	0.983 g	"	1 ml		154	175.6	4.573
16		#25B	1.041 g	"	1 ml		251	287.4	7.069 6.971
17		200 pg					245	1.2265	
18		BB					0.5		
19		#24	1.071 g	25.6 ml	0.5 ml		193	220.6	10.54
20		#23	1.081 g	"	"		137	156.0	7.389
21		#27 1.043 g	1.043 g	"	"		184	210.2	10.32
22		#30	1.155 g	"	"		36.0	39.55	1.753
23		#32	1.141 g	"	"		99.9	113.2	5.081
24		#19	1.159 g	"	"		12.5	12.45	0.5501
25		#25A	1.008 g	"	1 ml		235	269.0	6.832
26		#28	0.952 g	"	0.5 ml		307	352.0	18.93
27		#26	1.045 g	"	"		175	199.8	9.790
28		#31	1.048 g	"	"		30.5	33.21	1.622

Calculation Equation: $\frac{(400-1.7)\times 1.153 \times 25.6}{0.5 \times 1.166} = 20.17$ ng/g

Comments: after distillation and solvent extraction; #17A and #29 results on another sheet, please check.

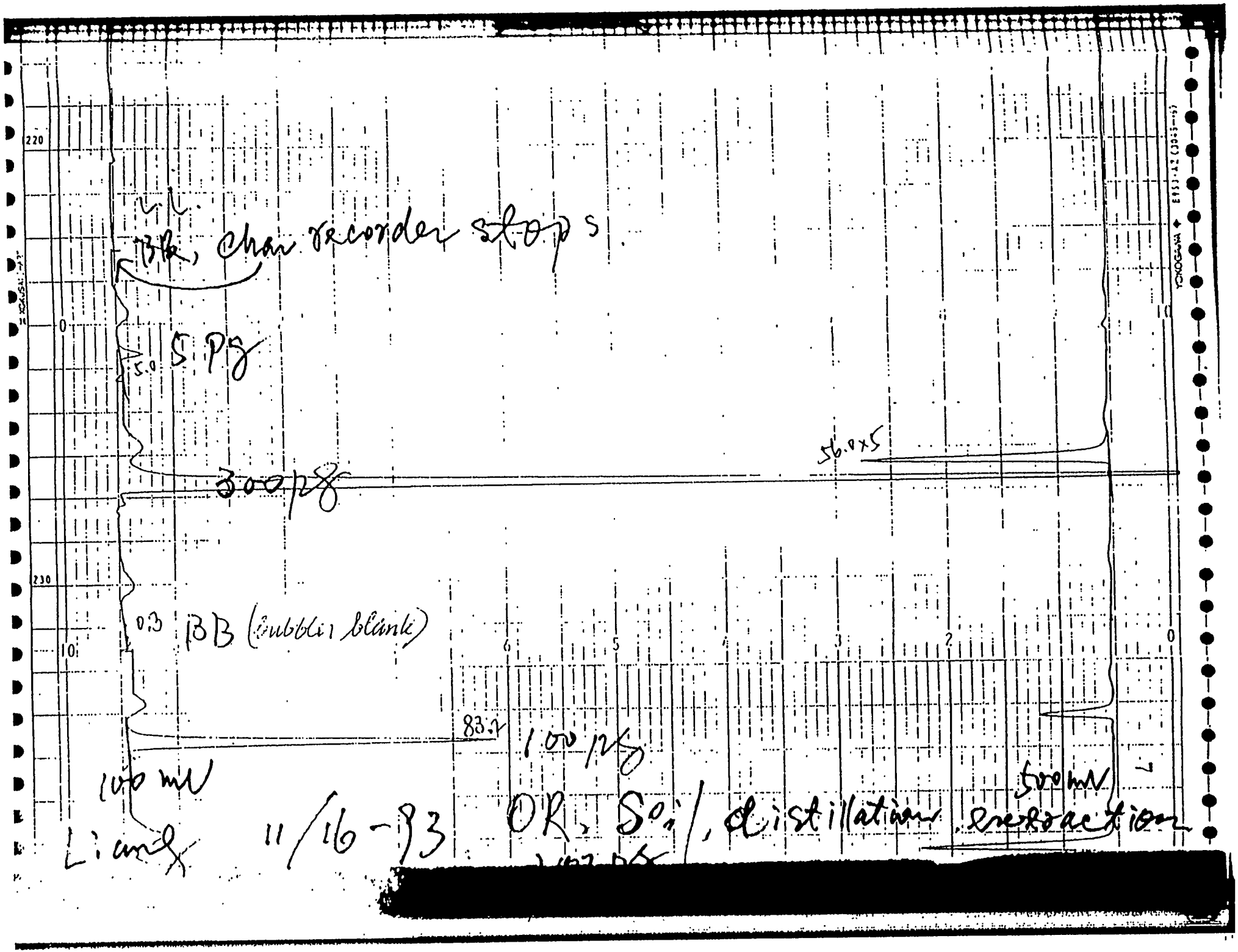
CRM, IAEA-356 Certified 5.257 ng/g.

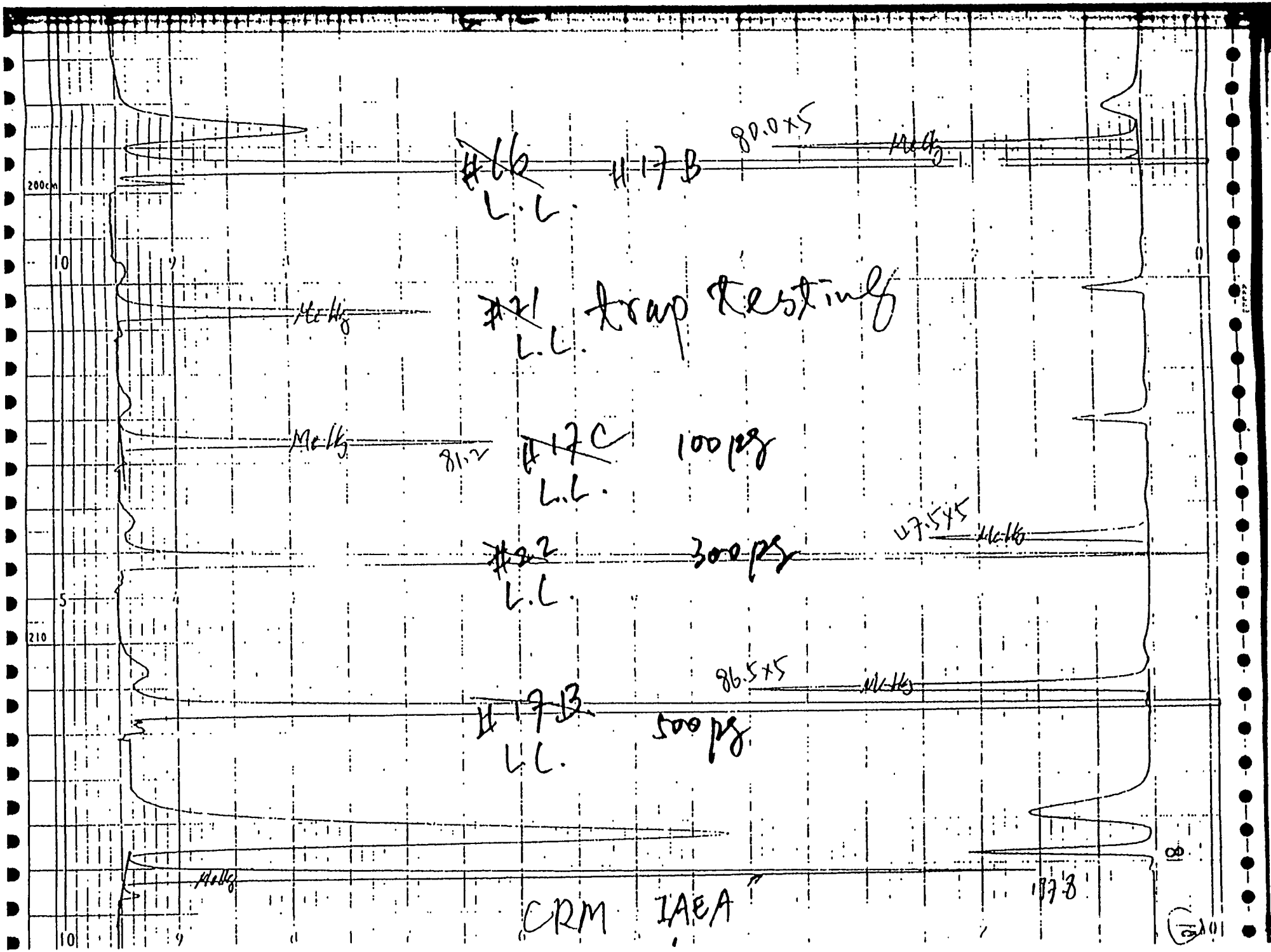
Hg Analysis Sheet

Project: OR. Soil Date: 11/16-93

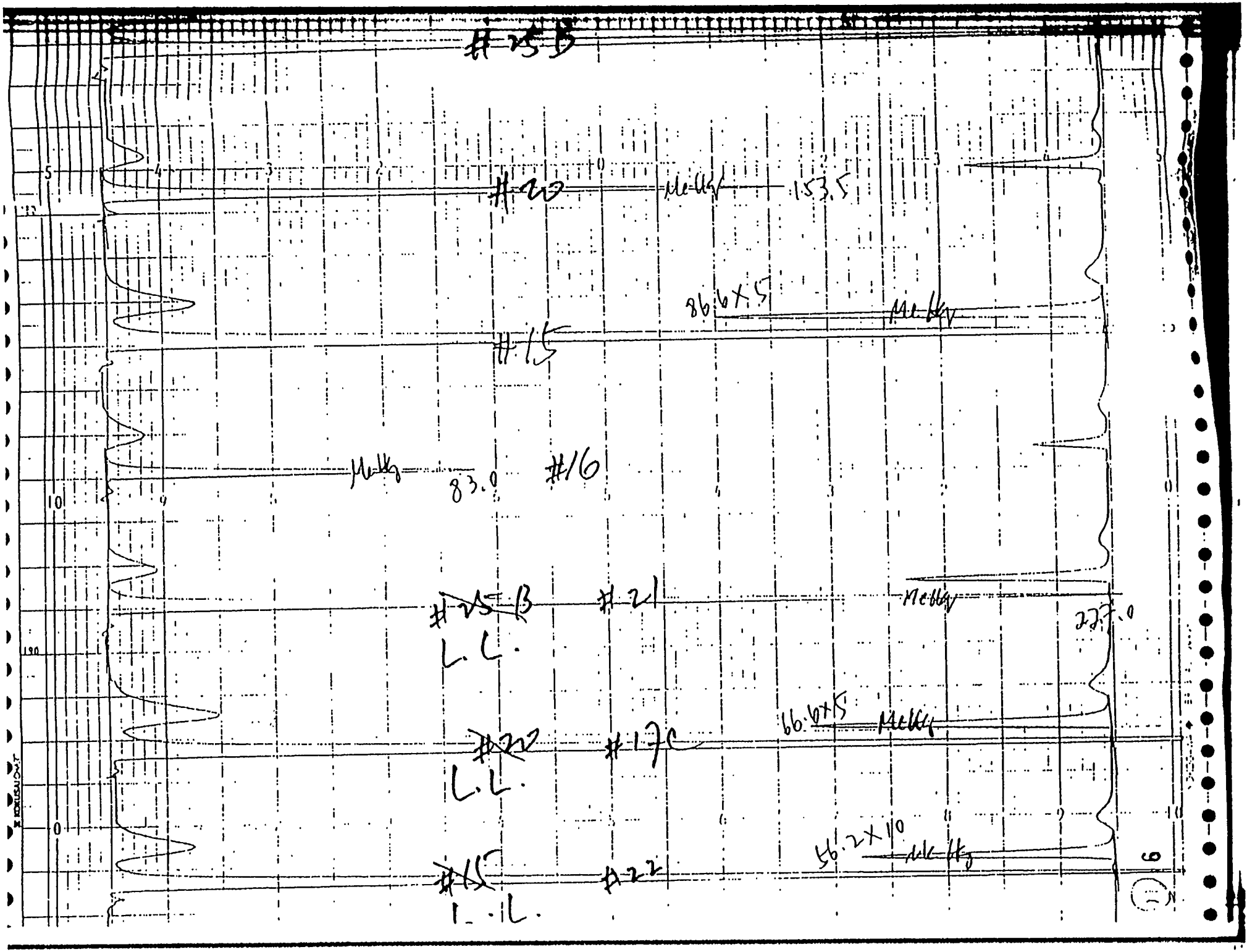
Run	Trap	Sample ID	Sample vol/wt.	Dilution Volume	Analyzed volume	BrCl (pg)	PH/PA	pg (x cor.fact.)	Result ng/g
1		#18	1.220	25.6 ml	0.5 ml		275	315.1	13.22
2		300 pg					285	1.0541	
3		5 pg					6.1		
4		RB					1.7		
5	G2	PT1-063+	44.2				110		
6	1	PT1-067	44.4				8.8		
7		PT1-034+	44.8				107		Gauge
8		PT1-034	44.10				4.0		Glass
9	✓	PT1-070+	44.1				103		
10		PT1-070	44.6				5.2		
11		100 pg					86	1.0461	
12		300 pg					261	1.1512	
13									
14									
15									
16									
17									
18									
19									
20									
21									
22									
23									
24									
25									
26									
27									
28									
29									
30									
31									
32									

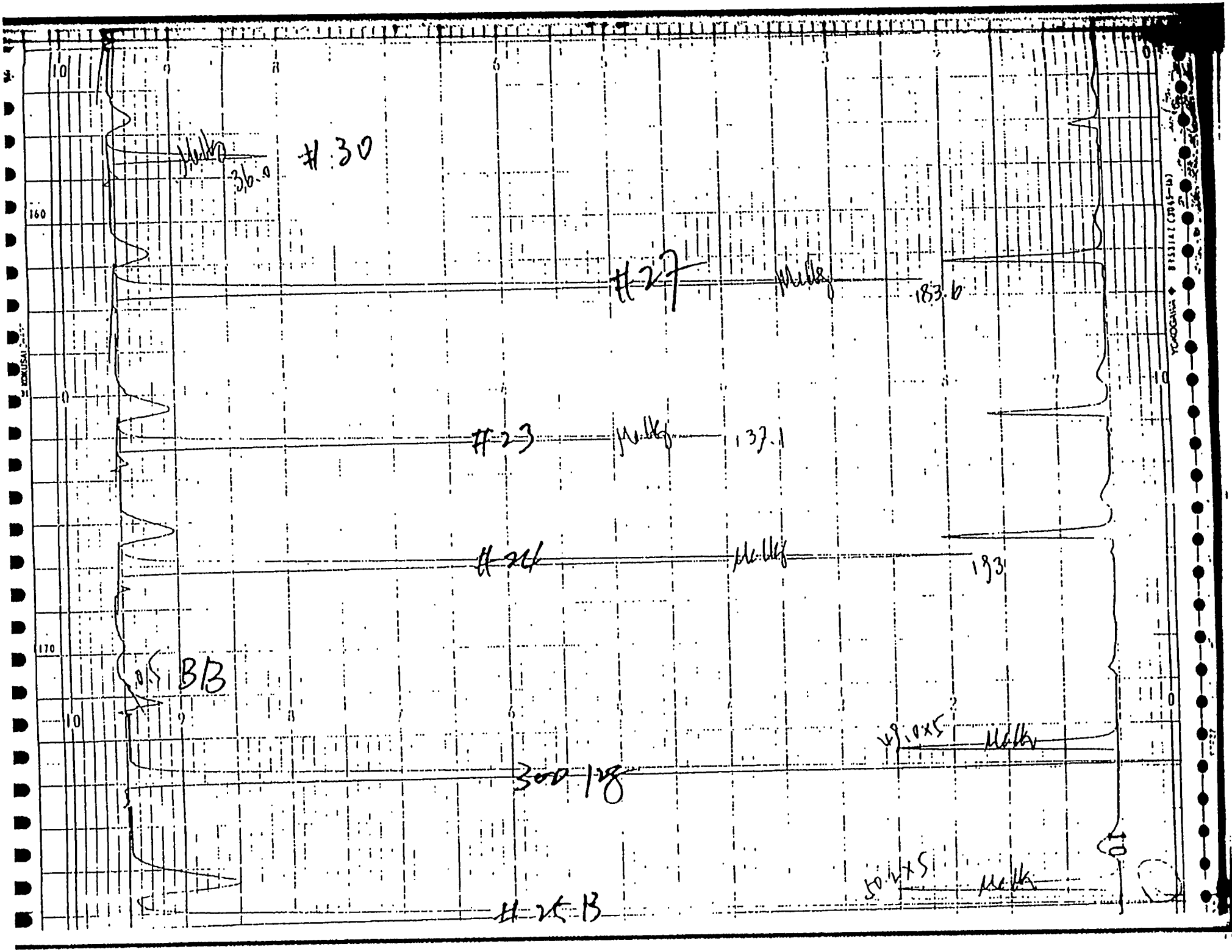
Comments:


220
230
0
10
6
5
4
3
2
0
BB, char recorder stops
5.0
5 pg
300 pg
56.0x5
0.3
BB (bubbler blank)
83.2
100 pg
100 mV
500 mV
Liang
11/16-93
OR, Soil, distillation, extraction

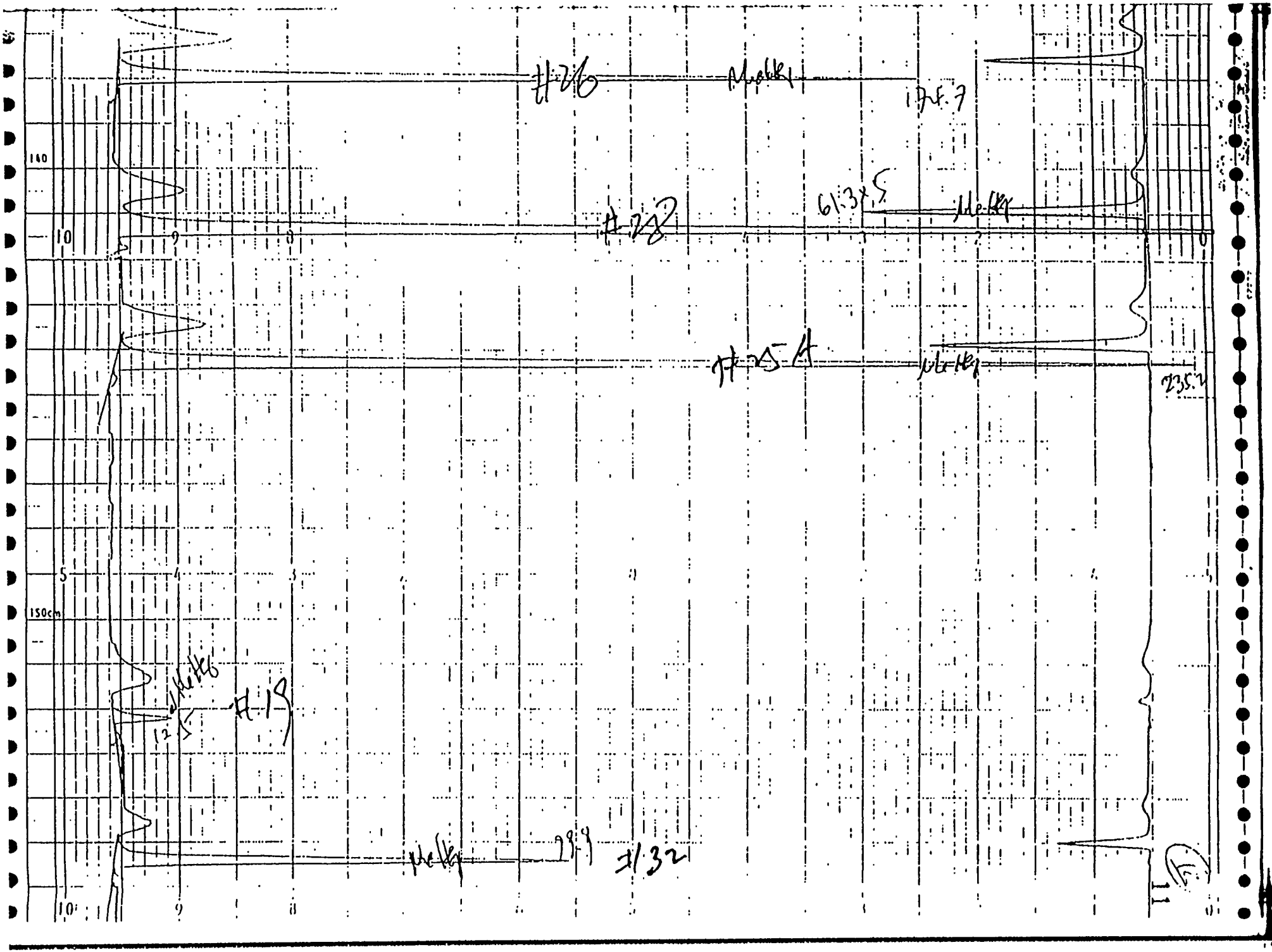


#16
L.L.
#17B
80.0x5
Melt
#21
L.L.
trap testing
Melt
Melt
81.2
#17C
L.L.
100 pg
#22
L.L.
300 pg
47.5x5
Melt
86.5x5
Melt
#17B
L.L.
500 pg
Melt
CRM IAEA
200cm
210
10
5
10


#25 B
#20
Mc Hg
153.5
86.6 x 5
Mc Hg
#15
Mc Hg
83.0
#16
#25 B
L.L.
#21
Mc Hg
227.0
66.6 x 5
Mc Hg
#22
L.L.
#17C
56.2 x 10
Mc Hg
#15
L.L.
#22



#30
36.0
#27
183.6
#23
137.1
#24
193
BB
300 lbs
#25 B



140
150cm
#26
#28
61.3x5
#25A
235.2
#19
12.5
#32

Date: 10-22-93 Distillation Technician: STEVE SENTER

Block Number	Sample I.D.	Distillation Time	Gas Flow Rate
1	Blank 2	1:35	10
2	#19	1:00	10
3	#18	1:00	10
4	#16	1:00	10
5	#15	1:15	10
6	Blank 1	1:25	10
7	#20	1:00	10
8	#17 A	1:00	10
9	B	1:00	10
10	C	1:00	10
11			
12			
13			
14			
15			

RADIAN Corp Soil Samples

Temperature(°C): 1-5: 44.6 6-10: 144.7 11-15: _____
Comments: 3P 3P
17A prepped out on 10-21
Blank prepped out on 10-22

Date: 10-27-93 Distillation Technician: STEVE SENTER

Block Number	Sample I.D.	Distillation Time	Gas Flow Rate
1	Distl.	1:15	10
2	North	1:00	1
3	IDEM-3534	1:02	1
4	IDEM-3543	1:02	1
5	PTI-004	2:55	1
6	PTI-333	3:15	1
7	PT-650	3:15	1
8	PT-204	3:15	1
9	PT-3316	3:15	1
10	PT-3316 D	4:10	1
11	Blank 1	3:55	1
12	Blank 2	3:35	1
13	PTI-709	3:35	1
14	PTI-951 Spike A	3:35	1
15	" Spike B	3:35	1

IN House Samples

OTHERS ARE FOR NYSDEC

100µl Stnd Spike

Temperature(°C): 1-5: 145.6 6-10: 144.7 11-15: 145.5
Comments:

15P 15 5/P 15 13/P

51

Date: 10-24-93 Distillation Technician: STEVE SEATER

Block Number	Sample I.D.	Distillation Time	Gas Flow Rate
1	#22	1:10	10
2	#25	1.00	"
3	#26	1.00	"
4	#24	1:05	"
5	#27	1.00	"
6	#28	1:07	"
7	#25A	1:10	"
8	#24	1:00	"
9	#23	:55	
10	#21	1:10	
11	BLANK 1	1:10	
12	BLANK 2	1:10	
13	#30	:55	"
14	#31	:55	"
15	#32	:55	"

Red Lion Soil Samples

Temperature(°C): 1-5: 145.2 (295 F) 6-10: 145.2 (295 F) 11-15: 146.7 (296 F)
Comments:

Date: 11-1-93 Distillation Technician: STEVE SEATER

Block Number	Sample I.D.	Distillation Time	Gas Flow Rate
1	PTI-129	2:40	10
2	PTI-114	2:45	"
3	BLANK 2	2:35	"
4	BLANK 1	2:40	"
5	PTI-110	2:25	"
6	PTI-122	3:25	"
7	PTI-143	3:15	"
8	PTI-106	3:15	"
9	PTI-118	3:20	
10	PTI-120	3:50	
11	PTI-516 A	3:45	
12	" B	3:20	
13	" C	3:30	
14	SPIKE 1	3:30	
15	SPIKE 2	3:45	"

PTI WATER Samples Dated 10-14-93

Temperature(°C): 1-5: 145.1 (293 F) 6-10: 145.2 (295 F) 11-15: 146.4 (296 F)
Comments:

15(1)

11/03/93
Page 1 of 2

Hg Analysis Results
110293T2.XLS

		Analyst:	Z. Huang	*Project:*	Radian		*Matrix:*	Soil	*Anal. Req.:*	Total Hg	
Sample Calculations											
Sample ID	Run	Batch # and Lab ID	Sample weight (g)	Dilution Volume (mL)	Analyzed Volume (mL)	DB	PH/PA	pg	Uncorr. Result (ug/g, wet)	dry wt. %	Corr. Result (ug/g, dry)
	Digestion Blanks										
	1.09	DB-1		25.6	5.0		332	86.4	442		
	1.10	DB-2		25.6	10.0		571	149	380		
								Average:	411		
	Samples										
ZE5441221	1.11	OR3-21	1.129	2560	0.025	411	6796	1768	160	75.1%	213
ZE5441423	1.12	OR3-22	1.125	2560	0.025	411	5954	1549	141	74.0%	190
ZE5442122	1.13	OR3-23	1.131	2560	0.025	411	4170	1085	98.2	76.9%	128
ZE5442324	1.14	OR3-24	1.010	2560	0.025	411	9987	2598	263	74.0%	356
ZN3373121	1.15	OR3-26	1.151	2560	0.025	411	9388	2442	217	72.7%	299
ZN3372129	1.21	OR3-25-A	1.152	2560	0.50	411	11347	2951	13.1	71.8%	18.3
ZN3372129	1.22	OR3-25-B	0.808	2560	0.50	411	7992	2079	13.2	71.8%	18.3
ZN3372129	1.23	OR3-25-C	1.138	2560	0.50	411	11733	3052	13.7	71.8%	19.1
								Average	13.3		18.6
								RSD:	3%		3%
ZN3341124	1.24	OR3-27	1.087	2560	0.025	411	8777	2283	215	61.0%	353
ZN3341225	1.25	OR3-28	1.119	2560	0.025	411	4271	1111	102	59.3%	171
ZN3341326	1.26	OR3-29	1.040	2560	0.25	411	8364	2175	21.4	70.4%	30.4
ZN3371228	1.27	OR3-32	1.056	2560	1.0	411	5358	1394	3.38	76.6%	4.41
ZN3341439	1.28	OR3-30	1.042	25.6	0.10	411	14181	3688	0.906	74.7%	1.21
ZN3371329	2.01	OR3-31-A	0.985	25.6	0.10	411	3008	782	0.203	76.9%	0.264
ZN3371329	2.02	OR3-31-B	0.995	25.6	0.10	411	3195	831	0.213	76.9%	0.277
								Average	0.208		0.271
								RSD:	4%		4%

Dorthy Spurling
Dorthy Spurling, QA Director

Hg Analysis Results
110293T2.XLS

Standards			Calibration		Blanks				
Run	pg	PH/PA	Coefficient		Run	PH/PA			
1.18	4000	15535	0.2575		1.01	5			
1.05	2500	9384	0.2664		1.08	5			
1.04	2000	7973	0.2508		1.20	5			
1.19	2000	7431	0.2691		2.06	5			
2.05	2000	7279	0.2748		Average:	5			
1.07	1500	5884	0.2549						
1.03	1000	3949	0.2532						
1.02	500	1969	0.2539						
2.04	20	108	0.1852		Method Detection Limit (pg)				
1.06	10	43	0.2326			Blanks:	3.90		
1.17	10	61	0.1639		Low pg standards:		26.2		
		Average:	0.2601						
		RSD:	3%						
		Slope:	0.2596						
		Intercept:	10.1531						
		RSQ:	0.9964						

Standard Reference Materials

Run	Sample ID	Sample weight (g)	Dilution Volume (mL)	Analyzed Volume (mL)	PH/PA	pg	Certified Value (ng/g)	Recovery (ng/g)	% Recovery
1.16	BEST-1	0.514	25.6	1.0	7000	1819	0.092	0.091	98%
2.03	BEST-1	0.772	25.6	0.5	5312	1380	0.092	0.092	100%

Dorthy Spurling

Dorthy Spurling, QA Director

Standard 270193-01 · 1.0 ng/mL Hg^{+2}
Exp date: 12/27/93

Hg Analysis Sheet

☒Total Hg ☐MeHg ☐Hg(II) ☐Other:_____ Project/Matrix:_____

Analyst: D. Huang
Date: 11/02/93
QA: High☒ Research☐

Blank $\bar{X}$: 407.9 pg
N= 2

Calibration Coefficient $\bar{X}$: 0.261
SD: 0.009
N=: 8

Run	Trap	Sample ID	Sample vol/wt.	Dilution Volume	Analyzed volume	BrCl (pg)	PH/PA	pg (x cor.fact.)	Result ng/g
1		Blank					<5		
2		500 pg					1969		0.255 pg/PA
3		1000 "					3949		0.254
4		2000 "					7973		0.251
5		2500					9386		0.267
6		10					43		
		1-500					5884		0.255
		Blank					<5		
		Digestion Blank ①		25.6	5.0		332	85.3	7
		②		25.6	10.0		571	147.7	407 pg/PA
		#21	1.129	2560	0.025		3796	1772	161 ng/g
		#22	1.125	"	"		5954	1553	141
		#23	1.131				4170	1087	98.4
		#24	1.010	"	"		9987	2605	264
		#26	1.151	"	"		9382	2449	218
		BEST-1	0.514	25.6	1.00		7000	1826	8.040 pg/PA
		10 pg					61		
		1000 "					15535		0.258 pg/PA
		2000 "					7431		0.269
		Blank					<5		
		#25 ①	1.152	2560	0.50		11347	2960	13.2 ng/g
		②	0.808	"	"		7992	2085	13.2 "
		③	1.138	"	"		11733	3061	13.8 "
		#27	1.001	"	0.025		8777	2291	216
		#28	1.119	"	0.025		4271	1113	102
		#29	1.040		0.25		8364	2182	21.5
		#32	1.056	"	1.00		5358	1397	3.39
		#30	1.042	25.6	0.10		~~3008~~	3700	0.908

Calculation Equation: Pg = (PA-5)*0.261
Comments: 14181

Conc. = (pg × $\frac{DV}{AV}$ − 407) / wt × 10^{-6} ng/g

Hg Analysis Sheet

Project: Radian Date: 10/02/93

Run	Trap	Sample ID	Sample vol/wt.	Dilution Volume	Analyzed volume	BrCl (pg)	PH/PA	pg (x cor.fact.)	Result ug/g
1		3i8	0.985	25.6	0.10		3008	784	0.204
2		"	0.995	25.6	0.10		3195	833	0.214
3		BEST-1	0.772	25.6	0.50		5312	1385	0.091
4		20 pg					10#		
5		2000 pg					7279		2.75 pg
6		Blank					<5		
7									
8									
9									
10									
11									
12									
13									
14									
15									
16									
17									
18									
19									
20									
21									
22									
23									
24									
25									
26									
27									
28									
29									
30									
31									
32									

Comments: BEST-1. 0.092 ug/g Hg

Radian Comparation
.1/02 [illegible] Total Hg

19

CHANNEL A INJECT 11/02/93 12:56:14

AZ 1

11/02/93 12:56:14 CH= "A" PS= 1.

