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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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 $\mu\text{g/g}$ 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

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-tetradecanoylphorbol-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, haloalkyl ether, haloaromatic, haloalkyl carbonyl, 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

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

EAST FORK POPLAR CREEK DRAINAGE

Figure 2

kilometer

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.

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-Ciocalteu 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 m μ , 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 m μ , 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 m μ . Addition of acetic acid solution resulted in a loss of color intensity whose absorbance was measured again at 500 m μ 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 $\pm$ SD (ppm)
Guard- house	1 Left	129.57 $\pm$ 39
	2 Middle	190.99 $\pm$ 18
	3 Right	197.05 $\pm$ 50
NOAA	4 Left	271.51 $\pm$ 40
	5 Middle	231.15 $\pm$ 38
	6 Right	100.71 $\pm$ 27
K-Mart	7 Left	191.47 $\pm$ 39
	8 Middle	162.55 $\pm$ 53
	9 Right	128.98 $\pm$ 16
Bruners	10 Left	190.52 $\pm$ 45
	11 Middle	124.68 $\pm$ 38
	12 Right	209.11 $\pm$ 25
Sewage Plant	13 Left	126.16 $\pm$ 18
	14 Middle	59.86 $\pm$ 12
	15 Right	69.81 $\pm$ 16
East Fork Turnpike Building	16 Left	168.98 $\pm$ 13
	17 Middle	90.09 $\pm$ 19
	18 Right	142.95 $\pm$ 40
Poplar Creek Perimeter Road	19 Left	99.10 $\pm$ 16
	20 Middle	73.00 $\pm$ 20
	21 Right	93.61 $\pm$ 17
Bear Creek	22 Left	111.25 $\pm$ 13
	23 Middle	101.29 $\pm$ 16
	24 Right	99.27 $\pm$ 17
Bear Creek	25 Left	100.69 $\pm$ 19
	26 Middle	107.74 $\pm$ 13
	27 Right	195.09 $\pm$ 14
Bear Creek	28 Left	127.04 $\pm$ 16
	29 Middle	101.78 $\pm$ 20
	30 Right	122.52 $\pm$ 23

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

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

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

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

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

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

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

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

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

TABLE 7
The Concentration of Mercury* in Soil
from East Fork Poplar Creek and Bear