FILE 1. METHOD 5. RUN *** INDEX ***

NAME	CONC	RT	AREA	BC	RF
TOTALS	0.				

Blank

CHANNEL A INJECT 11/02/93 12:59:25

AZ 1

.98

11/02/93 12:59:25 CH= "A" PS= 1.

FILE 1. METHOD 5. RUN *** INDEX ***

500 pg

NAME	CONC	RT	AREA	BC	RF
1	1969.452	0.98	1969452	01	1000.
TOTALS	1969.452		1969452		

CHANNEL A INJECT 11/02/93 13:02:16

AZ 1

.98

11/02/93 13:02:16 CH= "A" PS= 1.

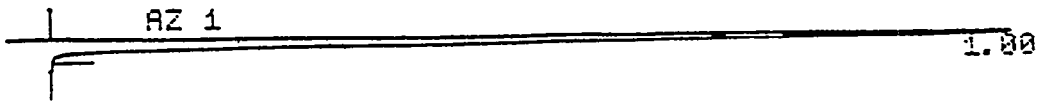
FILE 1. METHOD 5. RUN *** INDEX ***

1000 pg

NAME	CONC	RT	AREA	BC	RF
1	3949.231	0.98	3949231	01	1000.
TOTALS	3949.231		3949231		

111

CHANNEL A INJECT 11/02/93 13:05:52



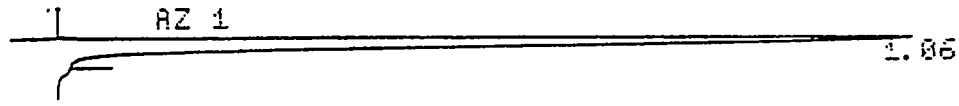
11/02/93 13:05:52 CH= "A" PS= 1.

FILE 1. METHOD 5. RUN *** INDEX ***

2000 pg

NAME	CONC	RT	AREA	BC	RF
1	7972.618	1.	7972618	01	1000.
TOTALS	7972.618		7972618		

CHANNEL A INJECT 11/02/93 13:08:34



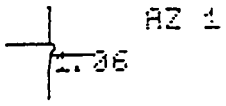
11/02/93 13:08:34 CH= "A" PS= 1.

FILE 1. METHOD 5. RUN *** INDEX ***

2500 pg

NAME	CONC	RT	AREA	BC	RF
1	9384.496	1.06	9384496	01	1000.
TOTALS	9384.496		9384496		

CHANNEL A INJECT 11/02/93 13:12:41



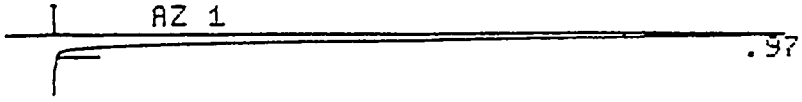
11/02/93 13:12:41 CH= "A" PS= 1.

FILE 1. METHOD 5. RUN *** INDEX ***

101 pg

NAME	CONC	RT	AREA	BC	RF
1	43.262	1.06	43262	01	1000.
TOTALS	43.262		43262		

CHANNEL A INJECT 11/02/93 13:16:09



11/02/93 13:16:09 CH= "A" PS= 1.

FILE 1. METHOD 5. RUN *** INDEX ***

1500 pg

NAME	CONC	RT	AREA BC	RF
1	5884.097	0.97	5884097 01	1000.
TOTALS	5884.097		5884097	

21 (23)

CHANNEL A INJECT 11/02/93 13:19:02

AZ 1

11/02/93 13:19:02 CH= "A" PS= 1.

FILE 1. METHOD 5. RUN *** INDEX ***

Blank

NAME	CONC	RT	AREA BC	RF
TOTALS	0.			

CHANNEL A INJECT 11/02/93 13:22:59

AZ 1

1.02

11/02/93 13:22:59 CH= "A" PS= 1.

FILE 1. METHOD 5. RUN *** INDEX *** Digital Blank

NAME	CONC	RT	AREA BC	RF
1	331.907	1.02	331907 01	1000.
TOTALS	331.907		331907	

CHANNEL A INJECT 11/02/93 13:25:48

AZ 1

1.03

11/02/93 13:25:48 CH= "A" PS= 1.

FILE 1. METHOD 5. RUN *** INDEX ***

D. Blank

NAME	CONC	RT	AREA BC	RF
1	571.007	1.03	571007 01	1000.
TOTALS	571.007		571007	

CHANNEL A INJECT 11/02/93 13:28:45

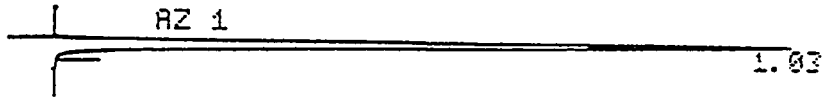
AZ 1

11/02/93 13:28:45 CH= "A" PS= 1.

FILE 1. METHOD 5. RUN *** INDEX *** #21

NAME	CONC	RT	AREA	BC	RF
1	6796.202	0.96	6796202	01	1000.
TOTALS	6796.202		6796202		

CHANNEL A INJECT 11/02/93 13:31:56



11/02/93 13:31:55 CH= "A" PS= 1.

FILE 1. METHOD 5. RUN *** INDEX *** #22

NAME	CONC	RT	AREA	BC	RF
1	5954.251	1.03	5954251	01	1000.
TOTALS	5954.251		5954251		

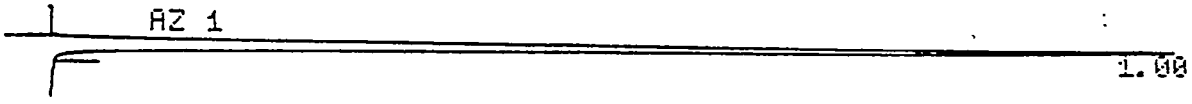
CHANNEL A INJECT 11/02/93 13:36:57

11/02/93 13:36:57 CH= "A" PS= 1.

FILE 1. METHOD 5. RUN *** INDEX *** #23

NAME	CONC	RT	AREA	BC	RF
1	4169.766	1.04	4169766	01	1000.
TOTALS	4169.766		4169766		

CHANNEL A INJECT 11/02/93 13:42:27

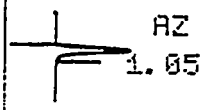


11/02/93 13:42:27 CH= "A" PS= 1.

FILE 1. METHOD 5. RUN *** INDEX *** #24

NAME	CONC	RT	AREA	BC	RF
1	9987.311	1.	9987311	01	1000.

CHANNEL A INJECT 11/02/93 13:54:34

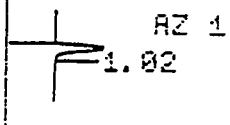


11/02/93 13:54:34 CH= "A" PS= 1.

FILE 1. METHOD 5. RUN *** INDEX ***

NAME	CONC	RT	AREA	BC	RF
1	546.962	1.05	546962	01	1000.
TOTALS	546.962		546962		

CHANNEL A INJECT 11/02/93 13:59:49

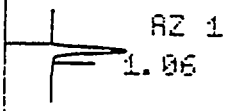


11/02/93 13:59:49 CH= "A" PS= 1.

FILE 1. METHOD 5. RUN *** INDEX ***

NAME	CONC	RT	AREA	BC	RF
1	402.945	1.02	402945	01	1000.
TOTALS	402.945		402945		

CHANNEL A INJECT 11/02/93 14:03:11

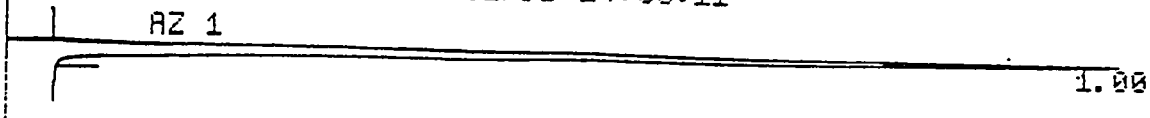


11/02/93 14:03:11 CH= "A" PS= 1.

FILE 1. METHOD 5. RUN *** INDEX ***

NAME	CONC	RT	AREA	BC	RF
1	593.706	1.06	593706	01	1000.
TOTALS	593.706		593706		

CHANNEL A INJECT 11/02/93 14:06:11

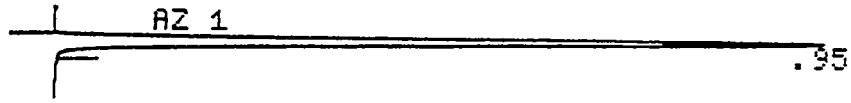


11/02/93 14:06:11 CH= "A" PS= 1.

FILE 1. METHOD 5. RUN *** INDEX ***

NAME	CONC	RT	AREA BC	RF
1	9387.732	1.	9387732 01	1000.
TOTALS	9387.732		9387732	

CHANNEL A INJECT 11/02/93 14:11:01



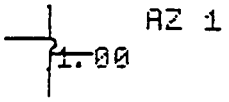
11/02/93 14:11:01 CH= "A" PS= 1.

FILE 1. METHOD 5. RUN *** INDEX ***

BEST-1

NAME	CONC	RT	AREA BC	RF
1	6999.973	0.95	6999973 01	1000.
TOTALS	6999.973		6999973	

CHANNEL A INJECT 11/02/93 14:16:34



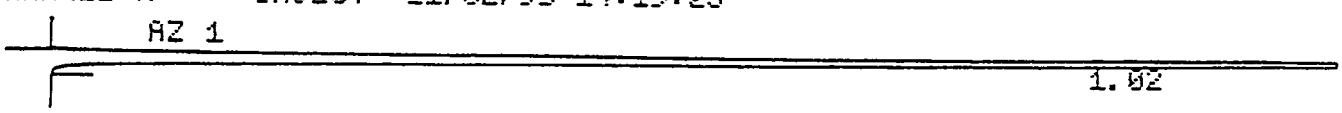
11/02/93 14:16:34 CH= "A" PS= 1.

FILE 1. METHOD 5. RUN *** INDEX ***

10 pg

NAME	CONC	RT	AREA BC	RF
1	60.615	1.	60615 01	1000.
TOTALS	60.615		60615	

CHANNEL A INJECT 11/02/93 14:19:25



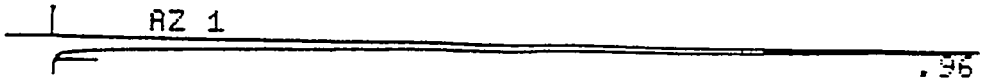
11/02/93 14:19:25 CH= "A" PS= 1.

FILE 1. METHOD 5. RUN *** INDEX ***

400 pg

NAME	CONC	RT	AREA BC	RF
1	15535.274	1.02	15535274 01	1000.
TOTALS	15535.274		15535274	

CHANNEL A INJECT 11/02/93 14:23:06



11/02/93 14:23:06 CH= "A" PS= 1.

FILE 1. METHOD 5. RUN *** INDEX ***

2000 pg 25 (27)

NAME	CONC	RT	AREA BC	RF
1	7430.677	0.96	7430677 01	1000.
TOTALS.	7430.677		7430677	

CHANNEL A INJECT 11/02/93 14:25:49

AZ 1

11/02/93 14:25:49 CH= "A" PS= 1.

FILE 1. METHOD 5. RUN *** INDEX ***

Blank

NAME	CONC	RT	AREA BC	RF
TOTALS	0.			

CHANNEL A INJECT 11/02/93 14:29:16

AZ 1

1.00

11/02/93 14:29:16 CH= "A" PS= 1.

FILE 1. METHOD 5. RUN *** INDEX ***

#25A 0.5/2560

NAME	CONC	RT	AREA BC	RF
1	11347.277	1.	11347277 01	1000.
TOTALS	11347.277		11347277	

CHANNEL A INJECT 11/02/93 14:33:17

AZ 1

.96

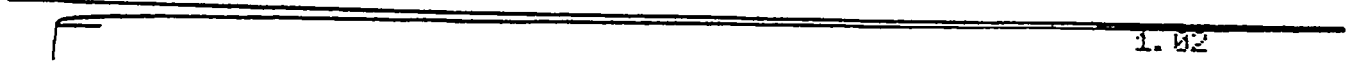
11/02/93 14:33:17 CH= "A" PS= 1.

FILE 1. METHOD 5. RUN *** INDEX ***

#25B

NAME	CONC	RT	AREA BC	RF
1	7992.277	0.96	7992277 01	1000.
TOTALS	7992.277		7992277	

CHANNEL A INJECT 11/02/93 14:36:08

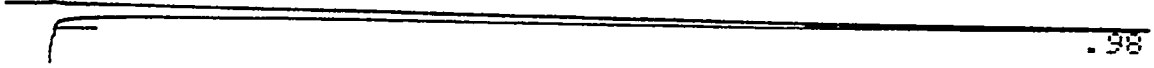


11/02/93 14:36:08 CH= "A" PS= 1.

FILE 1. METHOD 5. RUN *** INDEX ***

NAME	CONC	RT	AREA	BC	RF
1	11732.622	1.02	11732622	01	1000.
TOTALS	11732.622		11732622		

CHANNEL A INJECT 11/02/93 14:39:30

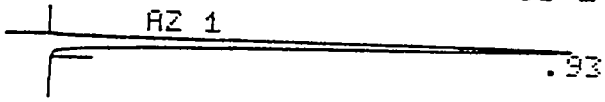


11/02/93 14:39:30 CH= "A" PS= 1.

FILE 1. METHOD 5. RUN *** INDEX ***

NAME	CONC	RT	AREA	BC	RF
1	8777.465	0.98	8777465	01	1000.
TOTALS	8777.465		8777465		

CHANNEL A INJECT 11/02/93 14:44:32



11/02/93 14:44:32 CH= "A" PS= 1.

FILE 1. METHOD 5. RUN *** INDEX ***

NAME	CONC	RT	AREA	BC	RF
1	4271.041	0.93	4271041	01	1000.
TOTALS	4271.041		4271041		

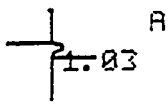
CHANNEL A INJECT 11/02/93 14:49:46

11/02/93 14:49:46 CH= "A" PS= 1.

FILE 1. METHOD 5. RUN *** INDEX ***

NAME				
1	850.288	1.03	850288 01	1000.
TOTALS	850.288		850288	

CHANNEL A INJECT 11/02/93 14:54:14

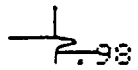


11/02/93 14:54:14 CH= "A" PS= 1.

FILE 1. METHOD 5. RUN *** INDEX ***

NAME	CONC	RT	AREA BC	RF
1	94.898	1.03	94898 01	1000.
TOTALS	94.898		94898	

CHANNEL A INJECT 11/02/93 14:57:04

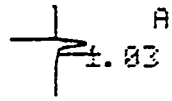


11/02/93 14:57:04 CH= "A" PS= 1.

FILE 1. METHOD 5. RUN *** INDEX ***

NAME	CONC	RT	AREA BC	RF
1	167.905	0.98	167905 01	1000.
TOTALS	167.905		167905	

CHANNEL A INJECT 11/02/93 15:02:48



11/02/93 15:02:48 CH= "A" PS= 1.

FILE 1. METHOD 5. RUN *** INDEX ***

NAME	CONC	RT	AREA BC	RF
1	216.1	1.03	216100 01	1000.
TOTALS	216.1		216100	

CHANNEL A INJECT 11/02/93 15:18:39

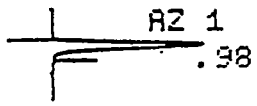
FILE 1. METHOD 5. RUN *** INDEX ***

#29

0.25/2560

NAME	CONC	RT	AREA	BC	RF
1	8364.364	0.96	8364364	01	1000.
TOTALS	8364.364		8364364		

CHANNEL A INJECT 11/02/93 15:21:30

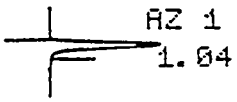


11/02/93 15:21:30 CH= "A" PS= 1.

FILE 1. METHOD 5. RUN *** INDEX ***

NAME	CONC	RT	AREA	BC	RF
1	1281.581	0.98	1281581	01	1000.
TOTALS	1281.581		1281581		

CHANNEL A INJECT 11/02/93 15:24:47

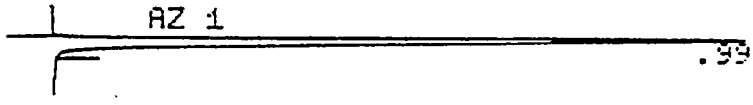


11/02/93 15:24:47 CH= "A" PS= 1.

FILE 1. METHOD 5. RUN *** INDEX ***

NAME	CONC	RT	AREA	BC	RF
1	940.022	1.04	940022	01	1000.
TOTALS	940.022		940022		

CHANNEL A INJECT 11/02/93 15:29:35



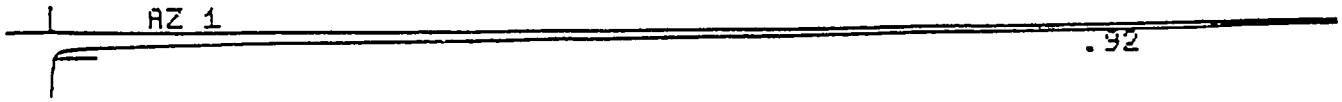
11/02/93 15:29:35 CH= "A" PS= 1.

FILE 1. METHOD 5. RUN *** INDEX ***

#32

1.0/2560

NAME	CONC	RT	AREA	BC	RF
1	5357.938	0.99	5357938	01	1000.
TOTALS	5357.938		5357938		



11/02/93 15:40:46 CH= "A" PS= 1.

29

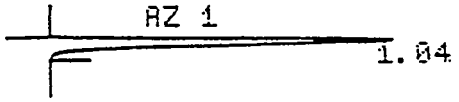
FILE 1. METHOD 5. RUN *** INDEX *** #30

NAME	CONC	RT	AREA	BC	RF
1	14181.304	0.92	14181304	01	1000.
TOTALS	14181.304		14181304		

C.10/25.6

0.10/25.6

CHANNEL A INJECT 11/02/93 15:43:40



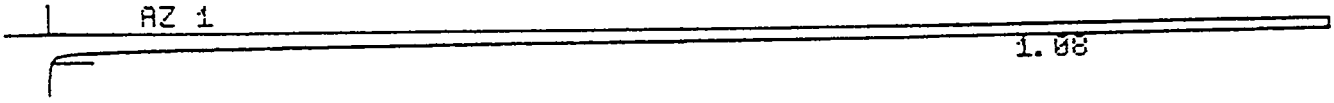
11/02/93 15:43:40 CH= "A" PS= 1.

FILE 1. METHOD 5. RUN *** INDEX *** #31

D.10/25.6

NAME	CONC	RT	AREA	BC	RF
1	3008.455	1.04	3008455	01	1000.
TOTALS	3008.455		3008455		

CHANNEL A INJECT 11/02/93 15:46:49

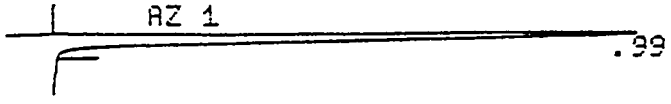


11/02/93 15:46:49 CH= "A" PS= 1.

FILE 1. METHOD 5. RUN *** INDEX ***

NAME	CONC	RT	AREA	BC	RF
1	38364.977	1.08	38364977	01	1000.
TOTALS	38364.977		38364977		

CHANNEL A INJECT 11/02/93 15:50:09



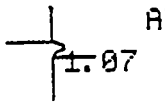
(15)

11/02/93 15:50:09 CH= "A" PS= 1.

FILE 1. METHOD 5. RUN *** INDEX *** BEST-1

1	5311.505	0.99	5311505 01	1000.
TOTALS	5311.505		5311505	

CHANNEL A INJECT 11/02/93 15:53:08 30

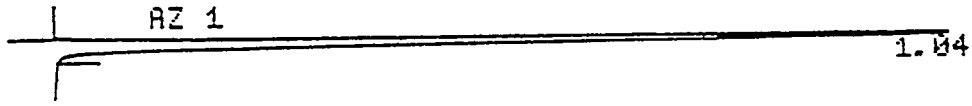


11/02/93 15:53:08 CH= "A" PS= 1.

FILE 1. METHOD 5. RUN *** INDEX *** 20 pg

NAME	CONC	RT	AREA BC	RF
1	107.759	1.07	107759 01	1000.
TOTALS	107.759		107759	

CHANNEL A INJECT 11/02/93 15:56:11

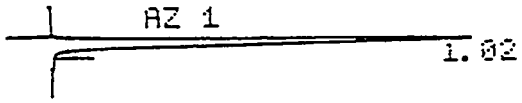


11/02/93 15:56:11 CH= "A" PS= 1.

FILE 1. METHOD 5. RUN *** INDEX *** 200 pg

NAME	CONC	RT	AREA BC	RF
1	7278.521	1.04	7278521 01	1000.
TOTALS	7278.521		7278521	

CHANNEL A INJECT 11/02/93 16:00:01



11/02/93 16:00:01 CH= "A" PS= 1.

FILE 1. METHOD 5. RUN *** INDEX *** 31#

0.10/25.6

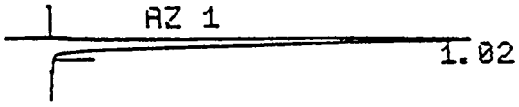
NAME	CONC	RT	AREA BC	RF
1	3194.74	1.02	3194740 01	1000.
TOTALS	3194.74		3194740	

CHANNEL A INJECT 11/02/93 16:02:43

(25) AZ 1

TOTALS 7278.521 7278521

CHANNEL A INJECT 11/02/93 16:00:01



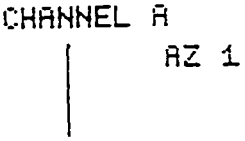
11/02/93 16:00:01 CH= "A" PS= 1.

FILE 1. METHOD 5. RUN *** INDEX *** 31#

NAME	CONC	RT	AREA	BC	RF
1	3194.74	1.02	3194740	01	1000.
TOTALS	3194.74		3194740		

0.10/25.6

CHANNEL A INJECT 11/02/93 16:02:43



11/02/93 16:02:43 CH= "A" PS= 1.

FILE 1. METHOD 5. RUN *** INDEX *** Blank

NAME	CONC	RT	AREA	BC	RF
TOTALS	0.				

(55)

11/1/93
Page 1 of 3

Hg Analysis Results
102793T1.XLS

		Analyst:	Z. Huang	Project:	Radian		Matrix:	Soil	Anal. Req.:	Total Hg	
				Batch#:	OR3						
	Sample Calculations										
Sample ID	Run	Batch and Lab ID	Sample weight (g)	Dilution Volume (mL)	Analyzed Volume (mL)	Dig. Blank	PH/PA	pg (x C.F.)	Uncorr. Result (ug/g, wet) (pg/blank)	Dry wt. %	Uncorr. Result (ug/g,dry)
Digestion Blanks											
	1.09	1		25.6	20		1171	301.0	385		
	1.10	2		25.6	10		559	143.7	368		
	1.11	3		25.6	10		454	116.7	299		
	1.12	4		25.6	10		390	100.2	257		
								Average:	327		
ZN3341439	1.13	OR3-5	1.012	25.6	0.025	327	7909	2276	2.30	73%	3.17
ZN3371228	1.14	OR3-6	1.041	25.6	0.025	327	9521	2740	2.69	79%	3.42
ZN3371329	1.15	OR3-7	1.157	25.6	0.500	327	12997	3740	0.165	83%	0.200
ZN3341326	1.16	OR3-3	1.196	256	0.025	327	12697	3654	31.3	67%	46.8
ZE5381324	1.17	OR3-16	0.922	256	0.025	327	12038	3464	38.5	81%	47.5
ZN3372129	1.18	OR3-13-A	0.989	256	0.025	327	12535	3607	37.3	71%	52.5
ZN3372129	1.19	OR3-13-B	0.999	256	0.025	327	13586	3910	40.1	71%	56.3
ZN3372129	1.20	OR3-13-C	1.235	256	0.025	327	17830	5131	42.5	71%	59.8
								Average:	40.0		56.2
								RSD:	6.5%		6.5%
ZN0001145	1.21	OR3-19	1.069	25.6	0.250	327	5897	1697	0.16	75%	0.22
ZN3341124	1.28	OR3-1-A	1.104	2560	0.050	327	23227	6684	310	66%	473
ZN3341124	2.01	OR3-1-B	1.000	2560	0.050	327	17578	5058	259	66%	395
ZN3341124	2.02	OR3-1-C	1.101	2560	0.050	327	22609	6506	303	66%	461
								Average:	291		443
								RSD:	9.5%		9.5%

Dorthy Spurling
Dorthy Spurling, QA Director

(32)

Hg Analysis Results
102793T1.XLS

ZN3341225	2.03	OR3-2	1.074	2560	0.025	327	4082	1175	112	63%	177
ZE5441221	2.04	OR3-8	1.179	2560	0.025	327	4003	1152	100	82%	122
ZE5441423	2.05	OR3-9	1.092	2560	0.025	327	5653	1627	153	80%	191
ZE5442112	2.06	OR3-10	0.935	2560	0.025	327	5234	1506	165	79%	209
ZE5442324	2.07	OR3-11	0.994	2560	0.025	327	5039	1450	149	82%	182
ZN3773121	2.08	OR3-14	1.058	2560	0.025	327	9257	2664	258	73%	351
ZE5382124	2.09	OR3-18	1.076	2560	0.025	327	6081	1750	167	74%	226
ZN3371331	2.10	OR3-20	1.147	2560	0.025	327	2495	718	64.1	98%	65.1
ZE5381223	2.11	OR3-15	1.052	2560	0.025	327	21848	6287	612	74%	823
ZE5381425	2.12	OR3-17-A	0.991	2560	0.025	327	10671	3071	317	73%	435
ZE5381425	2.13	OR3-17-B	1.061	2560	0.025	327	12564	3616	349	73%	478
ZE5381425	2.14	OR3-17-C	1.140	2560	0.025	327	20957	6031	542	73%	742
								Average:	403		552
								RSD:	30%		30%
					Average for runs 2.12 and 2.13 only:				333		456
					RSD for runs 2.12 and 2.13 only:				7%		7%

Dorthy Spurling
Dorthy Spurling, QA Director

Hg Analysis Results
102793T1.XLS

Standards			Calibration		Blanks	
Run	pg	PH/PA	Coefficient		Run	PH/PA
1.24	5000	21451	0.2331		1.01	5
1.23	4000	16212	0.2467		1.08	5
1.05	3000	12148	0.2470		1.27	5
1.04	2000	8422	0.2375		2.19	5
1.07	2000	7200	0.2778		Average:	5
2.18	2000	8637	0.2316		St.dev.:	0
1.26	1500	5643	0.2658			
1.03	1000	3812	0.2623			
1.02	500	1605	0.3115		Method Detection Limit (pg)	
1.25	40	139	0.2878		Blanks:	3.86
1.06	20	72	0.2778		Low pg standards:	29.6
2.17	20	73	0.2740			
		Average:	0.2570			
		RSD:	10%			
		Slope:	0.2295			
		Intercept:	162.8869			
		RSQ:	0.9944			

Standard Reference Materials

Run	Sample ID	Sample Weight (g)	Dilution Volume (mL)	Analyzed Volume (mL)	Dig. Blank	PH/PA	Certified Value (ng/g)	Recovered Value (ng/g)	% Recovery
1.22	BEST-1	0.608	25.6	1.00	327	8677	0.092	0.093	101%
2.15	BEST-1	0.826	25.6	1.00	327	12411	0.092	0.098	107%
2.16	BEST-1	0.749	25.6	1.00	327	10838	0.092	0.095	103%

Dorthy Spurling

Dorthy Spurling, QA Director

Standard
Exp. date : 12/27/93

Hg Analysis Sheet

Page 1 of 2

☒Total Hg ☐MeHg ☐Hg(II) ☐Other: _____ Project/Matrix: R.C./ Soil

Analyst: Z. Huang
Date: 10/27/93
QA: High☒ Research☐

Blank $\bar{X}$: 324 pg
N= 4

Calibration Coefficient $\bar{X}$: 0.257
SD: 0.026
N=: 9

Run	Trap	Sample ID	Sample vol/wt.	Dilution Volume	Analyzed volume	BrCl (pg)	PH/PA	pg (x cor.fact.)	Result ug/g
1		Blank					<5		
2		500 pg					1605		0.313 pg
3		1000 "					3812		0.263
4		2000 "					8422		0.238
5		3000 "					12143		0.247
6		20 "					72		
7		2000 "					7200		0.273
8		Blank					<5		
9		Digestion Blank (1)		25.6	20.		1171	300	384 pg
10		(2)		"	10		559	142	364 "
11		(3)		"	"		454	115	295 "
12		(4)		"	"		390	98.9	253 "
13		#5	1.012 g	"	0.025		7909	2031	2.05 ug
14		#6	1.041	"	0.025		9521	2446	2.41
15		#7	1.157	"	0.50		12997	3339	0.147
16		#3	1.196	256.	0.025		11697	3262	27.9
17		#16	0.922	"	"		12038	3092	34.3
18		#13C	0.959	"	"		12535	3220	33.3
19		(2)	0.999	"	"		13586	3490	35.8
20		(3)	1.235	"	"		17830	4581	33.0
21		#19	1.069	25.6	0.25		5897	1514	0.145
22		BCSI-1	0.603	25.6	1.00		8677	2229	0.093
23		4000 pg					16212		0.247
24		5000 pg					21451		0.233
25		40 pg					139		
26		~~Blank~~ 1500 pg					5643		0.266
27		~~24~~ 2 Blank					<5		
28		#10	1.101	2560	0.050		23227	5968	277

Calculation Equation: $pg = (PA - 5) * 0.257$, $Con. = \frac{(pg \times \frac{V_2}{V_3} - 324)}{W} \times 10^{-6}$ ug/g

Comments: _____

(12)

Hg

Hg Analysis Sheet

Project: Radian Corp. Date: 10/27/93

Run	Trap	Sample ID	Sample vol/wt.	Dilution Volume	Analyzed volume	BrCl (pg)	PH/PA	pg (x cor.fact.)	Result ng/g
1		#1 ②	1.000	560	0.050		17578	4516	231
2		③	1.101	560	0.050		22609	5809	270
3		#2	1.074		0.025		40882	1048	99.9
4		#8	1.179				40063	1027	89.2
5		#9	1.092				5653	1452	136
6		#10	0.935				5234	1344	147
7		#11	0.994				5039	1294	133
8		#12	1.058				9257	2378	230
9		#13	1.076				6081	1562	149
10		#22	1.147				2495	640	57.1
11		#15	1.052				21848	5614	546
12		#17 ①	0.991				10671	2741	283
13		④	1.061				12566	3228	312
14		⑤	1.140	〃	〃		20957	5385	484
15		BEST-1	0.826	25.6	1.00		12411	3188	0.093
16		BEST-1	0.749	25.6	1.00		10838	2784	0.095
17		20 pg					73		
18		200 pg					8637		0.232 pg/g
19		Blank					<5		
20									
21									
22									
23									
24									
25									
26									
27									
28									
29									
30									
31									
32									

Comments:

AZ 1

.91

ER 0

10/26/93 17:48:39 CH= "A" PS= 1.

FILE 1. METHOD 5. RUN *** INDEX ***

NAME	CONC	RT	AREA	BC	RF
1	72168.596	0.91	72168596	01	1000.
TOTALS	72168.596		72168596		

CHANNEL A INJECT 10/26/93 18:19:54

AZ 1

1.01

ER 0

10/26/93 18:19:54 CH= "A" PS= 1.

FILE 1. METHOD 5. RUN *** INDEX ***

NAME	CONC	RT	AREA	BC	RF
1	58436.655	1.01	58436655	01	1000.
TOTALS	58436.655		58436655		

10/27/93 Huang - Total Hg in Soil Radian Corp
High Blk

CHANNEL A INJECT 10/27/93 11:18:42

AZ 1

10/27/93 11:18:42 CH= "A" PS= 1.

FILE 1. METHOD 5. RUN *** INDEX ***

NAME	CONC	RT	AREA	BC	RF
TOTALS	0.				

Blank

CHANNEL A INJECT 10/27/93 11:27:50

AZ 1

.39

BZ

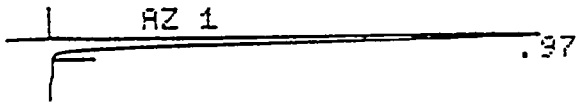
10/27/93 11:27:50 CH= "A" PS= 1.

FILE 1. METHOD 5. RUN *** INDEX ***

500 pg

NAME	CONC	RT	AREA BC	RF
1	1604.876	0.99	1604876 01	1000.
TOTALS	1604.876		1604876	

CHANNEL A INJECT 10/27/93 11:32:00



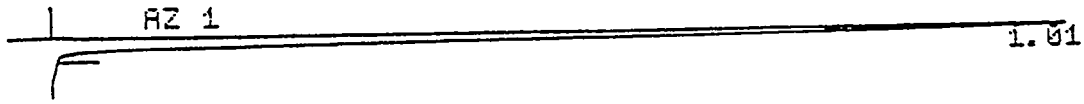
10/27/93 11:32:00 CH= "A" PS= 1.

FILE 1. METHOD 5. RUN *** INDEX ***

1000 pg

NAME	CONC	RT	AREA BC	RF
1	3811.796	0.97	3811796 01	1000.
TOTALS	3811.796		3811796	

CHANNEL A INJECT 10/27/93 11:37:37



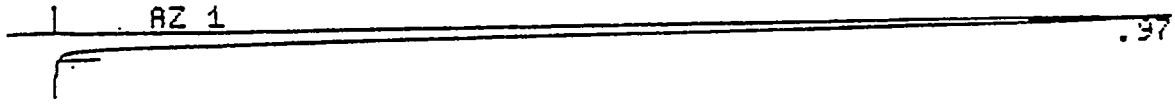
10/27/93 11:37:37 CH= "A" PS= 1.

FILE 1. METHOD 5. RUN *** INDEX ***

2000 pg

NAME	CONC	RT	AREA BC	RF
1	8422.288	1.01	8422288 01	1000.
TOTALS	8422.288		8422288	

CHANNEL A INJECT 10/27/93 11:41:56



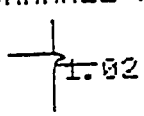
10/27/93 11:41:56 CH= "A" PS= 1.

FILE 1. METHOD 5. RUN *** INDEX ***

3000 pg

NAME	CONC	RT	AREA BC	RF
1	12147.804	0.97	12147804 01	1000.

CHANNEL A INJECT 10/27/93 11:45:33



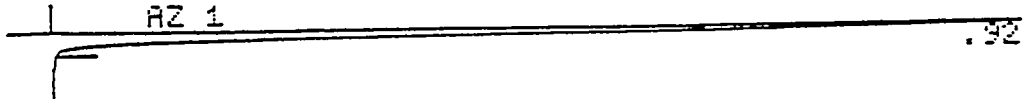
10/27/93 11:45:33 CH= "A" PS= 1.

FILE 1. METHOD 5. RUN *** INDEX ***

2 pg

NAME	CONC	RT	AREA BC	RF
1	72.052	1.02	72052 01	1000.
TOTALS	72.052		72052	

CHANNEL A INJECT 10/27/93 11:49:16



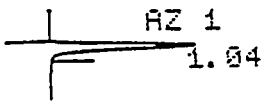
10/27/93 11:49:16 CH= "A" PS= 1.

FILE 1. METHOD 5. RUN *** INDEX ***

2000 pg

NAME	CONC	RT	AREA BC	RF
1	7200.32	0.92	7200320 01	1000.
TOTALS	7200.32		7200320	

CHANNEL A INJECT 10/27/93 11:52:17



10/27/93 11:52:17 CH= "A" PS= 1.

FILE 1. METHOD 5. RUN *** INDEX ***

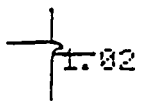
Digestion Blank

NAME	CONC	RT	AREA BC	RF
1	1171.053	1.04	1171053 01	1000.
TOTALS	1171.053		1171053	

CHANNEL A INJECT 10/27/93 11:55:16

10/27/93 11:55:16 CH= "A" PS= 1.

CHANNEL A INJECT 10/27/93 11:45:33



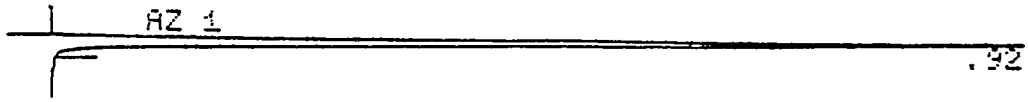
10/27/93 11:45:33 CH= "A" PS= 1.

FILE 1. METHOD 5. RUN *** INDEX ***

20 pg

NAME	CONC	RT	AREA BC	RF
1	72.052	1.02	72052 01	1000.
TOTALS	72.052		72052	

CHANNEL A INJECT 10/27/93 11:49:16



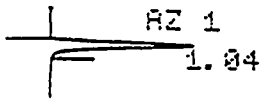
10/27/93 11:49:15 CH= "A" PS= 1.

FILE 1. METHOD 5. RUN *** INDEX ***

2000 pg

NAME	CONC	RT	AREA BC	RF
1	7200.32	0.92	7200320 01	1000.
TOTALS	7200.32		7200320	

CHANNEL A INJECT 10/27/93 11:52:17



10/27/93 11:52:17 CH= "A" PS= 1.

FILE 1. METHOD 5. RUN *** INDEX ***

Digestion Blank ①

NAME	CONC	RT	AREA BC	RF
1	1171.053	1.04	1171053 01	1000.
TOTALS	1171.053		1171053	

CHANNEL A INJECT 10/27/93 11:55:16

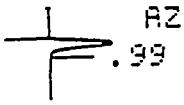
10/27/93 11:55:16 CH= "A" PS= 1.

FILE 1. METHOD 5. RUN *** INDEX ***

NAME	CONC	RT	AREA BC	RF
TOTALS	0.			

41

CHANNEL A INJECT 10/27/93 11:58:23

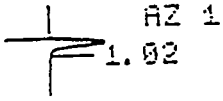


10/27/93 11:58:23 CH= "A" PS= 1.

FILE 1. METHOD 5. RUN *** INDEX *** Digestion Blank ①

NAME	CONC	RT	AREA BC	RF
1	558.663	0.99	558663 01	1000.
TOTALS	558.663		558663	

CHANNEL A INJECT 10/27/93 12:12:45

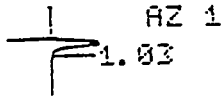


10/27/93 12:12:45 CH= "A" PS= 1.

FILE 1. METHOD 5. RUN *** INDEX *** Digestion Blank ③

NAME	CONC	RT	AREA BC	RF
1	454.02	1.02	454020 01	1000.
TOTALS	454.02		454020	

CHANNEL A INJECT 10/27/93 12:15:34

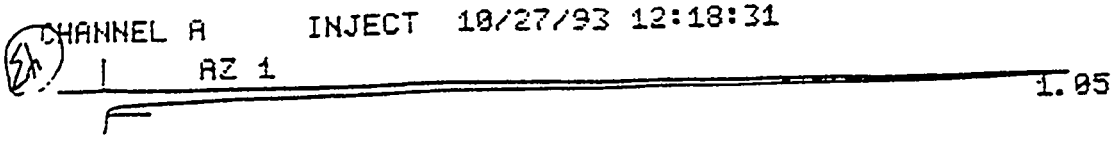


10/27/93 12:15:34 CH= "A" PS= 1.

FILE 1. METHOD 5. RUN *** INDEX *** D. Blank ④

NAME	CONC	RT	AREA BC	RF
1	389.513	1.03	389513 01	1000.
TOTALS	389.513		389513	

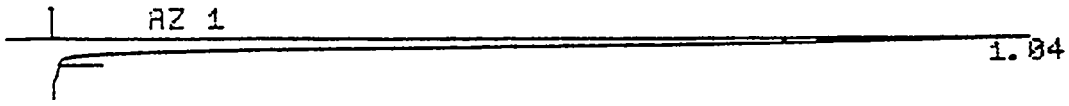
CHANNEL A INJECT 10/27/93 12:18:31



FILE 1. METHOD 5. RUN *** INDEX *** #5

NAME	CONC	RT	AREA	BC	RF
1	7908.709	1.05	7908709	01	1000.
TOTALS	7908.709		7908709		

CHANNEL A INJECT 10/27/93 12:22:05

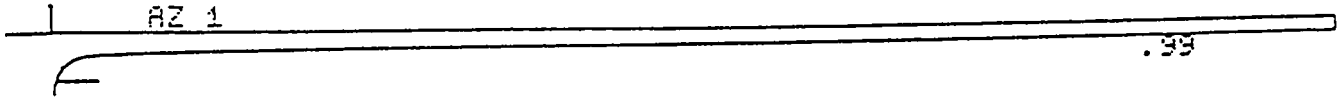


10/27/93 12:22:05 CH= "A" PS= 1.

FILE 1. METHOD 5. RUN *** INDEX *** #6

NAME	CONC	RT	AREA	BC	RF
1	9520.846	1.04	9520846	01	1000.
TOTALS	9520.846		9520846		

CHANNEL A INJECT 10/27/93 12:36:24

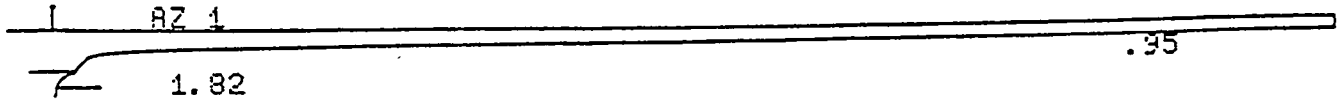


10/27/93 12:36:24 CH= "A" PS= 1.

FILE 1. METHOD 5. RUN *** INDEX ***

NAME	CONC	RT	AREA	BC	RF
1	54122.462	0.99	54122462	01	1000.
TOTALS	54122.462		54122462		

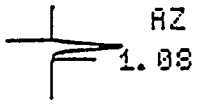
CHANNEL A INJECT 10/27/93 12:44:21



10/27/93 12:44:21 CH= "A" PS= 1.

FILE 1. METHOD 5. RUN *** INDEX ***

NAME	CONC	RT	AREA	BC	RF
1	48491.262	0.95	48491262	02	1000.
2	168.498	1.82	168498	03	1000.
TOTALS	48659.76		48659760		

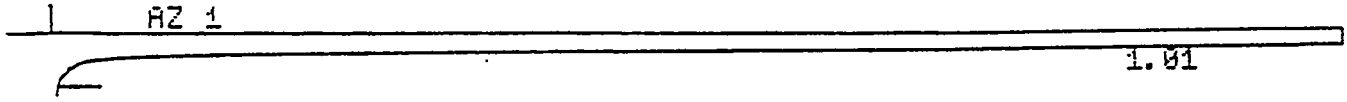


10/27/93 12:48:04 CH= "A" PS= 1.

FILE 1. METHOD 5. RUN *** INDEX ***

NAME	CONC	RT	AREA	BC	RF
1	584.561	1.08	584561	01	1000.
TOTALS	584.561		584561		

CHANNEL A INJECT 10/27/93 12:50:57

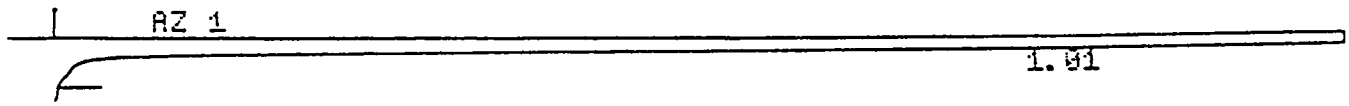


10/27/93 12:50:57 CH= "A" PS= 1.

FILE 1. METHOD 5. RUN *** INDEX ***

NAME	CONC	RT	AREA	BC	RF
1	61513.212	1.01	61513212	01	1000.
TOTALS	61513.212		61513212		

CHANNEL A INJECT 10/27/93 12:55:01

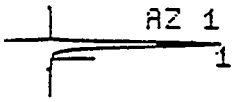


10/27/93 12:55:01 CH= "A" PS= 1.

FILE 1. METHOD 5. RUN *** INDEX ***

NAME	CONC	RT	AREA	BC	RF
1	44177.347	1.01	44177347	01	1000.
TOTALS	44177.347		44177347		

CHANNEL A INJECT 10/27/93 13:01:35



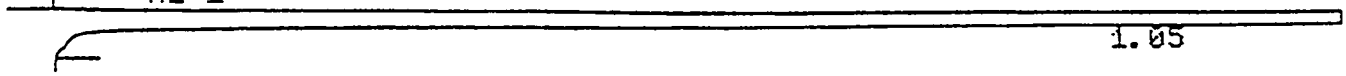
(SH)

10/27/93 13:01:35 CH= "A" PS= 1.

FILE 1. METHOD 5. RUN *** INDEX ***

1	1364.137	1.04	1364137	01	1000.
TOTALS	1364.137		1364137		

CHANNEL A INJECT 10/27/93 13:04:34

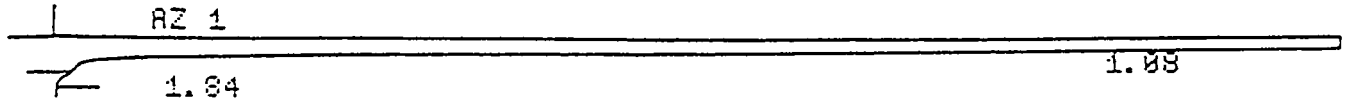


10/27/93 13:04:34 CH= "A" PS= 1.

FILE 1. METHOD 5. RUN *** INDEX ***

NAME	CONC	RT	AREA	BC	RF
1	48904.488	1.05	48904488	01	1000.
TOTALS	48904.488		48904488		

CHANNEL A INJECT 10/27/93 13:08:07

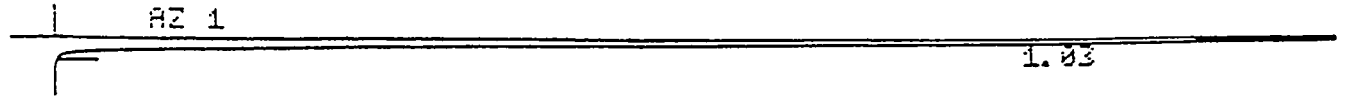


10/27/93 13:08:07 CH= "A" PS= 1.

FILE 1. METHOD 5. RUN *** INDEX ***

NAME	CONC	RT	AREA	BC	RF
1	47209.209	1.08	47209209	02	1000.
2	156.108	1.04	156108	03	1000.
TOTALS	47365.317		47365317		

CHANNEL A INJECT 10/27/93 13:12:12



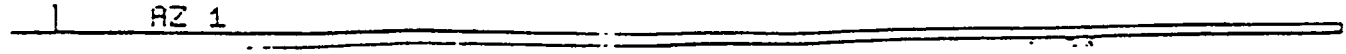
10/27/93 13:12:12 CH= "A" PS= 1.

FILE 1. METHOD 5. RUN *** INDEX ***

#7

NAME	CONC	RT	AREA	BC	RF
1	12996.795	1.0[illegible]	12996795	01	1000.
TOTALS	12996.795		12996795		

CHANNEL A INJECT 10/27/93 1[illegible]:15:59



10/27/93 13:15:59 CH= "A" PS= 1.

FILE 1. METHOD 5. RUN *** INDEX ***

NAME	CONC	RT	AREA	BC	RF
1	33247.26	1.04	33247260	01	1000.
TOTALS	33247.26		33247260		

CHANNEL A INJECT 10/27/93 13:18:49

AZ 1

1.05

10/27/93 13:18:49 CH= "A" PS= 1.

FILE 1. METHOD 5. RUN *** INDEX ***

#3

NAME	CONC	RT	AREA	BC	RF
1	12696.509	1.05	12696509	01	1000.
TOTALS	12696.509		12696509		

CHANNEL A INJECT 10/27/93 13:21:40

AZ 1

1.10

10/27/93 13:21:40 CH= "A" PS= 1.

FILE 1. METHOD 5. RUN *** INDEX ***

NAME	CONC	RT	AREA	BC	RF
1	37279.802	1.1	37279802	01	1000.
TOTALS	37279.802		37279802		

CHANNEL A INJECT 10/27/93 13:26:01

AZ 1

.99

ER 0

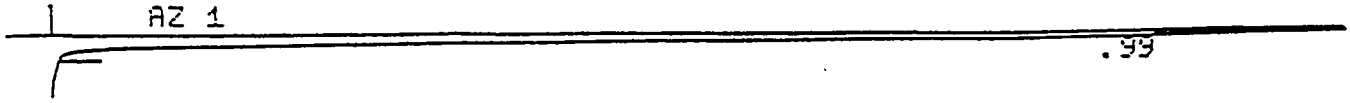
10/27/93 13:26:01 CH= "A" PS= 1.

FILE 1. METHOD 5. RUN *** INDEX ***

#16

NAME	CONC	RT	AREA	BC	RF
1	12038.462	0.99	12038462	01	1000.

CHANNEL A INJECT 10/27/93 13:29:23



10/27/93 13:29:23 CH= "A" PS= 1.

FILE 1. METHOD 5. RUN *** INDEX *** #13A

NAME	CONC	RT	AREA	BC	RF
1	12534.808	0.99	12534808	01	1000.
TOTALS	12534.808		12534808		

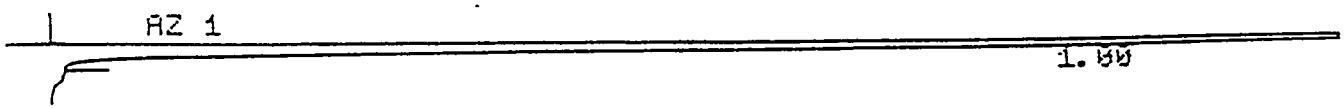
CHANNEL A INJECT 10/27/93 13:32:36

10/27/93 13:32:36 CH= "A" PS= 1.

FILE 1. METHOD 5. RUN *** INDEX *** #13B

NAME	CONC	RT	AREA	BC	RF
1	13586.168	1.03	13586168	01	1000.
TOTALS	13586.168		13586168		

CHANNEL A INJECT 10/27/93 13:35:32

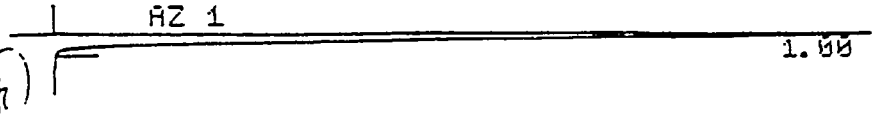


10/27/93 13:35:32 CH= "A" PS= 1.

FILE 1. METHOD 5. RUN *** INDEX *** #13C

NAME	CONC	RT	AREA	BC	RF
1	17830.253	1.	17830253	01	1000.
TOTALS	17830.253		17830253		

CHANNEL A INJECT 10/27/93 14:15:07



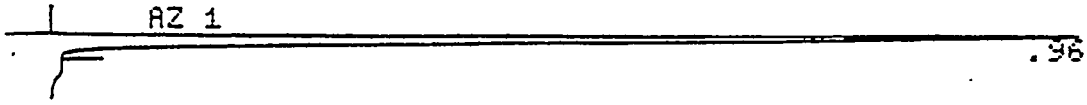
(OK)

10/27/93 14:15:07 CH= "A" PS= 1.

#19

NAME	CONC	RT	AREA	BC	RF
1	5897.426	1.	5897426	01	1000.
TOTALS	5897.426		5897426		

CHANNEL A INJECT 10/27/93 14:18:09



10/27/93 14:18:09 CH= "A" PS= 1.

FILE 1. METHOD 5. RUN *** INDEX ***

BEST-1

NAME	CONC	RT	AREA	BC	RF
1	8677.01	0.96	8677010	01	1000.
TOTALS	8677.01		8677010		

CHANNEL A INJECT 10/27/93 14:24:12

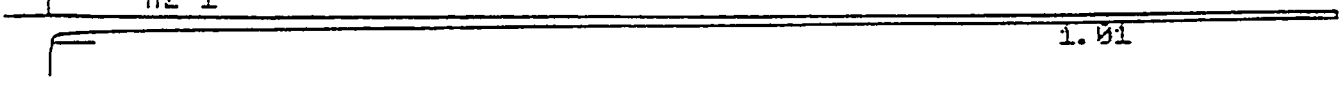
10/27/93 14:24:12 CH= "A" PS= 1.

FILE 1. METHOD 5. RUN *** INDEX ***

4000 pg

NAME	CONC	RT	AREA	BC	RF
1	16212.284	1.06	16212284	01	1000.
TOTALS	16212.284		16212284		

CHANNEL A INJECT 10/27/93 14:27:32



10/27/93 14:27:32 CH= "A" PS= 1.

FILE 1. METHOD 5. RUN *** INDEX ***

5000 pg

NAME	CONC	RT	AREA	BC	RF
1	21451.083	1.01	21451083	01	1000.
TOTALS	21451.083		21451083		

hh

CHANNEL A INJECT 10/27/93 14:34:20

10/27/93 14:34:20 CH= "A" PS= 1.

FILE 1. METHOD 5. RUN *** INDEX ***

40 pg

NAME	CONC	RT	AREA BC	RF
1	139.323	0.99	139323 01	1000.
TOTALS	139.323		139323	

CHANNEL A INJECT 10/27/93 14:37:36

10/27/93 14:37:36 CH= "A" PS= 1.

FILE 1. METHOD 5. RUN *** INDEX ***

1500 pg

NAME	CONC	RT	AREA BC	RF
1	5642.707	0.99	5642707 01	1000.
TOTALS	5642.707		5642707	

CHANNEL A INJECT 10/27/93 14:41:56

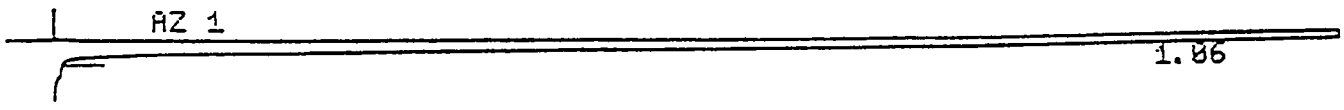
10/27/93 14:41:56 CH= "A" PS= 1.

FILE 1. METHOD 5. RUN *** INDEX ***

Blank

NAME	CONC	RT	AREA BC	RF
TOTALS	0.			

CHANNEL A INJECT 10/27/93 14:44:48



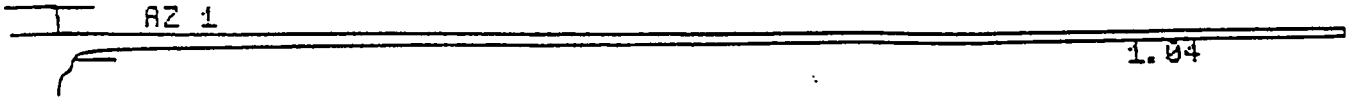
10/27/93 14:44:48 CH= "A" PS= 1.

FILE 1. METHOD 5. RUN *** INDEX ***

(65)

NAME	CONC	RT	AREA BC	RF
1	28603.418	1.06	28603418 01	1000.

CHANNEL A INJECT 10/27/93 14:48:02 49

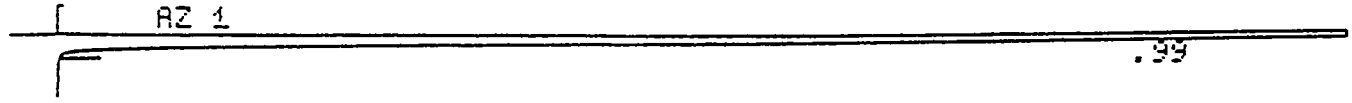


10/27/93 14:48:02 CH= "A" PS= 1.

FILE 1. METHOD 5. RUN *** INDEX ***

NAME	CONC	RT	AREA	BC	RF
1	31110.108	1.04	31110108	01	1000.
TOTALS	31110.108		31110108		

CHANNEL A INJECT 10/27/93 15:04:10



10/27/93 15:04:10 CH= "A" PS= 1.

FILE 1. METHOD 5. RUN *** INDEX *** #C/A

NAME	CONC	RT	AREA	BC	RF
1	23227.477	0.99	23227477	01	1000.
TOTALS	23227.477		23227477		

CHANNEL A INJECT 10/27/93 15:08:16

10/27/93 15:08:16 CH= "A" PS= 1.

FILE 1. METHOD 5. RUN *** INDEX *** #1B

NAME	CONC	RT	AREA	BC	RF
1	17577.888	0.91	17577888	01	1000.
TOTALS	17577.888		17577888		

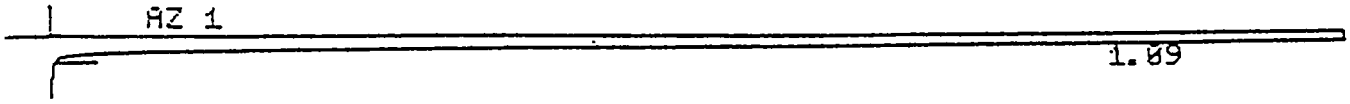
CHANNEL A INJECT 10/27/93 15:11:41

(15)

10/27/93 15:11:41 CH= "A" PS= 1.

NAME	CONC	RT	AREA	BC	RF
1	22609.366	1.05	22609366	01	1000.
TOTALS	22609.366		22609366		

CHANNEL A INJECT 10/27/93 15:15:07

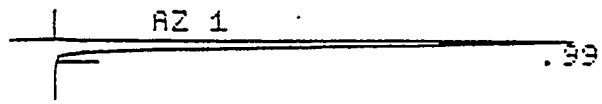


10/27/93 15:15:07 CH= "A" PS= 1.

FILE 1. METHOD 5. RUN *** INDEX ***

NAME	CONC	RT	AREA	BC	RF
1	34046.829	1.09	34046829	01	1000.
TOTALS	34046.829		34046829		

CHANNEL A INJECT 10/27/93 15:18:23



10/27/93 15:18:23 CH= "A" PS= 1.

FILE 1. METHOD 5. RUN *** INDEX ***

#2

NAME	CONC	RT	AREA	BC	RF
1	4082.292	0.99	4082292	01	1000.
TOTALS	4082.292		4082292		

CHANNEL A INJECT 10/27/93 15:23:27

10/27/93 15:23:27 CH= "A" PS= 1.

FILE 1. METHOD 5. RUN *** INDEX ***

#8

NAME	CONC	RT	AREA	BC	RF
1	4002.976	1.01	4002976	01	1000.
TOTALS	4002.976		4002976		

(25)

CHANNEL A INJECT 10/27/93 15:27:00

AZ 1

10/27/93 15:27:00 CH= "A" PS= 1.

FILE 1. METHOD 5. RUN *** INDEX *** #9

NAME	CONC	RT	AREA	BC	RF
1	5653.445	1.03	5653445	01	1000.
TOTALS	5653.445		5653445		

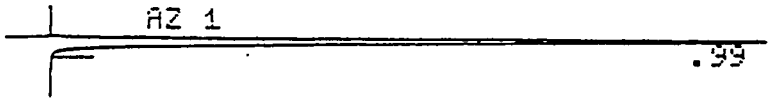
CHANNEL A INJECT 10/27/93 15:30:02

10/27/93 15:30:02 CH= "A" PS= 1.

FILE 1. METHOD 5. RUN *** INDEX *** #10

NAME	CONC	RT	AREA	BC	RF
1	5233.652	1.	5233652	01	1000.
TOTALS	5233.652		5233652		

CHANNEL A INJECT 10/27/93 15:33:27



10/27/93 15:33:27 CH= "A" PS= 1.

FILE 1. METHOD 5. RUN *** INDEX *** #11

NAME	CONC	RT	AREA	BC	RF
1	5038.614	0.99	5038614	01	1000.
TOTALS	5038.614		5038614		

CHANNEL A INJECT 10/27/93 15:37:23

10/27/93 15:37:23 CH= "A" PS= 1.

FILE 1. METHOD 5. RUN *** INDEX *** #14

NAME (99)	CONC	RT	AREA	BC	RF
1	9257.388	0.93	9257388	01	1000.

CHANNEL A INJECT 10/27/93 15:40:47

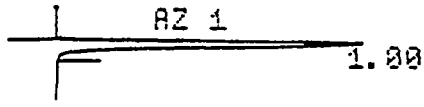
10/27/93 15:40:47 CH= "A" PS= 1.

FILE 1. METHOD 5. RUN *** INDEX ***

#18

NAME	CONC	RT	AREA	BC	RF
1	6081.406	1.01	6081406	01	1000.
TOTALS	6081.406		6081406		

CHANNEL A INJECT 10/27/93 15:43:38



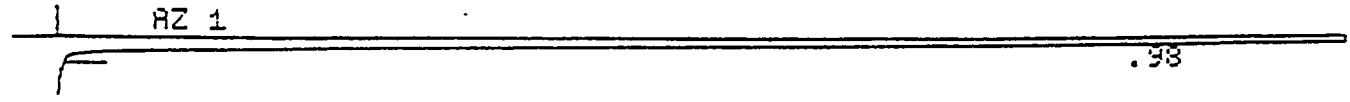
10/27/93 15:43:38 CH= "A" PS= 1.

FILE 1. METHOD 5. RUN *** INDEX ***

#20

NAME	CONC	RT	AREA	BC	RF
1	2495.177	1.	2495177	01	1000.
TOTALS	2495.177		2495177		

CHANNEL A INJECT 10/27/93 15:48:05



10/27/93 15:48:05 CH= "A" PS= 1.

FILE 1. METHOD 5. RUN *** INDEX ***

#15

NAME	CONC	RT	AREA	BC	RF
1	21848.049	0.98	21848049	01	1000.
TOTALS	21848.049		21848049		

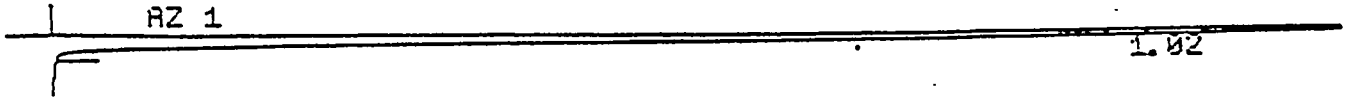
CHANNEL A INJECT 10/27/93 15:51:28

(#5)

10/27/93 15:51:28 CH= "A" PS= 1.

NAME	CONC	RT	AREA	BC	RF
1	10671.299	0.99	10671299	01	1000.
TOTALS	10671.299		10671299		

CHANNEL A INJECT 10/27/93 15:55:06



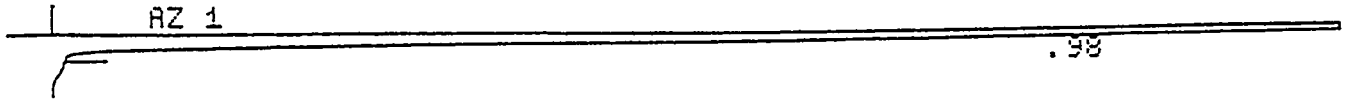
10/27/93 15:55:06 CH= "A" PS= 1.

FILE 1. METHOD 5. RUN *** INDEX ***

#17B

NAME	CONC	RT	AREA	BC	RF
1	12563.692	1.02	12563692	01	1000.
TOTALS	12563.692		12563692		

CHANNEL A INJECT 10/27/93 15:58:11



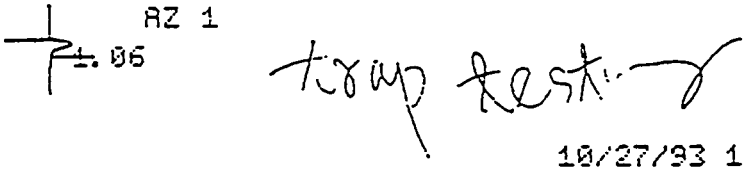
10/27/93 15:58:11 CH= "A" PS= 1.

FILE 1. METHOD 5. RUN *** INDEX ***

NAME	CONC	RT	AREA	BC	RF
1	20957.453	0.98	20957453	01	1000.
TOTALS	20957.453		20957453		

#17C

CHANNEL A INJECT 10/27/93 16:02:21



10/27/93 16:02:21 CH= "A" PS= 1.

FILE 1. METHOD 5. RUN *** INDEX ***

NAME	CONC	RT	AREA	BC	RF
1	207.967	1.06	207967	01	1000.
TOTALS	207.967		207967		

(25)

CHANNEL A INJECT 10/27/93 16:06:33

10/27/93 16:06:33 CH= "A" PS= 1.

FILE 1. METHOD 5. RUN *** INDEX ***

5d

NAME	CONC	RT	AREA BC	RF
TOTALS	0.			

CHANNEL A INJECT 10/27/93 16:14:28

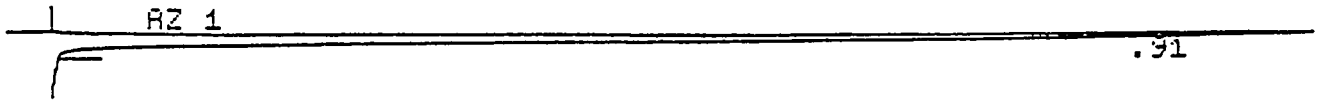
10/27/93 16:14:28 CH= "A" PS= 1.

FILE 1. METHOD 5. RUN *** INDEX ***

BEST 1

NAME	CONC	RT	AREA BC	RF
1	12411.03	1.02	12411030 01	1000.
TOTALS	12411.03		12411030	

CHANNEL A INJECT 10/27/93 16:17:41



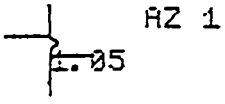
10/27/93 16:17:41 CH= "A" PS= 1.

FILE 1. METHOD 5. RUN *** INDEX ***

BEST-1

NAME	CONC	RT	AREA BC	RF
1	10838.322	0.91	10838322 01	1000.
TOTALS	10838.322		10838322	

CHANNEL A INJECT 10/27/93 16:20:46



10/27/93 16:20:46 CH= "A" PS= 1.

FILE 1. METHOD 5. RUN *** INDEX ***

20pg

NAME	CONC	RT	AREA BC	RF
1	72.541	1.05	72541 01	1000.
TOTALS	72.541		72541	

CHANNEL A INJECT 10/27/93 16:24:15

AZ 1

1.02

55 16

10/27/93 16:24:15 CH= "A" PS= 1.

FILE 1. METHOD 5. RUN *** INDEX ***

2000 pg

NAME	CONC	RT	AREA	BC	RF
1	8636.575	1.02	8636575	01	1000.
TOTALS	8636.575		8636575		

CHANNEL A INJECT 10/27/93 16:26:58

AZ 1

10/27/93 16:26:58 CH= "A" PS= 1.

FILE 1. METHOD 5. RUN *** INDEX ***

Blank

NAME	CONC	RT	AREA	BC	RF
TOTALS	0.				

(+5)

Dry Weight Determination

102293R1.XLS

Tag No.	Tare Wt.	Wet Wt. (Net g)	Dry Wt. (Gross g)	Dry Wt. (Net g)	Dry Wt. (%)
OR3-01	1.087	1.563	2.113	1.026	65.6%
OR3-02	1.090	1.609	2.105	1.015	63.1%
OR3-03	1.087	1.346	1.987	0.900	66.9%
OR3-05	1.080	1.609	2.250	1.170	72.7%
OR3-06	1.077	1.803	2.496	1.419	78.7%
OR3-07	1.084	1.414	2.251	1.167	82.5%
OR3-08	1.093	1.451	2.280	1.187	81.8%
OR3-09	1.089	1.458	2.251	1.162	79.7%
OR3-10	1.088	1.460	2.238	1.150	78.8%
OR3-11	1.096	1.381	2.228	1.132	82.0%
OR3-13	1.099	1.588	2.230	1.131	71.2%
OR3-14	1.084	1.499	2.185	1.101	73.4%
OR3-15	1.091	1.546	2.241	1.150	74.4%
OR3-16	1.077	1.728	2.476	1.399	81.0%
OR3-17	1.074	1.654	2.282	1.208	73.0%
OR3-18	1.095	1.586	2.265	1.170	73.8%
OR3-19	1.084	1.448	2.314	1.230	84.9%
OR3-20	1.099	1.038	2.120	1.021	98.4%

DJG

Radian Corp
Soil Samples
SET 2

(54) 57

Dry Weight Determination

Batch# OR-3 Tag No.	Tare Wt.	Wet Wt. (Net)	Dry Wt. (Gross)	Dry Wt. (Net)	Dry Wt. (%)
OR#-21	1.081	1.554	2.248	1.167	75.1%
#22	1.084	1.536	2.295	1.211	74.0%
#23	1.097	2.123	2.764	1.672	76.7%
#24	1.110	1.663	2.340	1.230	74.0%
#25	1.083	1.324	2.325	1.242	71.8%
#26	1.102	2.638	3.013	1.911	72.7%
*#27	1.093	2.453	2.590	1.497	61.0%
#28	1.114	1.598	2.109	0.995	54.3%
#29	.087	1.503	2.173	1.086	70.4%
#30	1.094	2.952	2.947	1.853	74.3%
#31	1.097	2.314	2.877	1.780	76.7%
#32	1.089	1.304	2.395	1.306	76.6%

Oven Temp 92°C
For 25:20 hours
} For all samples except #26, #27

Nov 3, 1993
Signed/Date

* Some material from #27 fell into #26
Will run dry weights over for these two.
Oven Temp 95°C
For 24:35 hours

Dry Weight Determination

110393R1.XLS

58 (69)

Tag No.	Tare Wt.	Wet Wt. (Net g)	Dry Wt. (Gross g)	Dry Wt. (Net g)	Dry Wt. (%)
OR3-21	1.081	1.554	2.248	1.167	75.1%
OR3-22	1.084	1.636	2.295	1.211	74.0%
OR3-23	1.092	2.173	2.764	1.672	76.9%
OR3-24	1.11	1.663	2.34	1.23	74.0%
OR3-25	1.083	1.729	2.325	1.242	71.8%
OR3-26	1.102	2.628	3.013	1.911	72.7%
OR3-27	1.093	2.453	2.59	1.497	61.0%
OR3-28	1.114	1.678	2.109	0.995	59.3%
OR3-29	1.087	1.543	2.173	1.086	70.4%
OR3-30	1.094	2.482	2.947	1.853	74.7%
OR3-31	1.097	2.314	2.877	1.78	76.9%
OR3-32	1.089	1.704	2.395	1.306	76.6%

DJS

Radian Corp
Soil Samples

(61)

Dry Weight Determination

Batch #: OR-3

Tag No.	Tare Wt.	Wet Wt. (Net)	Dry Wt. (Gross)	Dry Wt. (Net)	Dry Wt. (%)
OR-3-01	1.087	1.563	2.113	1.026	65.6%
02	1.090	1.609	2.105	1.015	63.1%
03	1.087	1.346	1.987	0.900	66.9%
05	1.080	1.609	2.250	1.170	72.7
06	1.077	1.803	2.496	1.419	78.7
07	1.084	1.414	2.251	1.167	82.5
08	1.093	1.451	2.280	1.187	81.8
09	1.089	1.458	2.251	1.162	79.7
10	1.088	1.460	2.238	1.150	78.8
11	1.096	1.381	2.228	1.132	82.0
13	1.099	1.588	2.230	1.131	71.2
14	1.084	1.499	2.185	1.101	73.4
15	1.091	1.546	2.241	1.150	74.4
16	1.077	1.728	2.476	1.399	~~80~~ 81.0
17	1.074	1.654	2.282	1.208	73.0
18	1.095	1.586	2.265	1.170	73.8
19	1.084	1.448	2.314	1.230	84.9
20	1.099	1.038	2.120	1.021	98.4

Oven Temp 85°C
For 24:00 hours

[signature] Oct 22, 1993
Signed/Date

Screening kiln

12/03/93
Page 1 of 1

Hg Analysis Results
110693T2.XLS

60

Analyst:	L. Liang	Project:	Oak Ridge	Matrix:	Soil	Anal. Req.:	T-Hg

Sample Calculations

Run	Sample ID	Sample Weight (g)	Sample Volume (mL)	Analyzed Volume (mL)	PA		
1.03	15		25.6	0.5			
1.04	17 C		25.6	0.5			
1.05	15		25.6	1.0	40837		
1.06	18		25.6	1.0	49530		
1.07	19		25.6	1.0	13279		
1.08	16		25.6	1.0	5244		
1.09	17 C	1.014		0.5	33709		
1.10	32	1.141		0.5	179.2		
1.11	24	1.071		0.5	25402		
1.12	31	1.048		0.5	27424		
1.13	23	1.081		0.5	13199		
1.14	26	1.045		0.5	34868		
1.15	28	0.952		0.5	15556		
1.16	25 B	1.041		0.5	2589		
1.17	22	0.982		0.5	25963		
1.18	27	1.043		0.5	42474		
1.19	30	1.155		0.5	2545		
1.20	21	1.056		0.5	26493		
1.21	25 A	1.008		0.5	8878		
1.22	20	0.983		0.5	3496		
1.23	29	1.120		0.5	4270		
1.24	17 A	1.150		0.5	43724		
1.25	J. Ealy (morning)			0.1	7460	6945	
1.26	R. Turner (morning)			0.1	5571	4851	
1.27	J. Ealy			0.1	7188	9729	
1.28	R. Turner			0.1	5041	7045	

Standards

Run	pg	PH/PA				
1.01	1000					
1.02	1000					
1.29	1000	12527	11299			
		Average:				
		St.dev.:				
		Slope:				
		Intercept:				
		RSQ:				

Dorthy Spurling, QA Director

Hg Analysis Sheet

☒Total Hg ☐MeHg ☐Hg(II) ☐Other:_____ Project/Matrix: distillate CR, Soil

Analyst: _____ Blank $\bar{X}$:_____ Calibration Coefficient $\bar{X}$:_____

Date: 11/6-7 N=_____ SD:_____

QA: High☐ Research☐ N=:_____

After distance Check of Concentrations

Run	Trap	Sample ID	Sample vol/wt.	Dilution Volume	Analyzed volume	BrCl (pg)	PH/PA	pg (x cor.fact.)	Result
1		1500 blk							
2		"							
3		#15	25.1-2		0.5 mL				
4		#17C	"		"				
5		#1C	"		0.1 mL		3186 4383		
6		#18	"		"		4558		
7		#19	"		"		13271		
8		#16	"		"		5002		
9		#17C, 1.010 g			0.5 mL		33707		
10		#32, 1.101 g			"		17972		
11		#24, 1.071 g			"		15402		
12		#31, 1.018 g			"		27452		
13		#23, 1.021 g			0.25 mL		13177		
14		#26, 1.005 g			0.25 mL		34367		
15		#33, 0.960 g			5.0 mL		15556		
16		#28B, 1.041 g			"		2531		
17		#22, 0.982 g			"		25763		
18		#27, 1.003 g			"		12424		
19		#30, 1.155 g			"		25455		
20		#21, 1.048 g			"		26073		
21		#25A 1.003 g			"		8878		
22		#20, 0.993 g			"		3496		
23		#29, 1.170 g			"		4260		
24		#17A, 1.155 g			"		16724		
25		T. Ealy Inorganic			0.1 mL		7660.6	0.25 ng/mL	
26		R. Tinsley "			"		5571 1981	4.6 ng/mL	
27		J. Ealy			"		7113 9999	8.163 ng/mL	
28		R. Tinsley			"		5041	2.911 ng/mL	

1 ng/g

1253, 1127 1193 ± 868

Calculation Equation:_____

Comments:_____

NEL A INJECT 11/06/93 13:14:36 62

AZ 1

56 .98

11/06/93 13:14:36 CH= "F" PS= 1.

1. METHOD 5. RUN *** INDEX ***

	CONC	RT	AREA BC	RF
	0.	0.56	35359 02	
	226.63	0.98	226630 02	1000.
LS	226.63		261989	

NEL A INJECT 11/06/93 13:18:20

AZ 1

1.29

ER 0

11/06/93 13:18:20 CH= "A" PS= 1.

1. METHOD 5. RUN *** INDEX ***

	CONC	RT	AREA BC	RF
	83082.169	1.29	83082169 01	1000.
LS	83082.169		83082169	

NEL A INJECT 11/06/93 13:21:31

AZ 1

1.51

11/06/93 13:21:31 CH= "A" PS= 1.

1. METHOD 5. RUN *** INDEX ***

	CONC	RT	AREA BC	RF
	1140.629	1.51	1140629 02	1000.

056

1	1140.[illegible]	[illegible]	[illegible]	[illegible]	[illegible]
2	1.669	1.77	1669 03	1000.	
TOTALS	1142.298		1142298		

63 (65)

CHANNEL A INJECT 11/06/93 13:24:33

AZ 1

1.12

ER 0

11/06/93 13:24:33 CH= "A" PS= 1.

FILE 1. METHOD 5. RUN *** INDEX ***

NAME	CONC	RT	AREA BC	RF
1	35689.546	1.12	35689546 01	1000.
TOTALS	35689.546		35689546	

CHANNEL A INJECT 11/06/93 13:27:20

AZ 1

1.01

11/06/93 13:27:20 CH= "A" PS= 1.

FILE 1. METHOD 5. RUN *** INDEX ***

NAME	CONC	RT	AREA BC	RF
1	40.546	1.01	40546 01	1000.
TOTALS	40.546		40546	

CHANNEL A INJECT 11/06/93 13:31:28

AZ 1

.98

11/06/93 13:31:28 CH= "A" PS= 1.

FILE 1. METHOD 5. RUN *** INDEX ***

NAME	CONC	RT	AREA BC	RF
1	14487.17	0.98	14487170 01	1000.
TOTALS	14487.17		14487170	

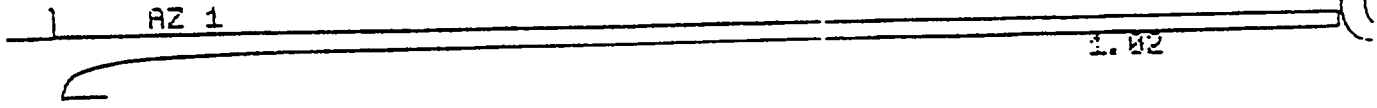
057

CHANNEL A INJECT 11/06/93 13:35:19

AZ 1

1.02

CHANNEL A INJECT 11/06/93 13:35:19

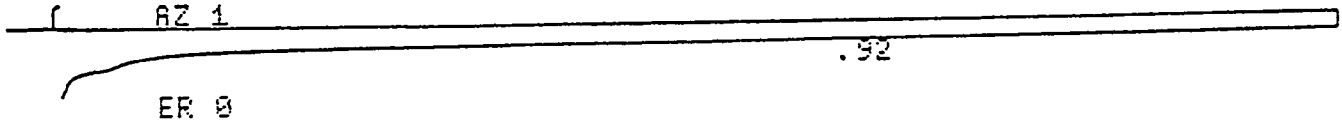


11/06/93 13:35:19 CH= "A" PS= 1.

FILE 1. METHOD 5. RUN *** INDEX ***

NAME	CONC	RT	AREA	BC	RF
1	59378.426	1.02	59378426	01	1000.
TOTALS	59378.426		59378426		

CHANNEL A INJECT 11/06/93 13:38:43

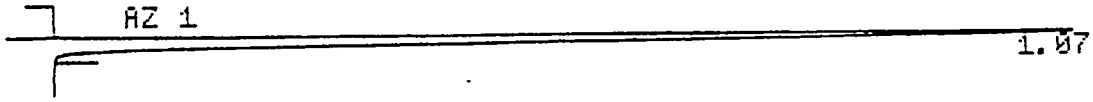


11/06/93 13:38:43 CH= "A" PS= 1.

FILE 1. METHOD 5. RUN *** INDEX ***

NAME	CONC	RT	AREA	BC	RF
1	65496.804	0.92	65496804	01	1000.
TOTALS	65496.804		65496804		

CHANNEL A INJECT 11/06/93 13:46:21

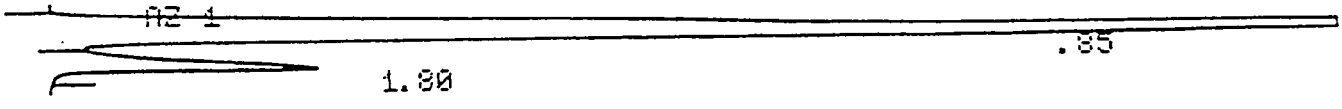


11/06/93 13:46:21 CH= "A" PS= 1.

FILE 1. METHOD 5. RUN *** INDEX ***

NAME	CONC	RT	AREA	BC	RF
1	8764.914	1.07	8764914	01	1000.
TOTALS	8764.914		8764914		

CHANNEL A INJECT 11/06/93 14:10:32



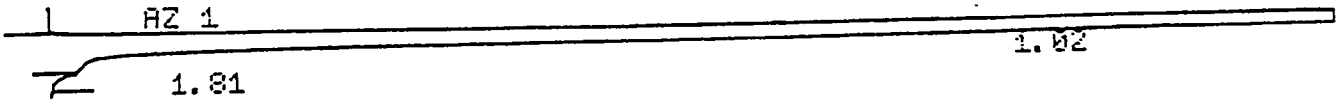
11/06/93 14:10:32 CH= "A" PS= 1.

FILE 1. METHOD 5. RUN *** INDEX ***

FILE 1. METHOD 5. RUN *** INDEX ***

NAME	CONC	RT	AREA	BC	RF
1	36933.661	0.85	36933661	02	1000.
2	3902.877	1.8	3902877	03	1000.
TOTALS	40836.538		40836538		

CHANNEL A INJECT 11/06/93 14:17:58

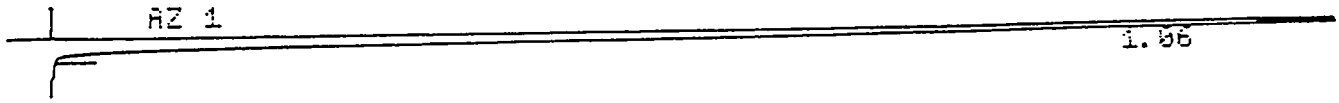


11/06/93 14:17:58 CH= "A" PS= 1.

FILE 1. METHOD 5. RUN *** INDEX ***

NAME	CONC	RT	AREA	BC	RF
1	49310.381	1.02	49310381	02	1000.
2	220.192	1.81	220192	03	1000.
TOTALS	49530.573		49530573		

CHANNEL A INJECT 11/06/93 14:21:24

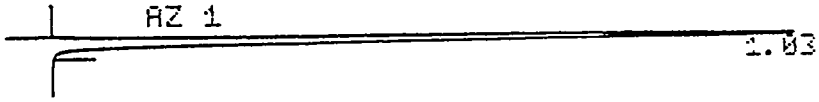


11/06/93 14:21:24 CH= "A" PS= 1.

FILE 1. METHOD 5. RUN *** INDEX ***

NAME	CONC	RT	AREA	BC	RF
1	13279.367	1.06	13279367	01	1000.
TOTALS	13279.367		13279367		

CHANNEL A INJECT 11/06/93 14:24:22



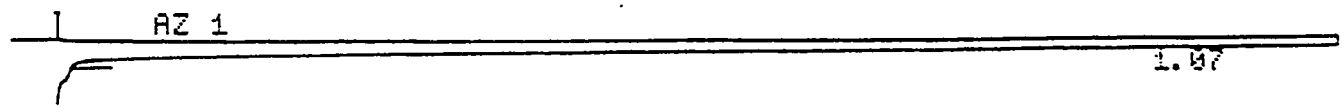
11/06/93 14:24:22 CH= "A" PS= 1.

FILE 1. METHOD 5. RUN *** INDEX ***

NAME	CONC	RT	AREA	BC	RF
1	5244.485	1.03	5244485	01	1000.
TOTALS	5244.485		5244485		

TOTALS	5244.485	5244485

CHANNEL A INJECT 11/06/93 14:29:40

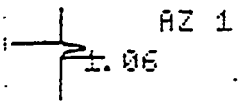


11/06/93 14:29:40 CH= "A" PS= 1.

FILE 1. METHOD 5. RUN *** INDEX ***

NAME	CONC	RT	AREA	BC	RF
1	33709.311	1.07	33709311	01	1000.
TOTALS	33709.311		33709311		

CHANNEL A INJECT 11/06/93 14:33:12

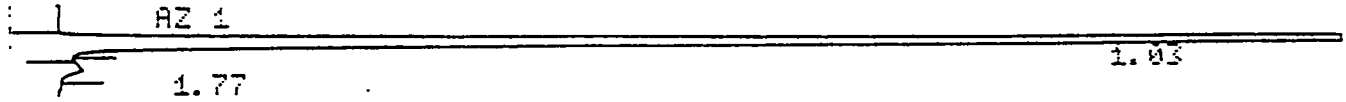


11/06/93 14:33:12 CH= "A" PS= 1.

FILE 1. METHOD 5. RUN *** INDEX ***

NAME	CONC	RT	AREA	BC	RF
1	179.274	1.06	179274	01	1000.
TOTALS	179.274		179274		

CHANNEL A INJECT 11/06/93 14:36:14

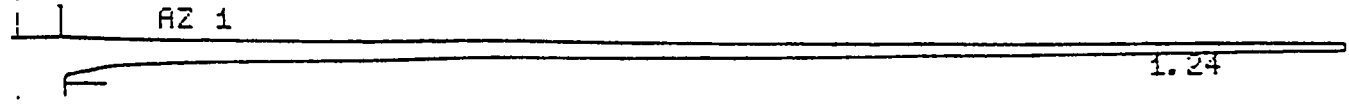


11/06/93 14:36:14 CH= "A" PS= 1.

FILE 1. METHOD 5. RUN *** INDEX ***

NAME	CONC	RT	AREA	BC	RF
1	25234.169	1.03	25234169	01	1000.
2	167.628	1.77	167628	01	1000.
TOTALS	25401.797		25401797		

CHANNEL A INJECT 11/06/93 14:39:11



1.24

11/06/93 1::39:11 CH= "A" PS= 1.

FILE 1. METHOD 5. RUN *** INDEX ***

NAME	CONC	RT	AREA	BC	RF
1	27423.527	1.24	27423527	01	1000.
TOTALS	27423.527		27423527		

CHANNEL A INJECT 11/06/93 14:43:18

AZ 1

.87

1.71

11/06/93 14:43:18 CH= "A" PS= 1.

FILE 1. METHOD 5. RUN *** INDEX ***

NAME	CONC	RT	AREA	BC	RF
1	13063.371	0.87	13063371	01	1000.
2	135.156	1.71	135156	01	1000.
TOTALS	13198.527		13198527		

CHANNEL A INJECT 11/06/93 14:48:24

AZ 1

1.14

11/06/93 14:48:24 CH= "A" PS= 1.

FILE 1. METHOD 5. RUN *** INDEX ***

NAME	CONC	RT	AREA	BC	RF
1	34868.138	1.14	34868138	01	1000.
TOTALS	34868.138		34868138		

CHANNEL A INJECT 11/06/93 14:51:31

AZ 1

1.06

1.77

11/06/93 14:51:31 CH= "A" PS= 1.

FILE 1. METHOD 5. RUN *** INDEX ***

NAME	CONC	RT	AREA	BC	RF

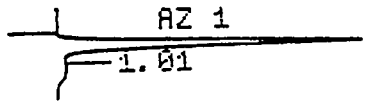
FILE 1. METHOD 5.

68

NAME	CONC	RT	AREA	BC	RF
1	15232.241	1.06	15232241	01	1000.
2	324.472	1.77	324472	01	1000.
TOTALS	15556.713		15556713		

061

CHANNEL A INJECT 11/06/93 14:54:34

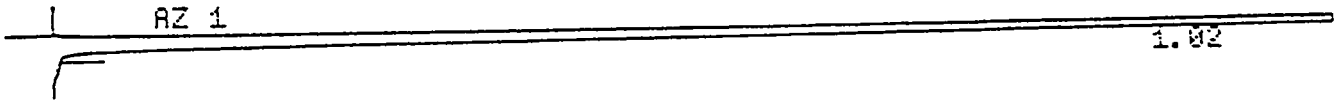


11/06/93 14:54:34 CH= "A" PS= 1.

FILE 1. METHOD 5. RUN *** INDEX ***

NAME	CONC	RT	AREA	BC	RF
1	2588.565	1.01	2588565	01	1000.
TOTALS	2588.565		2588565		

CHANNEL A INJECT 11/06/93 14:58:59

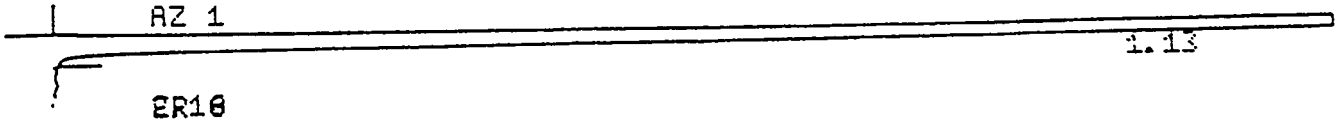


11/06/93 14:58:59 CH= "A" PS= 1.

FILE 1. METHOD 5. RUN *** INDEX ***

NAME	CONC	RT	AREA	BC	RF
1	25962.891	1.02	25962891	01	1000.
TOTALS	25962.891		25962891		

CHANNEL A INJECT 11/06/93 15:07:14



11/06/93 15:07:14 CH= "A" PS= 1.

FILE 1. METHOD 5. RUN *** INDEX ***

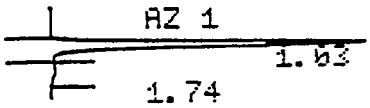
NAME	CONC	RT	AREA	BC	RF
1	42474.265	1.13	42474265	01	1000.
2	0.	2.16	23262	01	
TOTALS	42474.265		42497527		

1	42474.265	[illegible]	[illegible]
2	0.	2.16	23262 01
TOTALS	42474.265		42497527

(71)

69

CHANNEL A INJECT 11/06/93 15:10:09



11/06/93 15:10:09 CH= "A" PS= 1.

FILE 1. METHOD 5. RUN *** INDEX ***

NAME	CONC	RT	AREA	BC	RF
1	2495.571	1.03	2495571	01	1000.
2	49.455	1.74	49455	01	1000.
TOTALS	2545.026		2545026		

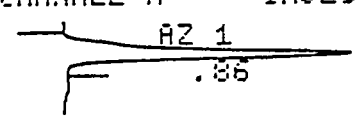
CHANNEL A INJECT 11/06/93 15:12:59

11/06/93 15:12:59 CH= "A" PS= 1.

FILE 1. METHOD 5. RUN *** INDEX ***

NAME	CONC	RT	AREA	BC	RF
1	26493.103	1.07	26493103	01	1000.
TOTALS	26493.103		26493103		

CHANNEL A INJECT 11/06/93 15:16:39

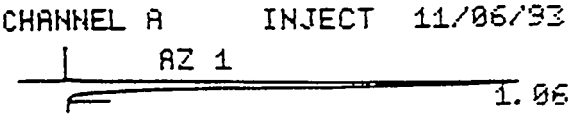


11/06/93 15:16:39 CH= "A" PS= 1.

FILE 1. METHOD 5. RUN *** INDEX ***

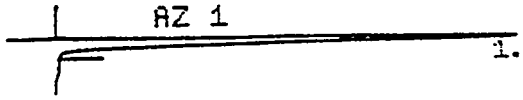
NAME	CONC	RT	AREA	BC	RF
1	3877.79	0.86	3877790	01	1000.
TOTALS	3877.79		3877790		

CHANNEL A INJECT 11/06/93 15:23:14



062

CHANNEL A INJECT 11/06/93 15:23:14



(42.)

70

11/06/93 15:23:14 CH= "A" PS= 1.

FILE 1. METHOD 5. RUN *** INDEX ***

NAME	CONC	RT	AREA BC	RF
1	3496.027	1.06	3496027 01	1000.
TOTALS	3496.027		3496027	

CHANNEL A INJECT 11/06/93 15:26:15

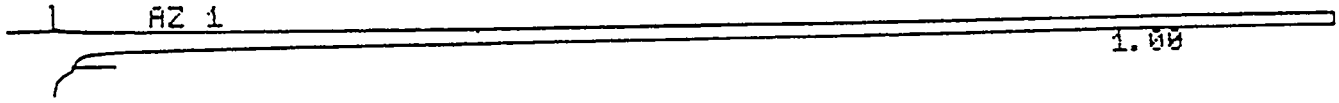
063

11/06/93 15:26:15 CH= "A" PS= 1.

FILE 1. METHOD 5. RUN *** INDEX ***

NAME	CONC	RT	AREA BC	RF
1	4139.635	1.07	4139635 01	1000.
2	136.045	1.69	136045 01	1000.
TOTALS	4275.68		4275680	

CHANNEL A INJECT 11/06/93 15:29:35

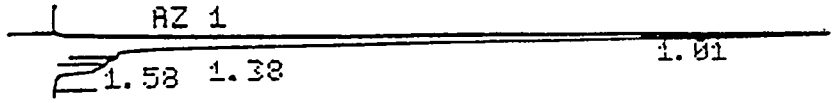


11/06/93 15:29:35 CH= "A" PS= 1.

FILE 1. METHOD 5. RUN *** INDEX ***

NAME	CONC	RT	AREA BC	RF
1	43723.571	1.	43723571 01	1000.
TOTALS	43723.571		43723571	

CHANNEL A INJECT 11/06/93 15:33:04



11/06/93 15:33:04 CH= "A" PS= 1.

FILE 1. METHOD 5. RUN *** INDEX ***

1.58 1.38

(73)

11/06/93 15:33:04 CH= "A" PS= 1.

71

FILE 1. METHOD 5. RUN *** INDEX ***

NAME	CONC	RT	AREA BC	RF
1	7460.117	1.01	7460117 02	1000.
2	521.818	1.38	521818 02	1000.
3	641.432	1.58	641432 03	1000.
TOTALS	8623.367		8623367	

CHANNEL A INJECT 11/06/93 15:36:45

1 AZ 1 1.10

11/06/93 15:36:45 CH= "A" PS= 1.

FILE 1. METHOD 5. RUN *** INDEX ***

NAME	CONC	RT	AREA BC	RF
1	0.	0.26	9249 01	
2	5571.125	1.1	5571125 01	1000.
TOTALS	5571.125		5580374	

CHANNEL A INJECT 11/06/93 15:43:01

1 AZ 1 .99

11/06/93 15:43:01 CH= "A" PS= 1.

FILE 1. METHOD 5. RUN *** INDEX ***

NAME	CONC	RT	AREA BC	RF
1	7117.618	0.99	7117618 01	1000.
TOTALS	7117.618		7117618	

CHANNEL A INJECT 11/06/93 15:45:44

1 AZ 1 1.00

11/06/93 15:45:44 CH= "A" PS= 1.

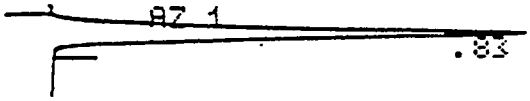
FILE 1. METHOD 5. RUN *** INDEX ***

064

FILE 1. METHOD 5. RUN *** INDEX ***

NAME	CONC	RT	AREA	BC	RF
1	5041.419	1.	5041419	01	1000.
TOTALS	5041.419		5041419		

CHANNEL A INJECT 11/06/93 15:51:31



11/06/93 15:51:31 CH= "A" PS= 1.

FILE 1. METHOD 5. RUN *** INDEX ***

NAME	CONC	RT	AREA	BC	RF
1	6945.103	0.83	6945103	01	1000.
TOTALS	6945.103		6945103		

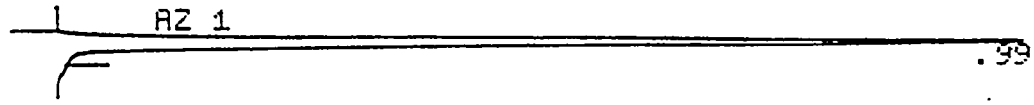
CHANNEL A INJECT 11/06/93 15:55:02

11/06/93 15:55:02 CH= "A" PS= 1.

FILE 1. METHOD 5. RUN *** INDEX ***

NAME	CONC	RT	AREA	BC	RF
1	4851.865	1.07	4851865	01	1000.
TOTALS	4851.865		4851865		

CHANNEL A INJECT 11/06/93 15:58:05



11/06/93 15:58:05 CH= "A" PS= 1.

FILE 1. METHOD 5. RUN *** INDEX ***

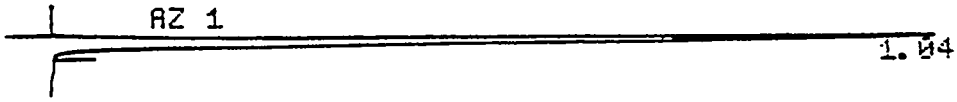
NAME	CONC	RT	AREA	BC	RF
1	9729.545	0.99	9729545	01	1000.
TOTALS	9729.545		9729545		

590

AME	CONC		AREA BC		RF
1	9729.545	0.99	9729545	01	1000.
OTALS	9729.545		9729545		

73

HANNEL A INJECT 11/06/93 16:01:10

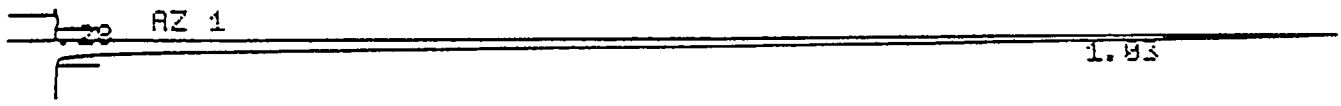


11/06/93 16:01:10 CH= "A" PS= 1.

ILE 1. METHOD 5. RUN *** INDEX ***

AME	CONC	RT	AREA BC		RF
1	7044.837	1.04	7044837	01	1000.
OTALS	7044.837		7044837		

HANNEL A INJECT 11/06/93 16:04:09

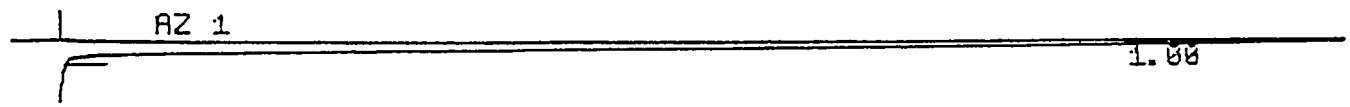


11/06/93 16:04:09 CH= "A" PS= 1.

ILE 1. METHOD 5. RUN *** INDEX ***

AME	CONC	RT	AREA BC		RF
1	0.	0.28	30160	01	
2	12527.126	1.03	12527126	01	1000.
OTALS	12527.126		12557286		

HANNEL A INJECT 11/06/93 16:07:27



11/06/93 16:07:27 CH= "A" PS= 1.

ILE 1. METHOD 5. RUN *** INDEX ***

AME	CONC	RT	AREA BC		RF
1	11299.158	1.	11299158	01	1000.
OTALS	11299.158		11299158		

996

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1, MESS-1, PACS-1, 1

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National Research
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ST-1.

SS-1 are estuarine
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S-1 is a sediment from
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The table overleaf show those constituents for which certified values have been established. Certified values are based on the results of determinations by at least two independent methods of analysis. The uncertainties represent 95 per cent confidence limits for an individual subsample. That is, 95 per cent of samples from any bottle would be expected to have concentrations within the specified range 95 per cent of the time.

To order this material, or for further technical information, contact:

National Research Council
Institute for Environmental Chemistry
Marine Analytical Chemistry Standards Program
Montreal Road
Ottawa, Ontario, Canada K1A 0R6

Telephone (613) 993-2359
Facsimile (613) 993-2451

October 1992

National Research Council Canada
Conseil national de recherches Canada

Marine Analytical Chemistry Standards Program

BCSS-1, MESS-1, PACS-1, BEST-1

Marine Sediment Reference Material for Trace Elements and Other Constitutents

Four sediments are available from the Marine Analytical Chemistry Standards Program of the National Research Council of Canada: MESS-1, BCSS-1, PACS-1, and BEST-1.

MESS-1 and BCSS-1 are estuarine sediments from the Gulf of St. Lawrence. PACS-1 is a sediment from Esquimalt harbour in British Columbia. BEST-1 is an estuarine sediment from the Beaufort Sea.

All sediments have been freeze dried, passed through a No. 120 (125 μm) screen, blended, bottled and radiation sterilized.

In general, the trace levels in MESS-1 and BCSS-1 are low to moderate, while those in the harbour sediment PACS-1 are relatively high . BEST-1 is certified for mercury only.

MESS-1 and BCSS-1 are no longer certified for mercury. A bottle of BEST-1 will be sent with all orders of MESS-1 and/or BCSS-1.

The bottles contain about 65 grams of MESS-1, 80 grams of BCSS-1, 60 grams of PACS-1, and 50 grams of BEST-1, respectively.

The table overleaf show those constituents for which certified values have been established. Certified values are based on the results of determinations by at least two independent methods of analysis. The uncertainties represent 95 per cent confidence limits for an individual subsample. That is, 95 per cent of samples from any bottle would be expected to have concentrations within the specified range 95 per cent of the time.

To order this material, or for further technical information, contact:

National Research Council
Institute for Environmental Chemistry
Marine Analytical Chemistry Standards Program
Montreal Road
Ottawa, Ontario, Canada K1A 0R6

Telephone (613) 993-2359
Facsimile (613) 993-2451

October 1992

BCSS-1, MESS-1, PACS-1, BEST-1

Trace Elements - (milligrams per kilogram)

	MESS-1	BCSS-1	PACS-1
Antimony	0.73 ± 0.08	0.59 ± 0.06	171 ± 14
Arsenic	10.6 ± 1.2	11.1 ± 1.4	211 ± 11
Beryllium	1.9 ± 0.2	1.3 ± 0.3	-
Cadmium	0.59 ± 0.10	0.25 ± 0.04	2.38 ± 0.20
Chromium	71 ± 11	123 ±14	113 ± 8
Cobalt	10.8 ± 1.9	11.4 ± 2.1	17.5 ± 1.1
Copper	25.1 ± 3.8	18.5 ± 2.7	452 ± 16
Lead	34.0 ± 6.1	22.7 ± 3.4	404 ± 20
Manganese	513 ± 25	229 ±15	470 ± 12
Mercury	-	-	4.57 ± 0.16
Molybdenum	-	-	12.9 ± 0.9
Nickel	29.5 ± 2.7	55.3 ± 3.6	44.1 ± 2.0
Selenium	0.34 ± 0.06	0.43 ± 0.06	1.09 ± 0.11
Strontium	-	-	277 ± 11
Tin	3.98 ± 0.44	1.85 ± 0.20	41.1 ± 3.1
Vanadium	72.4 ± 17	93.4 ± 4.9	127 ± 5
Zinc	191 ± 17	119 ±12	824 ± 22
Tributyltin (as Sn)	-	-	1.27 ± 0.22
Dibutyltin (as Sn)	-	-	1.16 ± 0.18
Monobutyltin (as Sn)	-	-	0.28 ± 0.17

Matrix and Minor Constituents - (Percent)

	MESS-1	BCSS-1	PACS-1
Al_2O_3	11.03 ± 0.38	11.83 ± 0.41	12.23 ± 0.22
C	2.99 ± 0.09	2.19 ± 0.09	3.69 ± 0.11
CaO	0.674 ± 0.064	0.76 ± 0.074	2.92 ± 0.13
Cl	0.82 ± 0.07	1.12 ± 0.05	2.39 ± 0.09
Fe_2O_3	4.36 ± 0.25	4.70 ± 0.14	6.96 ± 0.12
K_2O	2.24 ± 0.04	2.17 ± 0.04	1.50 ± 0.09
MgO	1.44 ± 0.09	2.44 ± 0.23	2.41 ± 0.09
Na_2O	2.50 ± 0.15	2.72 ± 0.21	4.40 ± 0.11
P_2O_5	0.146 ± 0.014	0.154 ± 0.016	0.233 ± 0.018
S	0.72 ± 0.05	0.36 ± 0.05	1.32 ± 0.08
SiO_2	67.5 ± 1.9	66.1 ± 1.0	55.7 ± 0.5
TiO_2	0.905 ± 0.028	0.734 ± 0.024	0.703 ± 0.011

BEST-1

Mercury 0.092 ± 0.009 milligrams per kilogram

In press, Appl. Organomet. Chem., 1993 (re 76

Certification of Methylmercury Compounds Concentration in Marine Sediment Reference Material, IAEA-356

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[4]*Research Centre, Julich, Applied Physical Chemistry, P.O. Box 1913, 5170 Jülich, (Germany),*
[5]*Swedish Environmental Research Institute, P.O.Box 47086, S-40258 Göteborg, (Sweden),*
[6]*GKSS - Research Institute, Institute of Physics, Marx-Planck-Strasse, D-2054 Geesthacht (Germany),*
[7]*Water Chemistry Program, University of Wisconsin - Madison, 660 North Park Street, Madison, WI 53706, (USA)*

An intercomparison exercise was organized between six laboratories using various isolation procedures (extraction, distillation, ion-exchange, and alkaline digestion) and detection systems (CV AAS, CV AFS, GC-ECD, and HPLC with UV detection) for determination of methylmercury compounds in sediment samples. The value for total methylmercury compounds concentration was certified to be 5.28 $ng.g^{-1}$, with the 95% confidence interval from 4.95 $ng.g^{-1}$ to 6.00 $ng.g^{-1}$. This is the first sediment reference material ever certified for methylmercury compounds concentration. Comparison of the data obtained by various methodologies has shown that the most critical step is the isolation of methylmercury compounds from binding sites. Acid leaching only, can not release methylmercury compounds quantitatively. Total release of methylmercury compounds could only be achieved by alkaline digestion or distillation. This simple intercomparison exercise has shown that since large numbers of laboratories world-wide are performing methylmercury compound analyses using various improved and specific separation methods and sensitive detection systems, certification of methylmercury compounds in different biological and environmental samples should not be a problem in the future.

Keywords: methylmercury, marine sediment, reference material, intercomparison

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* Author to whom correspondence should be addressed

Introduction

At the latest International Conference on "Mercury as a Global Pollutant" (Monterey, CA, June 1992) it was concluded that a lot of questions on the biogeochemical cycle of mercury in the natural environment have been answered, but there are still a lot of the gaps for its total understanding. One of the very important contributions in the latest research on biogeochemical cycles is the availability of specific and sensitive analytical methods, by which it was possible to perform studies under natural conditions. The importance of studies on exposure of humans to mercury through excessive consumption of food (particularly fish) is well known. The methylation of inorganic mercury is a key process in the biogeochemical mercury cycle. Net mercury methylation rates appear to be higher in sediments than in the water column, although the availability of methylmercury compounds formed in sediments to the food chain is unknown. Usually methylmercury concentration in sediments does not exceed 1.5% of the total Hg present. However, its concentration is very important in the interpretation of biogeochemical cycles of mercury in the aquatic environment. Mercury and methylmercury in this compartment of the environment is mainly associated with sulphide and organic substances (especially humic substances, amino acids and proteins).

It is obvious that a good quality assurance programme should be implemented in any of such studies. One way to control the accuracy of the results is by analyzing certified reference materials (CRMs). At present there are many CRMs available for total mercury concentration in various matrices (sediment, soil, ash, water, and tissues). Unfortunately, only five marine biological CRMs are certified for methylmercury compounds. Four are available from the National Research Council of Canada (NRCC)[1] and one is available from the International Atomic Energy Agency (IAEA)[2]. It is understood that these materials are not sufficient to satisfy the quality assurance control requirements in many laboratories performing methylmercury analyses. Therefore, apart from the analyses of CRMs, the accuracy of analytical procedures for determination of methylmercury were tested by intercomparison

exercises[3, 4] as well as comparison of the results obtained by various isolation and final measurement procedures[5, 6]. In some such studies it has been shown that when speciation of mercury compounds is required with insoluble samples (such as sediments and soils) it is very difficult to estimate the recovery of the isolation procedures. This is due to the fact that added methylmercury is not equivalent to the methylmercury originally present in the sample. It has been shown that conventional methods based on acid leaching of organomercury compounds prior to extraction of methylmercury compounds into an organic solvent is inadequate to release methylmercury compounds quantitatively. Recently it has been shown that total release of methylmercury compounds could so far only be achieved by alkaline digestion[7] or distillation[7, 8, 9].

A study of the effects of long-term storage and sample preparation on the stability of methylmercury compounds in sediments[7] and biological sample have already been performed[10]. The results of this study showed that methylmercury is very stable in most of matrices studied. In some currently available biological and sediment RMs certified for total mercury, methylmercury was also determined by a few laboratories using various analytical procedures[5, 6, 7]. Very good agreement of the results was obtained for biological samples[5, 6]. In addition, methylmercury was shown to be very stable under good long-term storage conditions[7,10].

The International Atomic Energy Agency (IAEA) has been providing analytical quality control service (AQCS) to its member states since 1960's. The AQCS programme includes distribution of reference materials, organization of intercomparison runs, providing training courses for quality assurance and chemical analyses of radioactive and partly also non-radioactive measurements of contaminants. For its part the IAEA - Marine Environment Laboratory in Monaco has been organizing intercomparison exercises on trace element analyses in marine biological and sediment samples since 1973. Laboratories (usually more than 100) from all over the world are invited to participate. The reported data are then statistically evaluated and where the consensus median value is very clearly determined and a

series of statistical criteria are fulfilled. the sample is certified as a reference material for use in the analytical quality control programme. A sediment intercomparison sample used for the present study has been prepared within a regular Analytical Data Quality Assurance Programme for trace metal analyses in polluted marine sediment. Preliminary measurements have shown that methylmercury compounds value is relatively high and therefore it was decided to distribute the sample for methylmercury analysis to some laboratories that were willing to participate. The reported results are therefore discussed in the present paper. Since all certification criteria were fulfilled the value for methylmercury was certified. This offers an excellent opportunity for many laboratories to compare their data with the certified value. Results for total mercury analyses are not discussed in this paper, as the value has been certified through a regular large scale intercomparison run[11].

EXPERIMENTAL

Description of the material:

About 30 Kg of sediment was collected from a contaminated bay in the Mediterranean. This sediment was deep frozen, freeze dried, ground and passed through a 250µm sieve and than thoroughly homogenized. Homogeneity of the material was tested by determining the concentration of selected trace metals in several samples taken randomly from the bulk of the powder. A one way analysis of the variance indicated that the material could be considered as homogeneous. The water content of the lyophilized material (as determined by drying to a constant weight at 105 °C) was found to be approximately 1.5%. All results reported are expressed on a dry weight basis.

Participating laboratories

An invitation to participate in a small scale intercomparison exercises on methylmercury analyses was sent to several laboratories that reported methylmercury values in the literature. Those that were willing to participate were given a bottle of approximately 50 g

of sample. A list of participants and the methods used together with relevant references are given in Table 1. Only a brief description of methods used is given below. As the organizer of this intercomparison exercise, the main purpose of the IAEA laboratory was to verify some of the conclusions drawn regarding the effectiveness of the acid leaching method compared to some other isolation techniques. Therefore, their methods are described more precisely.

Laboratory 1

The IAEA Marine Environment Laboratory has used three various approaches to separate methylmercury compounds from the sediment followed by GC with an electron capture detector.

- 1A: The first isolation method is based on distillation[6, 7] followed by solvent extraction method[12]. However, a slight modification was applied. Only approximately 0.5 g of sample was subjected to distillation. Distillate was then acidified with 4 mL 4M H_2SO_4(sat.$CuSO_4$) and 4 mL of 4M KBr. Methylmercury bromide was then extracted into 5 mL of toluene. This extraction was repeated twice. Combined toluene extracts were then purified by a clean-up step. Organic phase was first equilibrated with 3 mL of aqueous 1% cysteine solution and then back-extracted as methylmercury bromide (after acidification with 1.5 mL of 4M H_2SO_4(sat. $CuSO_4$) and 1.5 mL of 4M KBr) into 1 mL of toluene. The clean-up procedure was repeated again in order to reduce possibly present interfering peaks and also to concentrate methylmercury into a smaller volume of toluene. A strip of cysteine impregnated paper was inserted into the final toluene extract so that all methylmercury is trapped on the paper. The paper was rinsed once with a clean portion of toluene and then dried. The method is precisely described in another paper[12]. After acidification with 0.1 mL 4M H_2SO_4(sat. $CuSO_4$) and 0.1 mL of 4M KBr methylmercury bromide was back-extracted into 0.1 mL of toluene. In each separation step the mixture was shaken for 10 min. and centrifuged for 10 min. at 3000 rpm. 2µL of the final extract was then injected on the GC column (column temperature 160°C, 1.6 m long, 2 mm ID, packed with 5% DEGS on Supelcoport 100-120 mesh). An example of the chromatogram

is shown in Figure 1 (A). The recovery of the overall isolation procedure was 68 ± 2 % based on the overage of four spiking recovery tests.

- 1B: The second approach was the same as above except that the distillation step was replaced with the alkaline digestion. Approximately 2 g of the sample was digested with 6 mL of 25% KOH in methanol in closed Teflon vials for 3 hours at 75°C. When cooled it was acidified with 5 mL 4M H_2SO_4(sat. $CuSO_4$) and 5 mL of 4M KBr and extracted into 5 mL of toluene. The extraction was repeated twice. The same double clean-up procedure as described above was used. The volume of the final toluene extract was 0.5 mL. The recovery of spiked methylmercury was 50 % only. However, it was reproducible so that we could correct the results accordingly. An example of the chromatogram is given in Figure 1 (B).
- 1C: The third isolation procedure is based on acid leaching of methylmercury from the sediment. Approximately 2 g of the sediment was acidified with 4M H_2SO_4(sat. $CuSO_4$) and 5 mL of 4M KBr. MeHgBr was extracted into 5 mL of toluene. The extraction was repeated twice. A further procedure included the double clean-up step as described above (1A and 1B). The recovery of spiked methylmercury was 74 ± 1% based on four independent spiked runs. Very similar chromatograms as with the method 1B were obtained.

During each set of analysis two or three blanks were also run. An example of the chromatogram of the extraction procedure is shown in Figure 1 (C).

Laboratory 2:

Two different isolation procedures were applied : distillation[7] (2A) and alkaline digestion with 25% KOH in methano[7, 13, 14] (2B). An aliquot was then subjected to aqueous phase ethylation, gas liquid chromatography (GLC) separation and detection by cold vapour atomic fluorescence (CV AF)[14]. The recovery of spiked MeHg using distillation preseparation was 87.4%.

The laboratory has also provided total Hg results which were obtained by HNO_3/H_2SO_4 digestion in closed Teflon vials and determined by $SnCl_2$ reduction and gold amalgamation CV AF detection..

Laboratory 3

The methylmercury value was obtained by preseparation of methylmercury by distillation[7], followed by aqueous phase ethylation[13], GC separation and detection by CV AF[7, 13]. The recovery of the spiked methylmercury was 90.9 ± 5.1% based on three spiking recoveries. Total mercury was obtained after digestion of the sediment by hot aqua regia, $SnCl_2$ reduction, double amalgamation and CV AF detection[21].

Laboratory 4

Two methods were used for the isolation of methylmercury.

- 4A: The first is based on the distillation of MeHg from approximately 0.2g of the sample[6, 15]. Inorganic mercury from the distillate was removed by anion exchange resin [15, 16]. Methylmercury was destroyed by UV oxidation and measured as Hg^{2+} by $SnCl_2$ reduction, gold amalgamation and cold vapour atomic absorption (CV AA) detection[16]. Recovery of the spiked methylmercury was 80 % In order to confirm the methylmercury concentration obtained, sub aliquots of two distillates prior to UV irradiation were subjected to aqueous phase ethylation with $Na[B(C_2H_5)_4]$, collection on packed GC column at -194°C, separation at 120°C on a GC column, decomposition of separated compounds by pyrolyis and detection by CV AAS[22]. Very similar results were obtained which proved that the organic mercury in the distillates corresponded to methylmercury.

- 4B: The second method is based on acid leaching by 6M HCl, anion-exchange separation of organic and inorganic mercury, UV irradiation and measurement of organic

mercury as Hg^{2+} by $SnCl_2$ reduction, gold amalgamation and CV AA detection[16]. The recovery of spiked methylmercury was more than 98 %.

Total mercury was determined after acid digestion with 10 mL conc. HNO_3 at 150°C for 8 hours in closed quartz vessels[16]. Mercury was then measured by $SnCl_2$ reduction gold amalgamation and CV AA detection.

Laboratory 5

This laboratory has used the same methods as laboratories 2 and 3 [7, 8, 14, 17]. The reported recovery for spiked methylmercury was 82%.

Laboratory 6

Methylmercury was extracted with HCl followed by subsequent extraction with toluene. This step was repeated twice. The clean-up step included equilibration of methylmercury into aqueous thiosulphate solution. After separation thiosulphate solution was acidified and methylmercury chloride back-extracted into 1 mL of the organic solvent (toluene). About 25 µL were injected onto the octadecysilane HPLC column (200X3 mm i.d.) with gradient elution with methanol-water mixture. The absorbance of methylmercury compounds in the eluates was measured at 230 µm. The method is precisely described elsewhere[18, 19].

Laboratory 7

The same analytical method as in Labs 2, 3, and 5 was used.

Results and discussion

As mentioned, the overview of participating laboratories and methods used is given in Table 1. Various separation techniques were used. The very first step, when MeHg was released

from the binding sites three approaches were used: distillation, alkaline digestion and acid leaching. Further processing included additional separation using *anion exchange* separation of organic and inorganic mercury, *derivatization* by aqueous phase ethylation and ~~GLC~~ GC separation, and *solvent extraction* with clean up step using equilibration into aqueous cysteine or thiosulphate solution and back-extraction into organic solvent. Additionally, several detection systems were used: ~~GLC~~ GC combined with ~~CV AF~~ CVAFS, ~~CV AA~~ CVAAS and ECD detectors, and HPLC with UV detector. This study therefore really represents an intercomparison of various analytical methods. The results obtained are shown in Table 2. the aim of the study was to intercompare results for methylmercury compounds as obtained by different methods. However, some laboratories also reported total mercury values which are also reported in Table 2. The value for total Hg was certified on the basis of the results reported in the world-wide intercomparison exercise[11] and the certified value is 7.35.$\mu g.g^{-1}$ with the 95% confidence interval from 6.65 $\mu g.g^{-1}$ to 7.93 $\mu g.g^{-1}$. All values reported by participating laboratories are within the acceptable range. What range is acceptable ?

The laboratory's method means are plotted in ascending concentration values in Figure 2. As evident, results reported vary between 3.66 ng.g-1 to 6.83 ng.g-1. Although initially it appears that results are in good agreement, close examination of the results obtained by various methods have shown that differences can be related to the methods used. The results obtained by acid leaching followed by solvent extraction (Lab/meth. code 1A and 6) are lower than other reported results. This can be explained by the fact that acid leaching alone can not release methylmercury compounds from the sediment quantitatively, which is in agreement with recently published studies[6,7]. Even with the addition of Cu(II) ions quantitative release of methylmercury could not be achieved. Laboratory 1 has released methylmercury by H_2SO_4(sat.$CuSO_4$) and KBr, while the laboratory 6 has used 6 M HCl only. The final results are almost the same. Laboratory 1 has applied the same extraction procedure to distillates and alkaline digested samples and the value obtained were much higher 5.20 and 5.94, respectively. This clearly supports the conclusions drawn in other papers[6, 7] that when sediments rich in organic matter are to be analyzed, acid leaching releases only a certain fraction of methylmercury. Addition of Cu(II) ions can increase the amount of methylmercury

but not quantitatively. Similar conclusions were also reported from a methodological comparison study performed by Padberg et al. [4, 9, 15] for organic rich soil and sediment samples.

The highest result reported in this study was provided by Laboratory 2 using alkaline digestion followed by aqueous phase ethylation, room temperature precollection GLC separation and CV AE detection[13, 14]. It has been shown that when a sample with high concentration of inorganic mercury is analyzed using the above procedure a spurious formation of ethylmethylmercury is formed[7, 8]. This might certainly be a problem with bad quality ethylating reagent. Since the sediment sample IAEA-356 contains relatively high inorganic mercury concentration it can be concluded that higher methylmercury value was obtained due to this effect.

Interesting results were obtained by the Laboratory 4 which has applied non-specific measurement of methylmercury. This means that methylmercury was measured as inorganic after decomposition of MeHg by UV irradiation. As already mentioned in the experimental part the presence of methylmercury in the distillate was confirmed by derivatization of methylmercury into its volatile ethylmethylm mercury derivate and measured by GLC and CV AA detection[22]. The second method which is based on acid leaching and separation of organic and inorganic mercury has been shown to be very unspecific for determination of methylmercury compounds in samples such as sediments soils and organic rich water samples [4, 6, 8, 12]. Usually it resulted in too high organic mercury value. However, in this intercomparison the result obtained compares well with the rest of the results. It is believed that the value obtained do not correspond to methylmercury since the preseparation was performed by 6 M HCl which, as mentioned above, is not able to release methylmercury from this sample quantitatively. This is a typical example when a good agreement of the result obtained by a non-specific method may be misleading. Only a good agreement on one complex environmental sample, such as the sediment used in the present intercomparison, does not necessarily mean that the method in general gives accurate results.

Distillation was used by few laboratories as a preseparation technique. In most laboratories small sample weights (less than 0.50 g) were taken for distillation, since their final detection systems were relatively very sensitive. At the IAEA laboratory larger sample weights were taken in order to achieve necessary concentration of methylmercury in the final extract. Initially about 2 grams of sample was distilled. The recovery of spiked mercury was extremely low (< 5 %). With smaller sample weights a significant improvements in the recovery of spiked methyl mercury was obtained. For example spiking recovery increased from ~10% for 1.5 g of the sample to 75 % for 0.5 g of the sample. So, it is important to note that when separating methylmercury from this sediment by distillation sample weights should not exceed 0.5 g. Experiments on the effect of sample weight on spiking recovery have already been performed in earlier studies on various environmental and biological samples[6, 7]. Such strong interference of the sample weight had not been observed. This further suggests that when analysing such complex matrices as the present sediment precautions in many respects are necessary.

The certified value for total methylmercury compounds was therefore calculated taking into account the results obtained by Lab. code methods 1A, 1B, 2A, 3, 4A, 5, and 7, excluding the results obtained by acid leaching and solvent extraction(1C, 6), alkaline digestion (2B), followed by aqueous phase ethylation, and unspecific anion exchange separation and CV AAS detection (4B). Results obtained by those methods are, however, extremely important since they confirm the conclusions of previously published studies.

Conclusion

The value for total methylmercury compounds in reference material IAEA-356 has been certified. It can be concluded that good samples in terms of representing a "difficult" matrix was chosen. The intercomparison of the results obtained by well experienced laboratories have

confirmed conclusions drawn by previous studies, that when dealing with the analyses of complex matrixes such as sediments, a carefull evaluation of the results is necessary, particularly when using non-specific analytical methods. There is a need to organize similar intercomparison exercises on different kinds of sample matrixes with various methylmercury concentrations. This will be of great help for well experienced laboratories and those that have just started with such analyses.

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Table 1
List of participating laboratories and their analytical methods used for methylmercury compound determinations

Lab. No.*	Institute/ company	Lab./method code	Isolation procedure	Detection technique	DL * (ng/g)	References
1	IAEA	1A	Distillation/solvent extraction	GC ~~GKG~~, ECD	0.6	7, 12
		1B	Alkaline digestion/solvent extraction	GC ~~GLG~~, ECD	0.3	7, 13, 12
		1C	Acid leaching/solvent extraction	GC ~~GLG~~, ECD	0.3	12
2	Brooks Rand, Ltd	2A	Distillation/aqueous phase ethylation	GC GEC, CV AFS	~~0.001~~ 0.01	7, 8, 14
		2B	Alkaline digestion/ aqueous phase ethylation	GC GEC, CV AFS	0.1 1.0	13, 14
3	Frontier Geosciences	3	Distillation/ aqueous phase ethylation	GC GEC, CV AFS	0.001	7, 13, 14
4	Research Centre Julich	4A	Distillation/ion exchange	CV AAS	?	6, 15, 16
		4B	Acid leaching/ anion exchange	CV AAS	?	16
5	IVL, Sweden	5	Distillation,/aqueous phase ethylation	GC GLC, CV AFS	?	7, 8, 14, 17
6	GKSS	6	Acid leaching/solvent extraction	HPLC, UV detector	?	18, 19
7	University of Wisconsin	7	Distillation/ aqueous phase ethylation	GC GLC, CV AFS	?	7, 8, 14

* DL is detection limit of the method, expressed as three standard deviation of the procedural blank.

Table 2
Results for methylmercury compounds determined by various analytical methods used by participating laboratories

Lab. No.*	Lab./method code **	Methylmercury comp. conc. (ng/g)	n	Total Hg (µg/g)	n
1	1A	5.20 ± 0.61	6	6.87 ± 0.35	8
	1B	5.94 ± 0.71	4		
	1C	3.66 ± 0.37	4		
2	2A	5.87 ± 0.20	5	6.69 ± 0.27	3
	2B	6.83 ± 0.50	5		
3	3	5.28 ± 0.29	6	6.18 ±0.33	3
4	4A	6.15 ± 0.56	5	6.34 ±0.27	3
	4B	5.97 ± 0.65	9		
5	5	5.02 ± 0.19	5	n.r.	
6	6	3.87 ± 0.46	5	n.r.	
7	7	4.74 ± 0.54	16	n.r.	

* See Table 1
n.r. Value not reported
n Number of independent determinations

Figure 1

Chromatogram for 2 µL injection from 0.1 mL of the final toluene extract using distilation/solvent extraction procedure (A). Chromatogram for 2 µL injection from 0.5 mL of the final toluene extract using alkaline digestion and solvent extrcation procedure (B). Chromatogram for 2 µL injection of the blank of the extraction procedure (C). Chromatogram for 2 µL standard of 0.050 µgMeHg.mL^{-1}.and 0.050 µg.EtHg mL^{-1}, both expressed as mercury. Column: GP 5% DEGS-PS Supelcoport (100-120 mesh) operated at 160°C and 40 $mL.min^{-1}$ carrier gas-flow rate.

a) Methylmercury; b) Ethylmercury

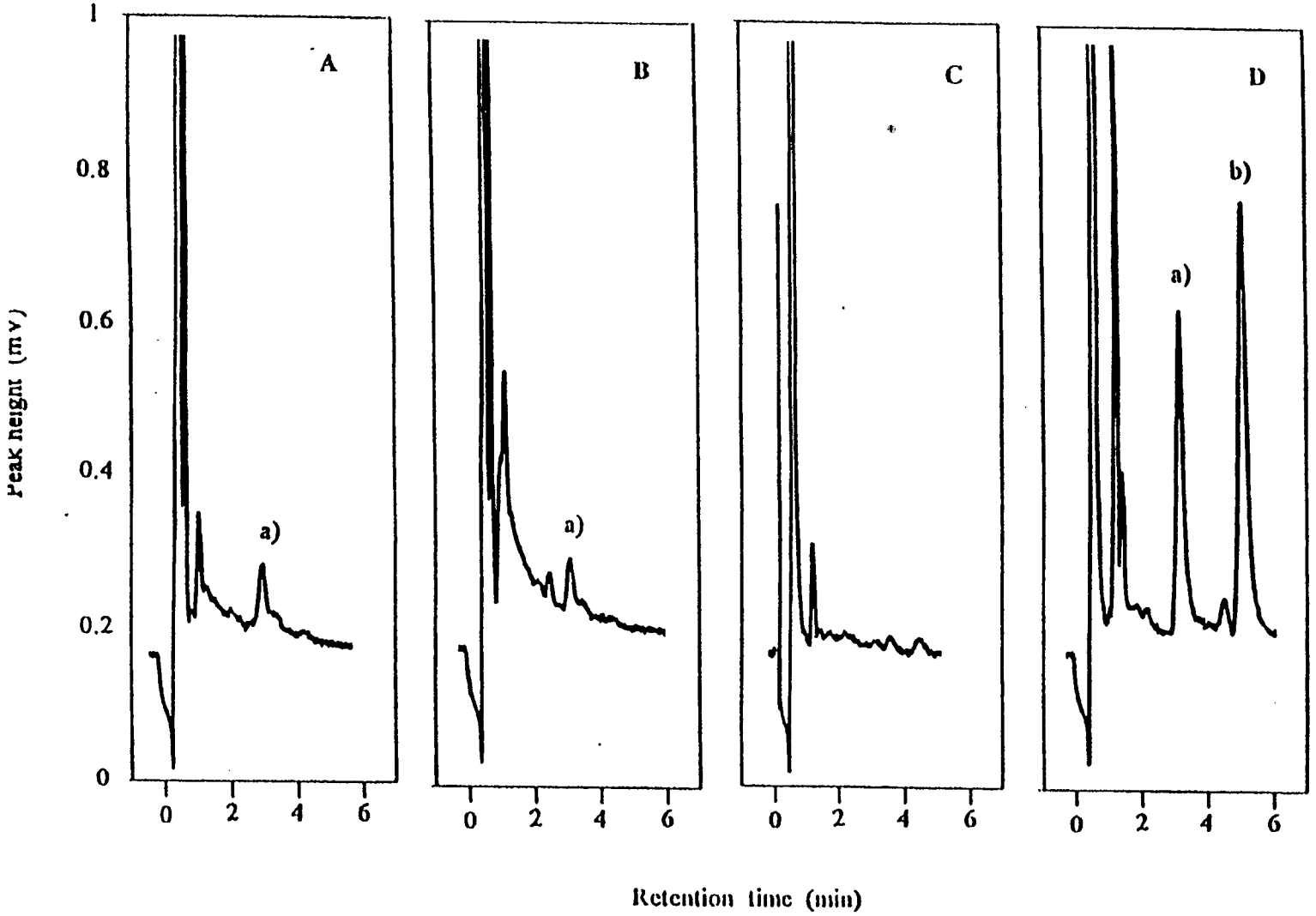
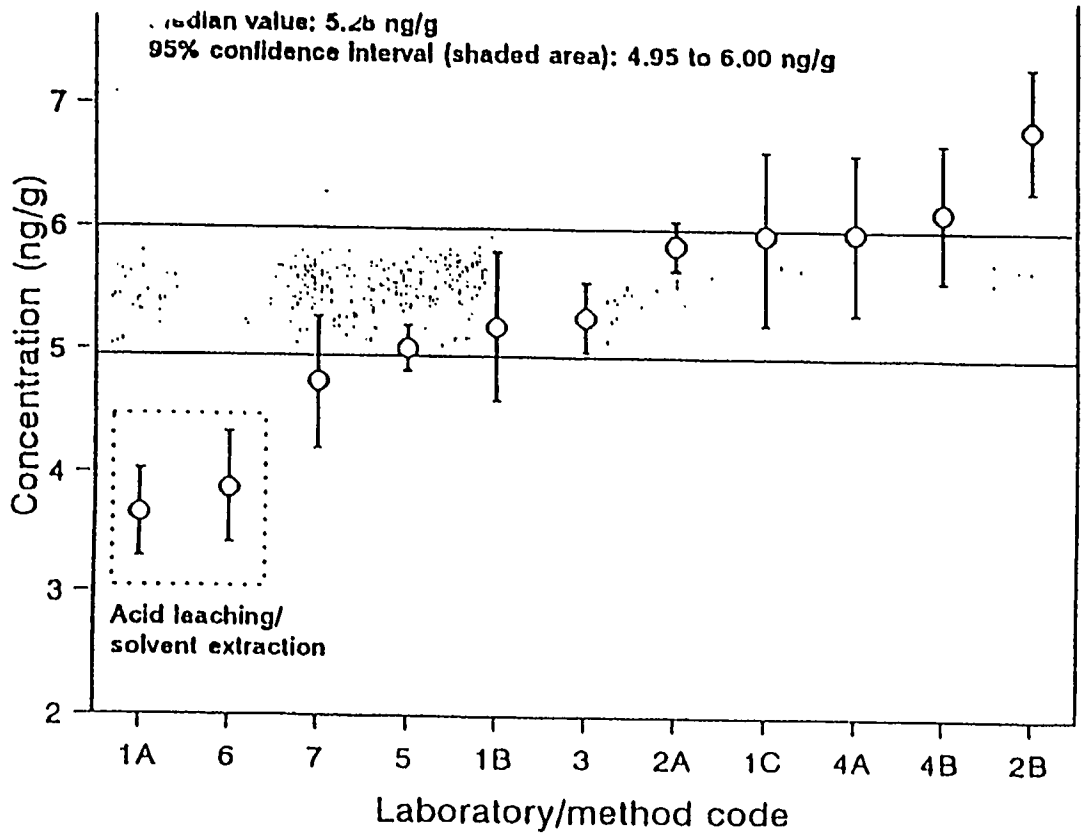

A
B
C
D
a)
a)
a)
b)
1
0.8
0.6
0.4
0.2
0
Peak height (mV)
0
2
4
6
Retention time (min)

Figure 2

The laboratory methods means plotted in ascending concentration on Y-axis and their respective laboratory method codes noted along X- axis. The respective S.D of the means are shown as error bars. Shaded area represent the 95 % confidence interval.


Median value: 5.26 ng/g
95% confidence interval (shaded area): 4.95 to 6.00 ng/g
Concentration (ng/g)
7
6
5
4
3
2
Acid leaching/
solvent extraction
1A
6
7
5
1B
3
2A
1C
4A
4B
2B
Laboratory/method code

Duplicate

BROOKS RAND LTD.
SAMPLE RECEIVING

95 96

Radian Corp (CUSTOMER) 10/19/93 (DATE)

C.O.C. # 02602, 02607, 02629, 02635 15:30 (TIME)

AIRBILL # 7938993366 CARRIER Fed Ex

SAMPLE SEAL INTACT? YES ( ) NO ( ) SEAL# _____

NAME _____ DATE _____

SHIPPING CONTAINER TYPE Cooler CONDITION intact

SAMPLE BOTTLES CONDITION Cool 4°C.

COLLECTION DATE See below

	TAG #	re-3-BOTTLE #	BOTTLE SIZE (ml)	ANALYSIS
1	ZN33 41124	BRL-EC1	9/24/93	T-Hg, Methyl Hg
2	41225	CR-3-02	"	"
3	41326	03	"	"
4	41431	05	"	"
5	71225	06	"	"
6	71329	07	"	"
7	ZC 5441221	08	9/24/93	"
8	1423	09 (*)	"	"
9	2122	10	"	"
10	2324	11	"	"
11	ZN 3372129	13		
12	3121	14	"	"
13	ZC 5351223	15	9/23/93	"
14	1324	16	"	"
15	1425	17	"	"
16	2124	18	"	"
17	ZN 0001145	19	10/1/93	"
18	ZN 3371351	20	10/1/93	"
19				
20				

DO ALL SAMPLE IDENTIFICATIONS MATCH THOSE ON C.O.C.? YES ( ) NO ( )

SAMPLE PRESERVATION _____

SAMPLE STORAGE LOCATION _____

COMMENTS: Check with Dad scientist regarding holding times

(*) 10/22/93 per phone call, samples collected 9/21/93 & 9/23/93 were to be discarded

see page 2 Book 6

SIGNATURE D. Spurling NAME D. Spurling DATE 10/19/93

97

RADIAN CORPORATION
120 S. Jefferson Circle • Oak Ridge, TN 37830

CHAIN-OF-CUSTODY RECORD

N Nº S 02602

PROJECT NAME: EFPC wetlands
PROJECT NUMBER: 380 07-0580 30
SAMPLERS (SIGNATURE): James Groton, Jr
James Groton, Jr.
PRINTED NAME

Lab# BRL	CLIENT ID (9 CHARACTERS) 1	2	3	4	5	6	7	8	9	No. of Bottles/Vials:	GC/MS: 624-8240	625-8270	CLP-VOA	CLP-SVOA	Other:	GC: 601-8010	602-8020	608-8080	8140	CLP Pest/PCBs	Herbicides	Other:	INORGANICS: Metals	Cyanide	CLP Metals	Other:	OTHER: TOC	TOX	Oil & Grease	Pet. Hydro.	Phenols	Neutron Activation	Hg, Methyl Hg			MATRIX: Water/Soil/Sediment	SAMPLING INFO: DATE	TIME	REMARKS: Brooks Rand Ltd. Lab
Rad 01	Z	N	3	3	4	1	1	2	4	1																							X			S	9/21/93	1100	Soil
Rad 02	Z	N	3	3	4	1	2	2	5	1																							X			S	9/21/93	1040	Soil
03	Z	N	3	3	4	1	3	2	6	1																							X			S	9/21/93	1110	Soil
~~04~~	~~Z~~	~~N~~	~~3~~	~~3~~	~~4~~	~~1~~	~~4~~	~~3~~	~~9~~	~~1~~																								X				~~9/21/93~~	~~1135~~	~~Soil~~
05	Z	N	3	3	4	1	4	3	9	1																							X			S	9/21/93	1515	Soil
06	Z	N	3	3	7	1	2	2	8	1																							X			S	9/21/93	1700	Soil
07	Z	N	3	3	7	1	3	2	9	1																							X			S	9/21/93	1715	Soil

RELINQUISHED BY:	Date/Time	RELINQUISHED BY:	Date/Time	RELINQUISHED BY:	Date/Time
James Groton, Jr	9/23/93 0810	[signature]	10/12/93 1325		
COMPANY NAME: SAIC		COMPANY NAME: ETE		COMPANY NAME:	
RECEIVED BY: [signature]	Date/Time 9/23/93 0810	RECEIVED BY: FEDERAL EXPRESS	Date/Time 10/12/93 1325	RECEIVED BY: D. Spurling	Date/Time 10/19/93 15:30
COMPANY NAME: ETE		COMPANY NAME: 7938993366		COMPANY NAME: BRL	
RELINQUISHED BY:	Date/Time	RELINQUISHED BY:	Date/Time	RELINQUISHED BY:	Date/Time
COMPANY NAME:		COMPANY NAME:		COMPANY NAME:	

SHIPPING INFORMATION
Number of Shipping Containers:
Method of Shipment
Special Handling Requirements

(201) 98

RADIAN CORPORATION
120 S. Jefferson Circle • Oak Ridge, TN 37830

CHAIN-OF-CUSTODY RECORD

Nº S 02607

PROJECT NAME: EFR Wetlands
PROJECT NUMBER: 07-0580
SAMPLERS (SIGNATURE): James Groton, Jr
PRINTED NAME: JAMES GROTON, JR

CLIENT ID (9 CHARACTERS)	No. of Bottles/Vials:	GC/MS: 624-8240	625-8270	CLP-VOA	CLP-SVOA	Other:	GC: 601-8010	602-8020	608-8080	8140	CLP Pest/PCBs	Herbicides	Other:	INORGANICS: Metals	Cyanide	CLP Metals	Other:	OTHER: TOC	TOX	Oil & Grease	Pet. Hydro.	Phenols	Neutron Activation	Hg, Methyl Hg				MATRIX: Water/Soil/Sediment	SAMPLING/INFO: DATE 1993	TIME	REMARKS: Brooks
1 2 3 4 5 6 7 8 9																													9/23/93		
08 ZE5441221	1																							X				S	9/23	0940	Soil
09 ZE5441423	1																							X				S	9/23	1010	Soil
10 ZE5442122	1																							X				S	9/23	1035	Soil
11 ZE5412324	1																							X				S	9/23	1105	Soil
17 ~~ZN5~~																															
13 ZN3372129	1																							X				S	9/23	1045	Soil
14 ZN3373121	1																							X				S	9/23	1055	Soil

RELINQUISHED BY:	Date/Time	RELINQUISHED BY:	Date/Time	RELINQUISHED BY:	Date/Time
James Groton, Jr	9/23/93	Sloy Hasel	10/12/93		
COMPANY NAME: JAR	1345	COMPANY NAME: ETE	1325	COMPANY NAME:	
RECEIVED BY: Sloy Hasel	Date/Time 9/23/93	RECEIVED BY: 7938993366	Date/Time 10/12/93	RECEIVED BY: D. Symbus	Date/Time 10/11/93
COMPANY NAME: ETE	1345	COMPANY NAME: Federal Express	1325	COMPANY NAME: BKL	11:3[illegible]
RELINQUISHED BY:	Date/Time	RELINQUISHED BY:	Date/Time	RELINQUISHED BY:	Date/Time
COMPANY NAME:		COMPANY NAME:		COMPANY NAME:	

SHIPPING INFORMATION
Number of Shipping Containers:
Method of Shipment
Special Handling Requirements

RADIAN CORPORATION

120 S. Jefferson Circle • Oak Ridge, TN 37830

CHAIN-OF-CUSTODY RECORD

NO S 02613

PROJECT NAME: EFPC Wetland #

PROJECT NUMBER: 07-0586

SAMPLERS (SIGNATURE): James Groton Jr

JAMES GROTON, JR.
PRINTED NAME

CLIENT ID (9 CHARACTERS)

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#5	Z	E	5	3	8	1	2	2	3	1																						X		S	9/27/93	1485	Brooks Road Ltd. Lab
#6	Z	E	5	3	8	1	3	2	4	1																						X		S		1430	
#7	Z	E	5	3	8	1	4	2	5	1																						X		S		1450	
#8	Z	E	5	3	8	2	1	2	4	1																						X		S		1530	

RELINQUISHED BY:	Date/Time	RELINQUISHED BY:	Date/Time	RELINQUISHED BY:	Date/Time	SHIPPING INFORMATION
James Groton Jr	9/27/93	Brian Henry	10/12/93			Number of Shipping Containers:
COMPANY NAME: SAIC	1730	COMPANY NAME: ETF	1325	COMPANY NAME:		Method of Shipment
RECEIVED BY: Brian Henry	Date/Time 9/27/93	RECEIVED BY: 793899 3366	Date/Time 10/12/93	RECEIVED BY: D. Spencer	Date/Time 10/13/93	
COMPANY NAME: ETF	1731	COMPANY NAME: FEDERAL EXPRESS	1325	COMPANY NAME: BKL	1630	
RELINQUISHED BY:	Date/Time	RELINQUISHED BY:	Date/Time	RELINQUISHED BY:	Date/Time	Special Handling Requirements
COMPANY NAME:		COMPANY NAME:		COMPANY NAME:		

RADIAN CORPORATION
120 S. Jefferson Circle • Oak Ridge, TN 37830

CHAIN-OF-CUSTODY RECORD

Nº S 02629

PROJECT NAME: EFPC Wetlands
PROJECT NUMBER: 07-0580
SAMPLERS (SIGNATURE): James Groton Jr
PRINTED NAME: JAMES GROTON, JR

CLIENT ID (9 CHARACTERS)	#	GC/MS					GC							INORGANICS				OTHER										SAMPLING/INFO		REMARKS
1 2 3 4 5 6 7 8 9	No. of Bottles/Vials:	624-8240	625-8270	CLP-VOA	CLP-SVOA	Other:	601-8010	602-8020	608-8080	8140	CLP Pest/PCBs	Herbicides	Other:	Metals	Cyanide	CLP Metals	Other:	TOC	TOX	Oil & Grease	Pet. Hydro.	Phenols	Neutron Activation	Hg, Methyl Hg				MATRIX: Water/Soil/Sediment	DATE 10/1/93	TIME	Brooks Rand Ltd. Lab
1 2 N 0 0 0 1 1 4 5	1																							X				S	1430		

RELINQUISHED BY:	Date/Time	RELINQUISHED BY:	Date/Time	RELINQUISHED BY:	Date/Time
James Groton Jr	10/1/93	[signature]	10/12/93		
COMPANY NAME: SAIC	1545	COMPANY NAME: ETE	1325	COMPANY NAME:	
RECEIVED BY: [signature]	Date/Time 10/1/93	RECEIVED BY: 7938993366	Date/Time 10/12/93	RECEIVED BY: D Spinks	Date/Time 10/19/93
COMPANY NAME: ETE	1545	COMPANY NAME: FEDERAL EXPRESS	1325	COMPANY NAME: BRL	15:30
RELINQUISHED BY:	Date/Time	RELINQUISHED BY:	Date/Time	RELINQUISHED BY:	Date/Time
COMPANY NAME:		COMPANY NAME:		COMPANY NAME:	

SHIPPING INFORMATION
Number of Shipping Containers:
Method of Shipment
Special Handling Requirements

101

RADIAN CORPORATION
120 S. Jefferson Circle • Oak Ridge, TN 37830

CHAIN-OF-CUSTODY RECORD

№ S 02635

PROJECT NAME: EFIY Wetlands
PROJECT NUMBER: 07-0580
SAMPLERS (SIGNATURE): James Groton, Jr
PRINTED NAME: JAMES GROTON, JR

OR-3 PP1 #

CLIENT ID (9 CHARACTERS) 1	2	3	4	5	6	7	8	9	#	GC/MS: No. of Bottles/Vials	GC/MS: 624-8240	GC/MS: 625-8270	GC/MS: CLP-VOA	GC/MS: CLP-SVOA	GC/MS: Other:	GC: 601-8010	GC: 602-8020	GC: 608-8080	GC: 8140	GC: CLP Pest/PCBs	GC: Herbicides	GC: Other:	INORGANICS: Metals	INORGANICS: Cyanide	INORGANICS: CLP Metals	INORGANICS: Other:	OTHER: TOC	OTHER: TOX	OTHER: Oil & Grease	OTHER: Pet. Hydro.	OTHER: Phenols	OTHER: Neutron Activation	OTHER: Total Hg, Methyl Hg				MATRIX: Water/Soil/Sediment	SAMPLING/INFO: DATE	SAMPLING/INFO: TIME	REMARKS
Z	N	3	3	7	1	3	3	1	1																								X				S	10/11/93	0940	Brooks Rand LH Lab

RELINQUISHED BY:	Date/Time	RELINQUISHED BY:	Date/Time	RELINQUISHED BY:	Date/Time
James Groton, Jr	10/11/93	[illegible]	10/12/93		
COMPANY NAME: SAIC	0950	COMPANY NAME: ETE	1325	COMPANY NAME:	
RECEIVED BY: [illegible]	10/11/93	RECEIVED BY: 7938993366	10/12/93	RECEIVED BY: D. [illegible]	10/19/93
COMPANY NAME: ETE	0950	COMPANY NAME: FEDERAL EXPRESS	1325	COMPANY NAME: ERL	15:30
RELINQUISHED BY:	Date/Time	RELINQUISHED BY:	Date/Time	RELINQUISHED BY:	Date/Time
COMPANY NAME:		COMPANY NAME:		COMPANY NAME:	

SHIPPING INFORMATION
Number of Shipping Containers:
Method of Shipment
Special Handling Requirements

FEDERAL EXPRESS

USE THIS AIRBILL FOR SHIPMENTS WITHIN THE CONTINENTAL U.S.A., ALASKA AND HAWAII
USE THE INTERNATIONAL AIR WAYBILL FOR SHIPMENTS TO PUERTO RICO AND ALL NON U.S. LOCATIONS
QUESTIONS? CALL 800-238-5355 TOLL FREE

PACKAGE TRACKING NUMBER 7938793366

RECIPIENT'S COPY

Date 10/12/93

From (Your Name) Please Print

Your Phone Number (Very Important)

Company

Department/Floor No.

Street Address

City State ZIP Required

To (Recipient's Name) Please Print: Patrick Moore

Recipient's Phone Number (Very Important): 206 432-6216

Company: Brooks Hand ...

Department/Floor No.

Exact Street Address (We Cannot Deliver to P.O. Boxes or P.O. Zip Codes.): 395 ... NW

City: Seattle State: WA ZIP Required: 98107

YOUR INTERNAL BILLING REFERENCE INFORMATION (optional) (First 24 characters will appear on invoice.)

3 PAYMENT 1 [X] Bill Sender 2 [ ] Bill Recipient's FedEx Acct. No. 3 [ ] Bill 3rd Party FedEx Acct. No. 4 [ ] Bill Credit Card
5 [ ] Cash/Check

H HOLD AT FEDEX LOCATION, Print FEDEX Address Here
Street Address
City State ZIP Required

4 SERVICES (Check only one box)

Priority Overnight (delivery by next business morning)
11 [X] OTHER PACKAGING
16 [ ] FEDEX LETTER*
12 [ ] FEDEX PAK*
13 [ ] FEDEX BOX
14 [ ] FEDEX TUBE

Standard Overnight (delivery by next business afternoon)
51 [ ] OTHER PACKAGING
56 [ ] FEDEX LETTER*
52 [ ] FEDEX PAK*
53 [ ] FEDEX BOX
54 [ ] FEDEX TUBE

Economy Two Day (delivery by second business day)
30 [ ] ECONOMY*

Government Overnight (Restricted to authorized users only)
46 [ ] GOVT LETTER
41 [ ] GOVT PACKAGE

Express Service (for packages over 150 lbs.)
70 [ ] OVERNIGHT FREIGHT** (Confirmed reservation required)
80 [ ] TWO-DAY FREIGHT**
† Delivery commitment may be later in some areas
** Declared Value Limit $500 Call for delivery schedule

5 DELIVERY AND SPECIAL HANDLING (Check services required)

Weekday Service
1 [ ] HOLD AT FEDEX LOCATION WEEKDAY (If box in Section H is checked)
2 [X] DELIVER WEEKDAY

Saturday Service
31 [ ] HOLD AT FEDEX LOCATION SATURDAY (If box in Section H is checked)
3 [ ] DELIVER SATURDAY (If also a charge) (Not available to all locations)
9 [ ] SATURDAY PICK-UP (If also charge)

Special Handling
4 [ ] DANGEROUS GOODS (If also charge)
6 [ ] DRY ICE (Dangerous Goods Shipper's Declaration not required)
By (no. of kgs) x ... kg per pkg
12 [ ] HOLIDAY DELIVERY (If offered) (If also charge)

6 | PACKAGES | WEIGHT In Pounds Only | YOUR DECLARED VALUE (See right)
Total | Total | Total

Emp. No Date
[ ] Cash Received
[ ] Return Shipment
[ ] Third Party [ ] Chg. To Del [ ] Chg. To Hold
Street Address
City State Zip
Received By: X
Date/Time Received FedEx Employee Number

7 Release Signature

REVISION DATE 12/92
FORMAT 0150
158

Batch# OR-3

105
103

2

SAMPLE RECEIVING

Radian Corporation (CUSTOMER) 10 30 A (TIME) Oct 26 1993 (DATE)

C.O.C. Present? Y[✓] N[ ] # 501037 and 501036

AIRBILL Present? Y[✓] N[ ] # 7938991665 Carrier Fed Exp

SAMPLE SEAL Present? Y[✓] N[ ] Intact? Y[✓] N[ ] # Nal

Broken by/Date _____ Signed by/Date _____

SHIPPING CONTAINER TYPE Cooler CONDITION Intact

SAMPLE BOTTLES CONDITION Cool, Intact 4°C COLLECTION DATE 10-25-93 [illegible] 10-22-93 [illegible]

	TAG #	BOTTLE #	MATRIX	BOTTLE SIZE	ANALYSIS
1	ZE5441221	OR-3 #21	Soil	100 ml Jar	[illegible] and [illegible]
2	ZE 1 1423	OR-3 #22			
3	2123	OR-3 #23			
4	3324	OR-3 #24			
5	ZN 3372129	OR-3 #25			
6	3121	OR-3 #26			
7	ZN3341124	OR-3 #27			
8	1225	OR-3 #28			
9	1326	#29			
10	1439	#30			
11	ZN3371329	#31			
12	1228	#32			
13					
14					
15					
16					
17					
18					
19					
20					

DO SAMPLE ID's MATCH THOSE ON C.O.C.? Y[✓] N[ ] ON S.A.R.? Y[ ] N[ ]

IF NO LIST NAME, TIME/DATE OF CLIENT CONTACT: _____

SAMPLE PRESERVATION Cooled to 4°C

SAMPLE STORAGE LOCATION Lower Lab Cabinet

COMMENTS: Radian #1-14 discarded as per client's instructions. ✗ High QA

SIGNATURE [signature] NAME Steve Senter DATE Oct 26, 1993

See page 95 Book 5

RADIAN CORPORATION

120 S. Jefferson Circle • Oak Ridge, TN 37830

CHAIN-OF-CUSTODY RECORD

No S 01037

PROJECT NAME: EFPC Wetlands

PROJECT NUMBER: 07-0580

SAMPLERS (SIGNATURE): James Gratton, Jr

JAMES GRATTON, JR
PRINTED NAME

CLIENT ID (9 CHARACTERS)	#	GC/MS					GC						INORGANICS				OTHER									SAMPLING/INFO		REMARKS
1 2 3 4 5 6 7 8 9	No. of Bottles/Vials:	624-8240	625-8270	CLP-VOA	CLP-SVOA	Other:	601-8010	602-8020	608-8080	8140	CLP Pest/PCBs	Herbicides	Other:	Metals	Cyanide	CLP Metals	Other:	TOC	TOX	Oil & Grease	Pet. Hydro.	Phenols	Neuron Activation	Total Hg/Methyl Hg	MATRIX: Water/Soil/Sediment	DATE	TIME	
																										10-25-93		Brooks Rand, Ltd. SOW #266
ZE5441212	1																											012-3
ZE5441423	1																						X		S	0925		1-L-211
ZE5442123	1																						X		S	0900		" 22
ZE5442323	1																						X		S	0940		" 23
ZN3377291	1																						X		S	0910		" 24
ZN3377312	1																						X		S	1040		" 25
																							X		S	1020		" 21

RELINQUISHED BY:	Date/Time	RELINQUISHED BY:	Date/Time	RELINQUISHED BY:	Date/Time
James Gratton Jr	10/25/93	Steve Kiser	10/25/93		
COMPANY NAME: SAIC	1125	COMPANY NAME: ETE	1324	COMPANY NAME:	
RECEIVED BY: Steve Kiser	Date/Time 10/25/93	RECEIVED BY: 7938991605	Date/Time 10/25/93	RECEIVED BY: Steve Seiter	Date/Time 10-26-93
COMPANY NAME: ETE	1125	COMPANY NAME: Federal Express	1324	COMPANY NAME: Brooks Rand LTD	1030
RELINQUISHED BY:	Date/Time	RELINQUISHED BY:	Date/Time	RELINQUISHED BY:	Date/Time
COMPANY NAME:		COMPANY NAME:		COMPANY NAME:	

SHIPPING INFORMATION

Number of Shipping Containers:

Method of Shipment

Special Handling Requirements

RADIAN CORPORATION

120 S. Jefferson Circle • Oak Ridge, TN 37830

CHAIN-OF-CUSTODY RECORD

NO S01036

PROJECT NAME: EFR Wetlands

PROJECT NUMBER: 07-0588

SAMPLERS (SIGNATURE): James Groton, Jr

JAMES GROTON, JR
PRINTED NAME

CLIENT ID (9 CHARACTERS)

1	2	3	4	5	6	7	8	9
Z	N	3	3	4	1	1	2	4
Z	N	3	3	4	1	2	2	5
Z	N	3	3	4	1	3	2	6
Z	N	3	3	4	1	4	3	9
Z	N	3	3	7	1	3	2	9
Z	N	3	3	7	1	2	2	8

#	No. of Bottles/Vials:	GC/MS: 624-8240	GC/MS: 625-8270	GC/MS: CLP-VOA	GC/MS: CLP-SVOA	GC/MS: Other:	GC: 601-8010	GC: 602-8020	GC: 608-8080	GC: 8140	GC: CLP Pest/PCBs	GC: Herbicides	GC: Other:	INORGANICS: Metals	INORGANICS: Cyanide	INORGANICS: CLP Metals	INORGANICS: Other:	OTHER: TOC	OTHER: TOX	OTHER: Oil & Grease	OTHER: Pet. Hydro.	OTHER: Phenols	OTHER: Neutron Activation	OTHER: Total Hg, Methyl Hg	MATRIX: Water/Soil/Sediment	SAMPLING INFO: DATE	SAMPLING INFO: TIME	REMARKS
																												Brooks Rand, Ltd. SOW #2266
																												OK - JG
																								X	S	1425		BRL #27
																								X	S	1430		#28
																								X	S	1440		#29
																								X	S	1450		#30
																								X	S	1510		#31
																								X	S	1520		#32

RELINQUISHED BY:	Date/Time	RELINQUISHED BY:	Date/Time	RELINQUISHED BY:	Date/Time
James Groton Jr	10/22/93 1610	[illegible]	11/2/93 1324		
COMPANY NAME: SAIC		COMPANY NAME: ETE		COMPANY NAME:	
RECEIVED BY: [illegible]	Date/Time 11/24/93 1610	RECEIVED BY: 793849 1645	Date/Time 11/25/93 1324	RECEIVED BY: STEVE SENTER	Date/Time 10-26-93 1015
COMPANY NAME: ETE		COMPANY NAME: Federal Express		COMPANY NAME: BROOKS RAND LTD	
RELINQUISHED BY:	Date/Time	RELINQUISHED BY:	Date/Time	RELINQUISHED BY:	Date/Time
COMPANY NAME:		COMPANY NAME:		COMPANY NAME:	

SHIPPING INFORMATION

Number of Shipping Containers:

Method of Shipment

Special Handling Requirements

107

FEDEX EXPRESS

USE THIS ARRIVAL FOR SHIPMENTS WITHIN THE CONTINENTAL U.S.A., MEXICO AND HAWAII
USE THE INTERNATIONAL AIR WAYBILL FOR SHIPMENTS TO PUERTO RICO AND ALL NON U.S. LOCATIONS
QUESTIONS? CALL 800-238-5355 TOLL FREE.

PACKAGE TRACKING NUMBER

7938991605

RECIPIENT'S COPY

1 Date

From (Your Name) Please Print

Company

Street Address

City State ZIP Required

Your Phone Number (Very Important)

Department/Floor No.

2 To (Recipient's Name) Please Print

Company

Exact Street Address (We Cannot Deliver to P.O. Boxes or P.O. Zip Codes)

City State ZIP Required

Recipient's Phone Number (Very Important)

Department/Floor No.

YOUR INTERNAL BILLING REFERENCE INFORMATION (optional) (First 24 characters will appear on invoice)

225-212-01-15 (EFIC Wetlands)

3 PAYMENT 1 [X] Bill Sender 2 [ ] Bill Recipient's FedEx Acct. No. 3 [ ] Bill 3rd Party FedEx Acct. No. 4 [ ] Bill Credit Card 5 [ ] Cash/Check

H IF HOLD AT FEDEX LOCATION, Print FEDEX Address Here

Street Address

City State ZIP Required

4 SERVICES (Check only one box)

Priority Overnight (Delivery by next business morning)

11 [X] OTHER PACKAGING
16 [ ] FEDEX LETTER*
12 [ ] FEDEX PAK*
13 [ ] FEDEX BOX
14 [ ] FEDEX TUBE

Standard Overnight (Delivery by next business afternoon for Saturday delivery)

51 [ ] OTHER PACKAGING
56 [ ] FEDEX LETTER*
52 [ ] FEDEX PAK*
53 [ ] FEDEX BOX
54 [ ] FEDEX TUBE

Economy Two Day (Delivery by second business day)

30 [X] ECONOMY*

Government Overnight (Restricted for authorized users only)

46 [ ] GOV'T LETTER
41 [ ] GOV'T PACKAGE

Freight Service (for packages over 150 lbs.)

70 [ ] OVERNIGHT FREIGHT** (Confirmed reservation required)
80 [ ] TWO-DAY FREIGHT**

* Delivery commitment may be later in some areas
** Declared Value Limit $500 Call for delivery schedule

5 DELIVERY AND SPECIAL HANDLING (Check services required)

Weekday Service

1 [ ] HOLD AT FEDEX LOCATION WEEKDAY (Fill in Section H)
2 [X] DELIVER WEEKDAY

Saturday Service

31 [ ] HOLD AT FEDEX LOCATION SATURDAY (Fill in Section H)
3 [ ] DELIVER SATURDAY (Extra charge) (Not available in all locations)
9 [ ] SATURDAY PICK-UP (Extra charge)

Special Handling

4 [ ] DANGEROUS GOODS (Extra charge)
6 [ ] DRY ICE Dangerous Goods Shipper's Declaration not required
[ ]
12 [ ] HOLIDAY DELIVERY (Extra charge)

6 PACKAGES | WEIGHT in Pounds Only | YOUR DECLARED VALUE (See right)

Total | Total | Total

Emp. No | Date

[ ] Cash Received
[ ] Return Shipment
[ ] Third Party [ ] Chg. To Del [ ] Chg. To Hold

Street Address

City State Zip

Received By:

X

Date/Time Received FedEx Employee Number

7 Release Signature

REVISION DATE 12/92
FORMAT #158

158

106

(108)

#8

COMPARISON OF MERCURY SPECIATION METHODS

M. O. Barnett, R. R. Turner and K. Misra[1]

Environmental Sciences Division
Oak Ridge National Laboratory[2]
Oak Ridge, TN 37831

INTRODUCTION

The floodplain of East Fork Poplar Creek (EFPC) in Oak Ridge, Tennessee, is contaminated with mercury from historical releases from the U.S. Department of Energy Y-12 Plant located in the headwaters. The creek and its floodplain are currently being investigated as a Superfund site. Selection of appropriate remedial actions will depend in part on information concerning the speciation, or chemical form of mercury, in the floodplain soils. The history of studies of the speciation of mercury in these soils has been reviewed in detail elsewhere (Sect. 2.1 of this addendum). Revis et al. (1989a,b), in one of the earliest attempts to characterize the forms of mercury in EFPC soils, developed a sequential extraction procedure for mercury in soils. The results of applying this extraction procedure to EFPC soils with a range of total mercury concentrations indicated that mercuric sulfide was the predominant mercury form in these soils, accounting for 63 to 100% of the mercury in the soil. Evidence to support Revis' work has been provided by the results from a variety of electron and X-ray beam techniques [scanning electron microscopy (SEM), X-ray diffraction (XRD), transmission electron microscopy (TEM)/selected area electron diffraction (SAED)] applied to EFPC soil as discussed in Sect. 2.1.

Recently, a new sequential extraction procedure (Miller 1993), developed for the Environmental Protection Agency (EPA) at the Environmental Monitoring Systems Laboratory (EMSL) in Las Vegas, Nevada, was used to characterize a suite of 20 soils from EFPC (results discussed in Sect. 2.1). The soils were not the same soils analyzed by Revis et al. Although mercuric sulfide was suggested by the EMSL results to be a significant species in some soils, metallic mercury or mercury amalgams were suggested as the predominant forms in the soils tested (EPA 1994).

To resolve the discordance between the Revis and EMSL results, a study was initiated in late April 1994 to perform both the Revis and EMSL extraction procedures on the same set of EFPC soils. An additional sequential extraction procedure (Sakamoto et al. 1992), never before used for EFPC soil, was also included in this comparison. Finally, estimates of the presence and relative fraction of metallic mercury in EFPC soils were made using sample headspace measurements and losses of mercury from the soils due to thermal treatment.

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[1] Department of Geological Sciences, University of Tennessee, Knoxville, Tennessee.

[2] Managed for the U.S. Department of Energy by Martin Marietta Energy Systems, Inc. under contract DE-AC05-84OR21400.

METHODS

A subset of the soil samples collected in January 1994 and used for the EMSL extraction study were used for the present comparison. The samples were collected from a variety of locations within the EFPC floodplain, homogenized in stainless steel bowls, and refrigerated in the naturally moist state at ORNL in sealed containers until use. Four soils (ZN5470424, ZE3340211, ZN3181013, ZN5150729) were selected from the original group, and a fifth soil (ZN3210127) from the original location and depth in the floodplain was resampled on April 24, 1994. The fresh sample exhibited a very similar total mercury concentration (2400 mg/kg) to the earlier sample (2700 mg/kg) from the same location. In the discussion and tables that follow, the soils are identified by reference only to the last three digits of the sample identifier as underlined above.

Small (≤ 100 g) subsamples of each soil were removed and further homogenized. One portion (~ 3 g) of each soil was air dried to determine percent moisture, ground, and submitted for mercury analysis by EPA Method 7471 (SW-846). Aliquots of each moist soil were sequentially extracted in 50-mL centrifuge tubes in accordance with the methods outlined in each of the three procedures (some slight deviations are discussed below). Triplicate aliquots were run for sample 729 for each procedure to quantify the reproducibility of results. In addition, metacinnabar, the form of mercuric sulfide identified unequivocally in EFPC soil by TEM/SAED (Sect. 2.1), was spiked into the soil with the lowest total mercury concentration (013) to measure recovery of a known mercury species of interest. The metacinnabar was precipitated in the laboratory and submitted for XRD verification of crystal form. The supernatant from each extraction was preserved with 17.5 mL of 0.7% potassium dichromate in 1:1 nitric acid and diluted to ~ 250 mL prior to analysis for total mercury by EPA Method 7470 (SW-846). Process blanks were carried through each procedure and analyzed for total mercury. The residual soil left after the last extraction step was also recovered, dried, weighed, ground, and submitted for total mercury analysis by EPA Method 7471 (SW-846). National Institute for Standards and Technology SRM 2710, Montana Soil (certified mercury value = 32.6 ± 1.8 mg/kg) was submitted blind to the analytical laboratory as a check on accuracy. All mercury analyses were performed by the Y-12 Plant Laboratory.

There were a few deviations between the sequential extraction protocols, as described by their respective authors, and the manner in which they were carried out by us. All extraction protocols were run using field-moist soils in contrast to the EMSL specification of dried and pulverized soils. However, all results were corrected to a dry weight basis. Revis et al. used field-moist soils and Sakamoto et al. did not clearly specify whether samples were first dried or not. Drying (especially at 45 to 50°C) and machine pulverizing the soils, as was done by EMSL, was thought by us to potentially alter mercury speciation. Ten-gram portions, as opposed to 20-g portions, of soil for the EMSL procedure were used to avoid overflow problems due to sample foaming noted in a trial run with 20 g in the 50-mL centrifuge tubes. Solid/solution and solid/extractant ratios were, however, maintained at their specified values. The supernatants from each of the centrifuged extracts were filtered using 0.2- or 0.4-μm pore size filters to avoid any possible transfer of soil into the extracts (some soils produced a floating fraction after extraction and centrifugation). Finally, the determination of "organic" mercury in each procedure was deleted because both the Revis et al. and EMSL results, as well as other data, have not indicated that organic mercury constitutes a significant fraction in the EFPC soils (typically < 0.1% of total mercury).

to calculate mercury [illegible]

Revis et al. (1989a,b) estimated the fraction of metallic mercury in EFPC soils by the loss of mercury from soils heated to 150°C for 5 d. Landa (1978), using Montana soils exposed to mercury vapor, had shown previously that metallic mercury "sorbed" to soils is quantitatively volatilized between 100 to 200°C over a period of several days. Revis et al. (1989a,b) recovered 100% of metallic mercury dosed into soils and held at 150°C for 5 d. We applied the Revis et al. method of estimating the fraction of metallic mercury to all 20 EFPC soil samples previously characterized by the EMSL speciation protocol. One-gram aliquots (moist weight) were air dried and placed in 125-mL glass reagent bottles fitted with gas-purging closures. The outlet side of each closure was fitted with an iodated charcoal mercury sorbent tube (MSA Part No. 459003). After placing the bottles in an oven, the inlet side of each closure was connected to a mercury-free air supply (60 cc/min) using silicone tubing. The oven was brought to temperature (150 ± 5°C) and held for 3 d. After 3 d the oven and air flow were turned off long enough to change the charcoal tubes and then restarted for an additional 2 d of thermal treatment. After cooling, the soil in each bottle was recovered for total mercury analysis by EPA Method 7471 (SW-846). The charcoal sorbent were analyzed by a modified version of Method 7471.

In addition to applying the Revis et al. protocol for metallic mercury, we also measured mercury vapor in the headspace of 1-L glass jars containing 1.0 gram aliquots of air-dried and ground soils from the same group of 20 EFPC soils characterized by EMSL and used in the bioavailability study (Sect. 2.1). Soils were equilibrated for 24 h prior to headspace mercury vapor measurements using a Jerome Model 431-X Gold Film Mercury Analyzer (Arizona Instruments Inc, Tempe, Arizona). The highest of three successive 10-second (125 cc of headspace air) measurements was recorded. Measurements were conducted at room temperature (23°C) and 50°C.

RESULTS AND DISCUSSION

Sakamoto Protocol

Results for the protocol developed by Sakamoto et al. (1992) are summarized in Table A.1. Mercury and material balances for this protocol were relatively good (mean mercury recovery of 98%) with the possible exception of that for Soil 013 (70%). The total mercury value for this soil, obtained by analysis of the aliquot used for moisture determination, may be unrepresentative. Earlier analyses of mercury in this soil by both us and EMSL gave values between 28 and 34 mg/kg, or 66 to 81% of the value (42 mg/kg) shown in Table A.1. Using the lower value to calculate mercury balance would yield much better overall recovery.

Virtually no mercury was extracted from the soils by the 0.05 molar sulfuric acid solution intended by developers of this protocol to extract mercuric oxide. The cuprous chloride solution, intended as a selective extractant for mercuric sulfide, extracted significant fractions (63 to 112%) of the soil mercury for all samples except for the sample dosed with metacinnabar (which released only ~24% of its mercury into this extractant).

Revis Protocol

Considerable difficulty was experienced in analyzing the sodium sulfide extracts from this procedure. The entire procedure was carried out twice for the study soils without resolving the

Table A.1. Results of application of Sakamoto et al. (1992) mercury speciation protocol to EFPC soils

		0.05 *M* H_2SO_4		CuCl in 1 *M* HCl		Residue			Hg recovery %
Soil sample	Total Hg mg/kg	Solution Hg mg/L	Percent total Hg	Solution Hg mg/L	Percent total Hg	Weight g	Residue Hg mg/kg	Percent total Hg	
013	42	0.0002	0.04	0.36	66	3.55	1.8	4.2	70
127	2400	0.0071	0.0007	19	63	3.49	750	30	93
211	270	0.0002	0.006	3.4	105	3.21	34	12	117
424	1300	0.0013	0.009	12	83	3.25	180	14	98
729-1	840	0.0002	0.002	11	112	3.34	86	10	122
729-2	840	0.0002	0.002	8.6	89	3.10	72	7.9	97
729 Mean			0.002		100			9.1	110
Total Mean			0.011		84			14	98
HgS Spike	4540	0.0002	0.000	14	24	3.60	3000	65	89

difficulty. Acidification of the sodium sulfide extracts caused elemental sulfur to precipitate in the extracts. The presence of elemental sulfur in turn caused low recovery of the mercury. Partial results for the protocol are summarized in Table A.2. Although the results are incomplete, sufficient results are available to conclude that substantially more mercury (average of 54% with range from 19 to 99%) was released from the soils by 12 *M* nitric acid than expected (~15%) based on the findings of Revis et al. (1989a). The relatively low residue fractions (mean of 4.2%) for most of the soils, and the metacinnabar-spiked soil (mean of 1.5%), suggest that the bulk of the unaccounted for mercury should have been in the sodium sulfide extracts that could not be analyzed. Assuming reasonable mass balance, the sodium sulfide extracts should have accounted for 1 to 76% of total mercury. Contrary to expectation based on results from Revis et al. (1989a,b), the concentrated nitric acid extracted ~12 and 31% of the metacinnabar in the duplicate spiked soils.

EMSL Protocol

Results for the protocol developed by EMSL (Miller 1994) are summarized in Table A.3. Overall mass balance was reasonably good with the same tendency noted by EPA (1994) for the sum of the fractions to exceed 100%. The notable exception to this trend is Soil 013 which indicated only 65% mercury recovery. As noted for the Sakamoto results, the total mercury value (42 mg/kg) used to calculate the species fractions may be unrepresentative (and too high) of the soil actually extracted. Mercury remaining in the residual soils after the last sequential extraction was low, accounting for from 2 to 7% of the total soil mercury and indicating relatively complete extraction of all the mercury from the soils by the complete sequence.

The potassium sulfate/chloride solution extracted almost no mercury (<0.1%) from the soils. The dilute nitric acid extracted an average of ~6% of soil mercury, with Soil 127 exhibiting the maximum fraction (22%) for this extractant. Metacinnabar was essentially insoluble in both the potassium sulfate/chloride and dilute nitric acid solutions.

The 4 *M* nitric acid solution was particularly effective in extracting mercury (average of 72%) from all the soils, including from the soil spiked with metacinnabar (45%). EMSL attributed the mercury extracted by this solution to metallic or amalgamated mercury. The average fraction of soil mercury extracted by aqua regia was 30%, with a range from 6 to 46%. Compared to the Sakamoto and Revis protocols, the EMSL protocol was the most destructive of the soil, with ~40% of the soil mass lost in the overall extraction compared with ~10 to 15% in the Revis protocol.

Discussion of Extraction Results

Although all three mercury speciation protocols underwent some form of validation by the respective authors, it is clear that the extractants are not as selective in removing a given form of mercury as implied by the original publications describing each protocol. Sakamoto et al. (1992) did not propose a selective extractant for metallic/amalgamated mercury, and the effectiveness of the cuprous chloride in removing metallic mercury was not evaluated by the original authors or by us. Nonetheless, the cuprous chloride solution was notably ineffective (only 24%) in removing the very form of mercury claimed by the protocol developers to be extracted with this reagent. The EMSL protocol suffered the opposite problem: the extractant targeted at metallic/amalgamated mercury actually extracted almost one half (45%) of the

Table A.2. Partial results of application of Revis et al. (1989b) mercury speciation protocol to EFPC soils (First run/Second run)

		12 *M* HNO_3		Saturated Na_2S		Residue			Percent mercury recovery[b]
Soil sample	Total Hg mg/kg	Solution Hg mg/L	Percent total Hg	Solution Hg mg/L	Percent total Hg	Weight g	Residue Hg mg/kg	Percent total Hg	
013	42/29	0.068/0.073	64/99	—[a]	—[a]	0.84/0.65	1.0/0.5	2.7/1.6	66/101
127	2400/2300	2.5/3.0	41/52	—[a]	—[a]	0.97/73	74/80	4.2/3.6	46/56
211	270/270	0.57/0.64	88/96	—[a]	—[a]	0.81/0.62	5.0/5.3	2.2/1.8	91/98
424	1300/1100	0.56/0.52	19/21	—[a]	—[a]	0.86/0.61	52/35	5.4/3.1	26/24
729-1	840/810	0.58/0.58	30/31	—[a]	—[a]	0.93/0.65	105/10	17/1.2	48/32
729-2	840/810	0.60/0.57	31/30	—[a]	—[a]	0.94/0.59	140/16	23/1.7	55/32
729-3	840/810	0.57/0.64	29/33	—[a]	—[a]	0.90.0.62	52/13	8.3/1.5	38/35
729 Mean			30/31		—[a]			16/1.5	47/33
Total Mean			48/60		—[a]			6.1/2.3	55/62
HgS Spike	3340/1240	1.0	12/31	—[a]	—[a]	0.84/0.65	52/12	2.1/0.9	14/32

[a] Results rejected because of low recovery of mercury after elemental sulfur precipitated in samples.
[b] Based on recovery by 12 *M* HNO_3 and in residue only.

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Table A.3. Results of application of EMSL mercury speciation protocol to EFPC soils

		0.01 *M* K_2SO_4/KCl		0.2 *M* HNO_3		4 *M* HNO_3		Aqua Regia		Residue			
Soil sample	Total Hg mg/kg	Solution Hg mg/L	Percent total Hg	Solution Hg mg/L	Percent total Hg	Solution Hg mg/L	Percent total Hg	Solution Hg mg/L	Percent total Hg	Weight g	Residue Hg mg/kg	Percent total Hg	Percent mercury recovery
013	42	0.00004	0.004	0.0003	0.03	0.62	58	0.065	6.1	6.45	0.81	1.7	66
127	2400	0.01	0.017	13	22	22	36	24	41	6.63	190	7.3	106
211	270	0.0002	0.003	0.0026	0.04	6.2	95	0.47	7.5	5.70	6.2	1.9	105
424	1300	0.0013	0.004	1.6	5.5	20	68	13	46	5.73	100	6.9	126
729-1	840	0.0008	0.004	0.33	1.7	16	81	5.0	26	5.69	35	3.5	112
729-2	840	0.0005	0.003	0.40	2.0	21	104	4.8	25	6.22	35	3.9	135
729-3	840	0.0005	0.003	0.37	1.9	24	120	5.1	27	6.16	39	4.3	153
729 Mean			0.003		1.9		102		26			3.9	133
Total Mean			0.006		5.8		72		25			4.3	107
HgS Spike	1530	0.00003	0.0001	0.0006	0.002	18	45	32	84	6.51	84	4.9	134

mercury in a metacinnabar-spiked soil. Both the EMSL and Revis protocols under-predicted the amount of mercuric sulfide in the metacinnabar spike soil. The results for the protocol (Revis) which was used originally to suggest that mercury occurred predominantly in the sulfide form clearly contradict the original findings (average of 85% mercuric sulfide). One unpublished report (Revis et al. 1989c) to DOE by the Revis group does show results obtained using 12 *M* nitric acid applied to 21 soils and sediments from EFPC ranging in total mercury concentration from 2.0 to 1800 mg/kg. For this group of soils the percent of total mercury which was soluble in the nitric acid ranged from 12 to 92%, with an average of 43%. This range and average agrees much better with the results obtained here using the Revis protocol.

Because we employed a subset of essentially the same soils used by EMSL in their recent characterization (EPA 1994) of mercury speciation, it is instructive to compare these interlaboratory (EMSL versus ORNL) results (Table A.4). As noted in the Methods section, we employed naturally moist soils instead of dried and ground soils to avoid any possibility of changing the in situ speciation of mercury in the soils. We might expect drying and grinding to improve recovery of some forms of mercury in some extractants by virtue of the greater surface area exposed to leaching. On the other hand, dry grinding can cause reactions and phase changes among otherwise unreactive components (e.g., metallic mercury and elemental sulfur). One reference in a handbook of preparative chemistry notes that metacinnabar can be converted to cinnabar by grinding, and we have produced metacinnabar by gentle grinding of a mixture of elemental sulfur and metallic mercury.

With some notable exceptions, the results given in Table A.4 are remarkably similar in many cases (e.g., Soils 127 and 424) and within the uncertainty expected given the differences in sample processing prior to extraction. No consistent laboratory bias appears to be present even where significant differences exist between laboratories (e.g., results for 0.2 *M* nitric acid). We experienced considerable difficulty achieving and maintaining the target pH value specified by EMSL for the 0.2 *M* nitric acid, which may explain the large discrepancies between laboratories in these results. EFPC soils are known to have considerable acid neutralizing capacity. Even anticipating this issue, it was still a challenge to maintain pH during the heating portion of this step. We contend that "adsorbed" mercury, rather than mercuric oxide, is being removed during this step and thus solution pH is critical to control within close limits.

Thermal Release and Sample Headspace Measurements

Results of thermal treatment to release metallic mercury in accordance with the method of Revis et al. (1989b) are given in Table A.5 and compared with EMSL results for metallic/amalgamated mercury in Fig. A.1. In most cases, but not all, the EMSL results are higher than the Revis results. The two negative percentages probably result from sample inhomogeneity due to using the total mercury analyses for air-dried and ground soils as the basis for the initial concentration. There is no correlation between the EMSL extraction results and the thermal release results. The results based on mercury trapped on the charcoal tubes show 5 and 24% soil losses, respectively, for the two samples which yielded negative recoveries. Unfortunately, either method (thermal or chemical) of estimating this mercury fraction could be in error. Estimates based on thermal release could be low due to presence of mercury amalgams or high due to thermal degradation of non-elemental forms. The charcoal tubes which collected mercury being released from the soils on thermal treatment days 4 and 5 typically contained only ~10% of the total mercury volatilized, indicating that most of the volatilization occurred in the

Table A.4. Comparison of results of EMSL mercury speciation protocol conducted at EMSL and at ORNL[a]

Soil sample	Total Hg mg/kg[b]	0.01 *M* K_2SO_4/KCl		0.2 *M* HNO_3		4 *M* HNO_3		Aqua Regia	
		EMSL %	ORNL %	EMSL %	ORNL %	EMSL %	ORNL %	EMSL %	ORNL %
013	28/42	0.1	<0.1	71	<0.1	21	58	7.7	6.1
127	2700/2400	0.3	<0.1	7.1	22	35	36	57	41
211	270/270	<0.1	<0.1	<0.1	<0.1	92	95	8.4	7.5
424	1300/1300	0.1	<0.1	14	5.5	49	68	37	46
729	900/840	0.1	<0.1	11	1.9	56	102	33	26

[a] Soils were from same sampling event (1/31/94), except for Sample 127 which was resampled (4/24/94) for ORNL effort. Expressed as percent (dry weight basis) of total mercury soluble in each extracting solution. EMSL used pulverized dry soil while ORNL used soil in naturally moist state.

[b] First value is for dry ground aliquot from "Bioavailability Study"; second value from dry ground aliquot for "% moisture."

Table A.5. Results of thermal treatment of EFPC soils and sample headspace measurements, including results from EMSL for metallic/amalgamated mercury

Soil sample	Total Hg mg/kg	150°C Total Hg mg/kg	150°C Percent volatilized[a]	EMSL percent metallic[b]	23°C Headspace mg/m³	50°C Headspace mg/m³
ZN3210115	260	200	23/21	74	<0.001	<0.001
ZN3210127	2700	1300	52/40	35	0.152	0.806
ZN3340211	270	240	11/13	92	<0.001	<0.001
ZN3340223	1900	1600	16/4.0	25	0.005	0.069
ZN3340312	230	160	30/19	94	<0.001	<0.001
ZN3340324	2100	1800	14/10	36	<0.001	<0.001
ZE5470412	85	32	62/53	88	<0.001	<0.001
ZE5470424	1300	1200	8/5.5	49	<0.001	<0.001
ZE5380512	67	32	52/37	91	<0.001	<0.001
ZE5380524	2100	1400	33/4.3	32	<0.001	<0.001
ZE5340619	140	61	56/47	90	<0.001	<0.001
ZE5340621	1200	1600	-33/5.2	57	<0.001	<0.001
ZE5150717	230	66	71/52	83	<0.001	<0.001
ZE5150729	900	700	22/8.3	56	<0.001	<0.001
ZE3770810	480	420	13/8.4	81	<0.001	<0.001
ZE3770822	15	19	-27/24	76	<0.001	<0.001
ZE3740918	55	19	65/60	26	<0.001	<0.001
ZE3740920	780	560	28/19	66	<0.001	<0.001
ZE3181013	28	15	46/36	21	<0.001	<0.001
ZE3181025	180	180	54/16	36	<0.001	<0.001

[a] First value is based on loss from soil; second value is based on mercury trapped on charcoal.
[b] EPA (1994).

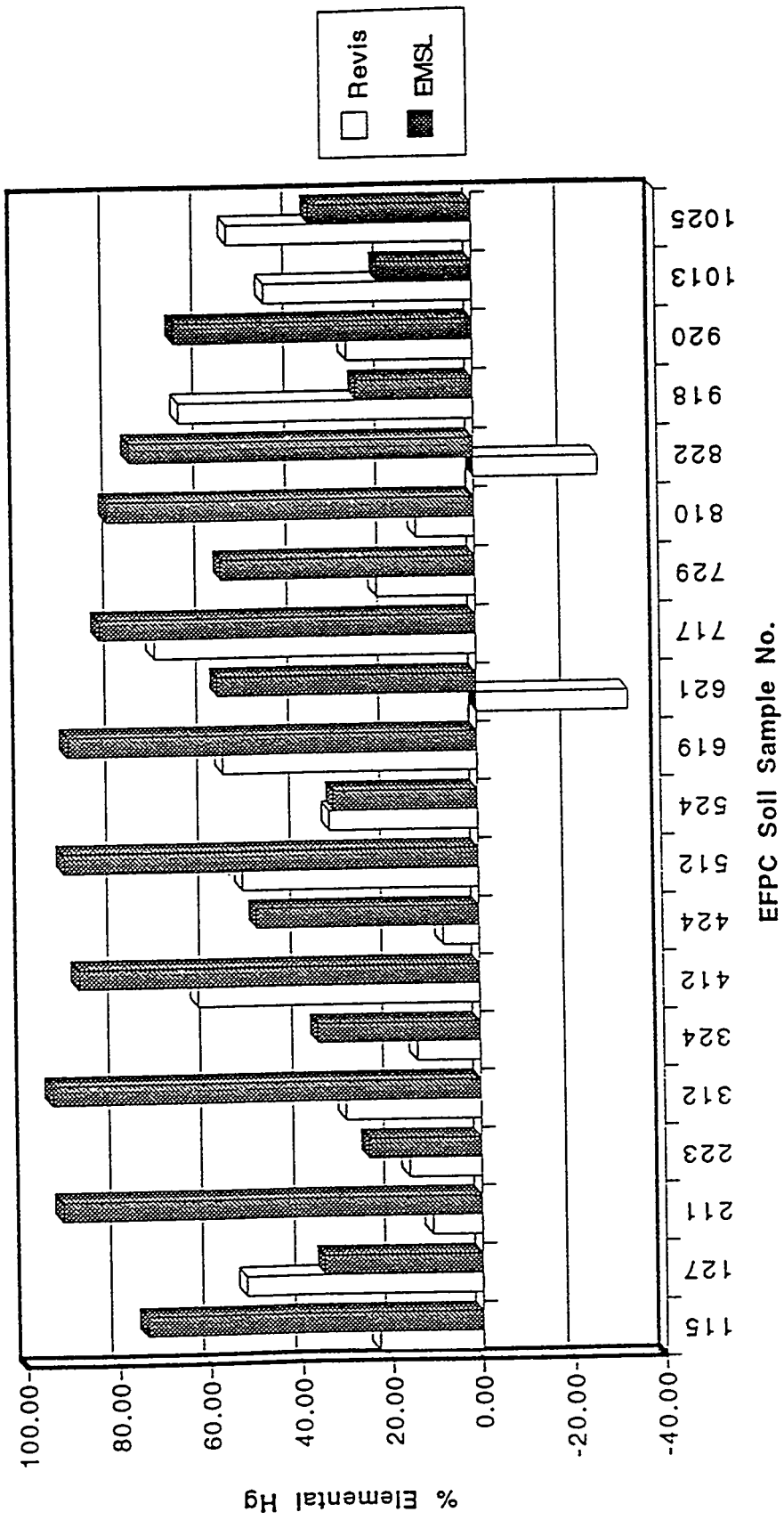

Fig. 1. Comparison of estimates of elemental mercury fractions in EFPC soils using thermal treatment (Revis) and chemical extraction (EMSL).

first 3 d. The notable exceptions to this pattern included soils 621, 729, 810, 822, 920, and 1025, which typically showed 30 to 40% of the total release occurring in treatment days 4 and 5. The latter pattern is suggestive of slow conversion of the mercury in these soils to a volatile form. Results from the Pilot Thermal Desorption work performed by IT Corporation and several literature citations suggest that metacinnabar does not begin thermal decomposition until temperatures above 200°C are reached. As discussed above, the EMSL extractant targeted at metallic/ amalgamated mercury extracted a substantial fraction (45%) of mercury in the soil spiked with metacinnabar, the form of mercury identified unequivocally as being present in EFPC soils.

Sample headspace measurements of mercury vapor can reveal the presence of metallic mercury in soil if sufficient metallic mercury is present to overcome the natural sorptive capacity of the soil. Landa (1978) suggested that soil organic matter plays a key role in binding metallic mercury vapor in soils. Willett et al. (1992) observed that sample headspace mercury vapor concentration increased as the quantity of metallic mercury dosed into a soil (2.08% organic carbon) increased from 100 to 1000 to 10000 mg/kg. Thus, while the absence of detectable mercury vapor in soil sample headspace does not preclude presence of metallic mercury in the soil, detection of mercury vapor in the headspace is unambiguous evidence of presence in the soil. Of the 20 soils tested for headspace vapor only two showed detectable concentrations at room temperature and 50°C (Table A.5). These soils represented two of the four soils with the highest total mercury concentrations (0.2% or more total mercury). Paradoxically, neither of these soils display atypical percentages of metallic mercury as measured thermally (Revis) or by extraction (EMSL).

CONCLUSIONS

The results of this comparison of three mercury speciation protocols challenge the notion that selective/sequential chemical extractions can provide unambiguous identification and quantification of mercury forms in soil. There was insufficient concordance among results from the methods to support any general statement about the fraction of any given mercury species in the soil. The authors of all three methods have reported that samples spiked with the target pure mercury compounds were recovered quantitatively by the individual procedures. However, such calibration does not mean that the target mercury forms are unequivocally present in study soils, only that mercury forms which seem to behave like the target form are present. Thus, mercuric oxide and mercury adsorbed to soil minerals and organic matter may both extract in 0.2 *M* acid, but the true nature of indigenous soil mercury cannot presently be discriminated. Similarly, hot 4 *M* nitric acid is likely to extract both metallic mercury and mercury incorporated into mineral and organic matter in soils. The intercomparison results do support the hypothesis that mercury in the EFPC floodplain soils is generally insoluble in all but the most harsh chemical extractants. Metallic mercury is definitely present in some soils but resists volatilization unless heated to temperatures (>50°C) well beyond the range expected to occur on the EFPC floodplain.

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In press - Water, Air & Soil Pollution

#7

CHARACTERIZATION OF MERCURY SPECIES IN CONTAMINATED FLOODPLAIN SOILS

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(Received 10 July 1994; accepted 20 August 1994)

Abstract. The chemical form or speciation of mercury (Hg) in the floodplain soils of the East Fork Poplar Creek in Oak Ridge, Tennessee, a site contaminated from past industrial activity, was investigated. The speciation of Hg in the soils is an important factor in controlling the fate and effect of Hg at the site and in assessing human health and ecological risk. Application of three different sequential extraction speciation schemes indicated the Hg at the site was predominantly relatively insoluble mercuric sulfide or metallic Hg, though the relative proportions of each did not agree well between procedures. Application of X-ray and electron beam studies to site soils confirmed the presence of metacinnabar, a form of mercuric sulfide, the first known evidence of authigenic mercuric sulfide formation in soils.

1. Introduction

During the 1950s, approximately 100 t of Hg were discharged from the U. S. Department of Energy facilities in Oak Ridge, Tennessee to East Fork Poplar Creek (EFPC). Much of the Hg has remained trapped in the floodplain soils which are currently being investigated under U.S. Superfund legislation. The chemical form or speciation of Hg in the soils is a controlling factor in the fate and effect of Hg at the site and influences the site risk assessment and required remedial actions.

A sequential extraction methodology for the speciation of Hg in soils (method 1) indicated the Hg in the floodplain soils was almost entirely inorganic, of which approximately 85% was mercuric sulfide (Revis *et al.*, 1989 a,b). Since several investigators have shown mercuric sulfide is a relatively insoluble and non-bioavailable form of Hg (Fagerstrom and Jernelov, 1971; Gillespie and Scott, 1971; Engler and Patrick, 1975; Rogers, 1979; Willett *et al.*, 1992), the study was considered significant in terms of the fate and effect of Hg at the site. Subsequently, X-ray fluorescence (XRF) dot maps obtained from a scanning electron microscope (SEM) examination of twenty soils revealed a consistent elemental association between Hg and sulfur (S) (Harris *et al.*, in press). To support the characterization effort, another sequential extraction methodology in development for characterization of Hg in soils was applied to twenty floodplain soils (method 2) (Miller, 1993). The results of this analysis indicated that although mercuric sulfide was a significant form, elemental Hg (or Hg amalgams) was the predominant form of Hg in the floodplain (Dobb *et al.*, 1994).

Although the speciation results from two different sequential extraction methodologies did not agree, the analyses were not performed on the same soils. In order to resolve the discordance, the two previously utilized sequential extraction methodologies as well as a third method (method 3) (Sakamoto *et al.*, 1992) were each tested on five soils. In addition, supplemental X-ray and electron beam studies were initiated to further characterize the species of Hg in the floodplain.

2. Methods

Soil samples were collected from the floodplain, composited in stainless steel bowls and refrigerated in the laboratory in sealed containers until use. Small (<100 g) subsamples of five soils were taken and further homogenized. One portion (approximately 3 g) of each soil was air dried to determine percent moisture, ground and analyzed for Hg by SW-846 Method 7470. Field-moist portions of each soil were sequentially extracted in 50 ml centrifuge tubes by the methods outlined in each of the three procedures. The determination of organic Hg in each analysis was omitted because the results of all studies have consistently indicated organic Hg is an insignificant fraction in EFPC soils. The supernatant from each extraction was filtered and analyzed for total Hg by SW-846 Method 7471. In addition, one soil was spiked with metacinnabar, the form of mercuric sulfide identified in EFPC soils as discussed below.

For the X-ray and electron beam studies, one of the most Hg-contaminated soils was selected because of the relatively high minimum detectable concentrations of some of the methods (i.e. nominally 1% for X-ray diffraction). Further enhancements of Hg concentration were achieved by particle size separation by differential sedimentation/centrifugation (Jackson, 1975). Soils were characterized by a JEOL 840 SEM, a JEOL 2000FX transmission electron microscope (TEM) with select area electron diffraction (SAED) using an accelerating voltage of 100 kV and a beam current of 15 μA and a Phillips high angle diffractometer with a graphite crystal monochromator using CuKα (λ=1.5418 angstroms) radiation operated at 40 kV and 40 μA.

3. Results and Discussion

The concentration of Hg in the five soils ranged from 42 to 2400 μg/g. The results of the sequential extraction studies are shown in Figure 1. Metallic Hg and mercuric sulfide accounted for greater than 70% on average of the total Hg in the five soils in all methods. The percentage of metallic Hg detected in each of the soils by methods 1 and 2 are not in agreement (method 3 did not have a determination for metallic Hg). The average percentage of metallic Hg in the soils by method 1 was 28% compared to 72% by method 2 and differences within individual soils were even more pronounced. The percentage of mercuric sulfide detected by the three procedures also did not agree. For the five natural soils, method 1 detected an average of 46% mercuric sulfide compared to 25% for method 2 and 83% for method 3. For all five soils, the percentage of mercuric sulfide detected was highest for method 3 followed by method 1 and then method 2. The recoveries of the metacinnabar spike were 87% for method 1, 84% for method 2 and only 24% for method 3.

A series of X-ray/electron beam studies were initiated to further refine the Hg speciation at the site. Hg-rich soil fractions with a concentration of 0.89% (2 - 5 μm) and 0.96% (<2 μm) were obtained by particle size separation from a 2670 μg/g soil and further analyzed by SEM energy dispersive spectroscopy (EDS) and wavelength dispersive spectroscopy (WDS), X-ray diffraction (XRD) and TEM with SAED. EDS/WDS of numerous Hg-rich soil particles showed consistent stoichiometric relationships between soil Hg and mercuric sulfide standards, particularly metacinnabar. TEM with SAED confirmed the presence of sub-micron crystals of metacinnabar, often in close association with the clay matrix (Stevenson *et al.*, 1994). As shown in Figure 2, the XRD patterns of the enriched soil fractions have peaks indicative of metacinnabar. The relatively small, broad peaks are caused by the sub-micron crystallite size and concentrations of Hg near the

Fig. 1. Comparison of speciation from different sequential extraction procedures for metallic Hg (left) and mercuric sulfide (right)

Fig. 2. Comparison of XRD patterns for Hg rich size fractions with as-received soil and with synthetic metacinnabar.

nominal detection limit, but are pronounced when compared to the as-received soil.

The causes for the differences in the results of each sequential extraction procedure is unclear. The methods were developed with the use of pure Hg compounds spiked in soils, though the behavior of Hg in soils may not be well represented by pure compounds. The behavior of sorbed Hg, in particular, was not investigated in either of the methods. There is supporting evidence of the presence of mercuric sulfide from X-ray/electron beam studies, while there has been no additional evidence of metallic Hg. Nonetheless, X-ray/electron beam studies have been limited to a few soils making extrapolation of the results for validation of the sequential extraction schemes difficult.

4. Conclusions

The results of three different sequential extraction studies concurred the Hg in EFPC soils consists primarily of inorganic metallic Hg and mercuric sulfide, though the relative percentages of each did not agree well between methodologies. Therefore, the use of sequential extraction procedures alone for the speciation of Hg in soils is considered problematic at present (further development is underway). The presence of sub-micron, crystalline mercuric sulfide (metacinnabar) in soils was confirmed by TEM with SAED and XRD. This is the first known evidence of the formation of authigenic mercuric sulfide in soils.

Acknowledgments

This research was sponsored by the U. S. Department of Energy's Environmental Restoration Division. Oak Ridge National Laboratory and K25 are managed for the U. S. DOE under contract DE-AC05-84OR21400 with Martin Marietta Energy Systems, Inc. Environmental Sciences Division Publication No. 4328.

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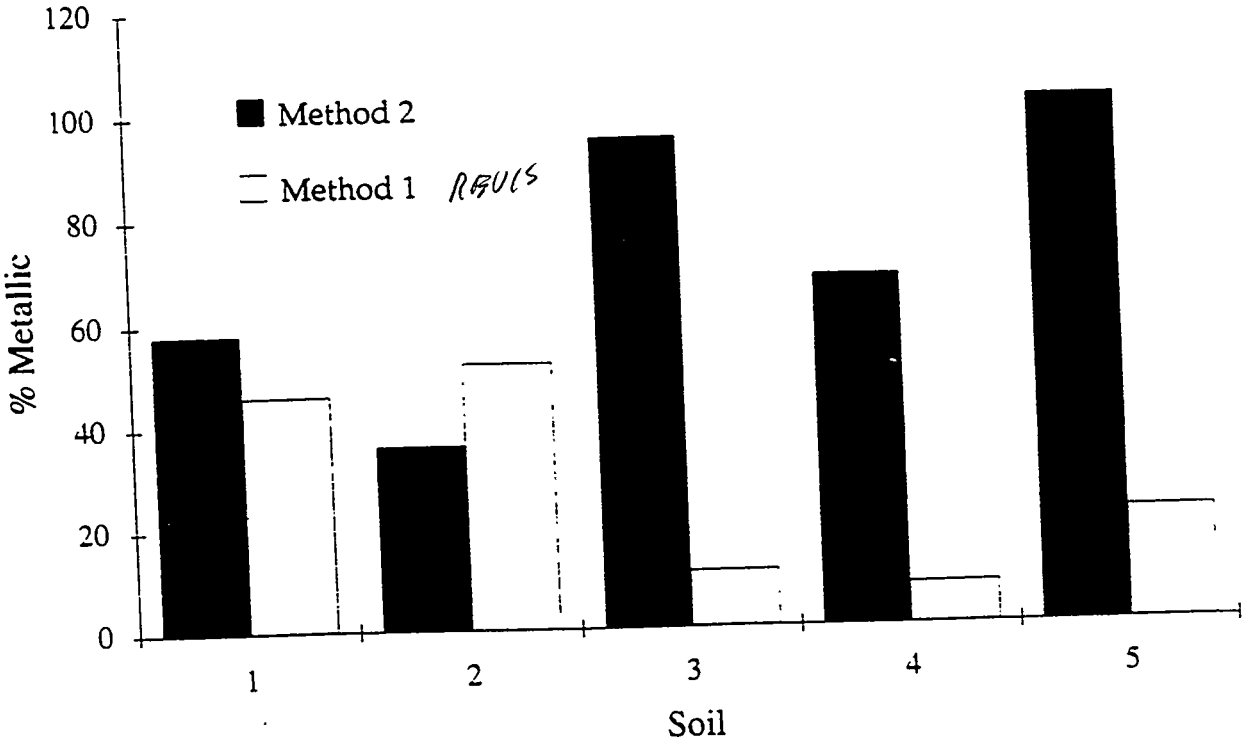
120
100
80
60
40
20
0
% Metallic
Method 2
Method 1
1
2
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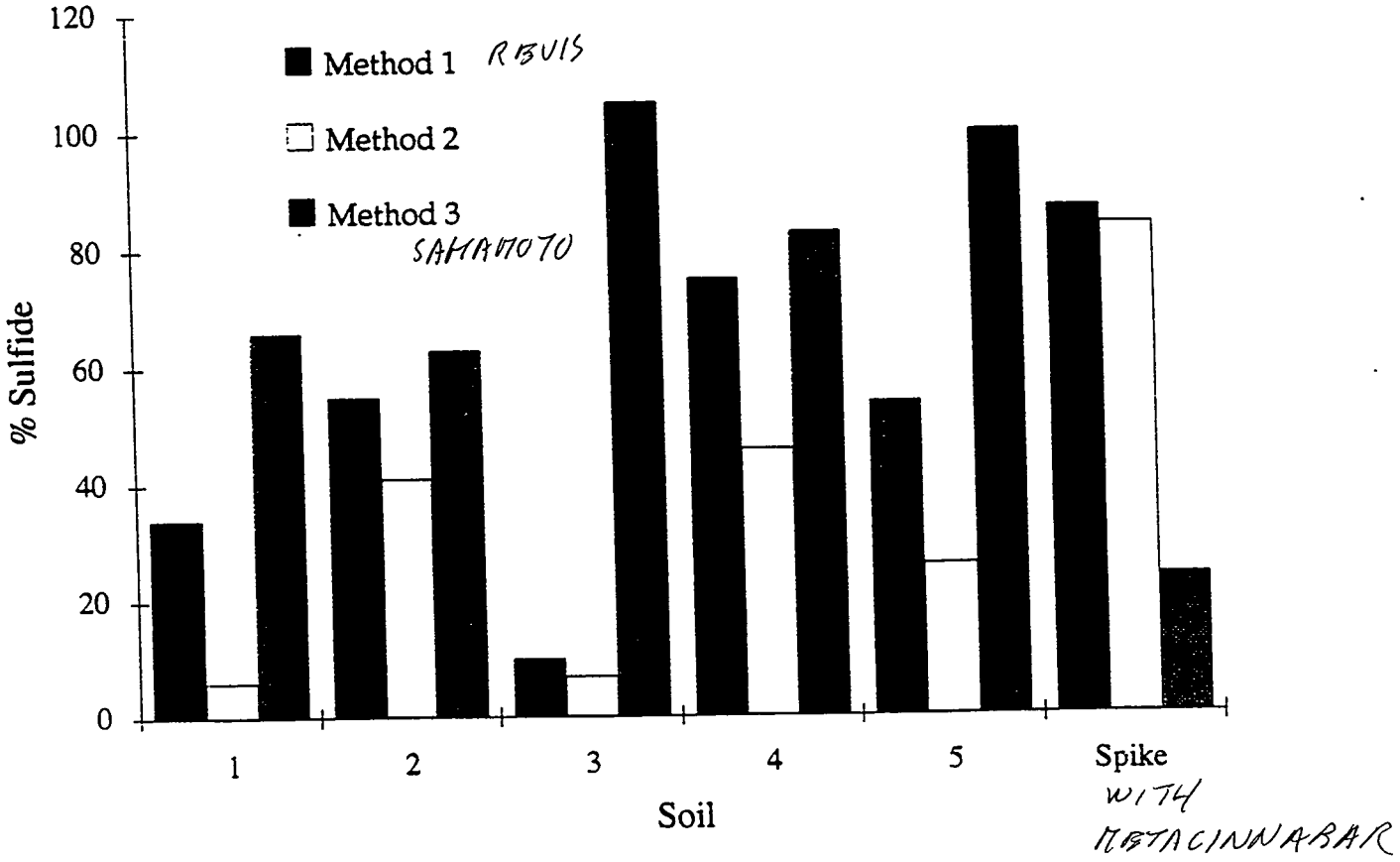
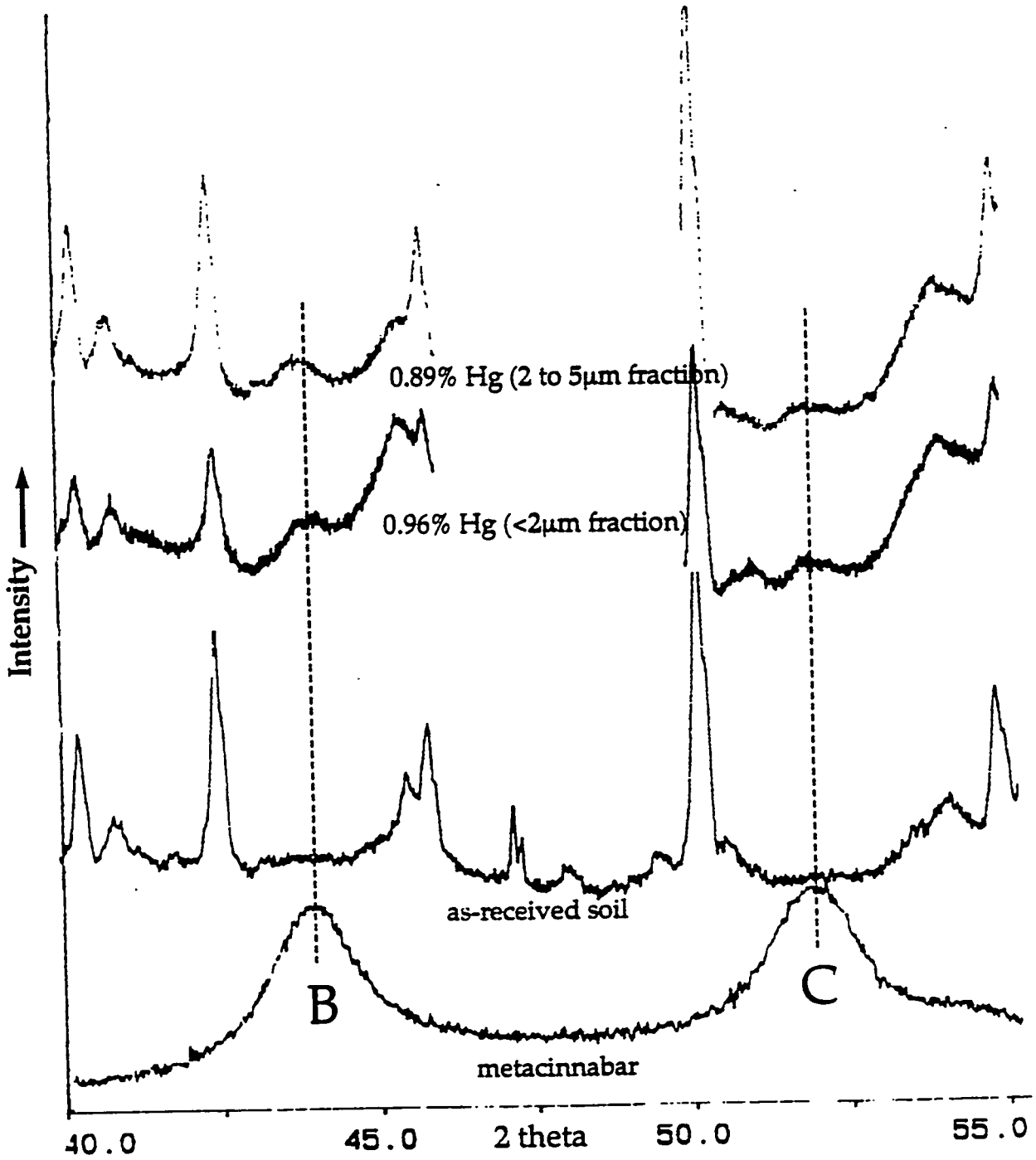
Soil


120
100
80
60
40
20
0
% Sulfide
Method 1
Method 2
Method 3
1
2
3
4
5
Spike
Soil


0.89% Hg (2 to 5μm fraction)
0.96% Hg (<2μm fraction)
as-received soil
metacinnabar
B
C
Intensity
40.0
45.0
2 theta
50.0
55.0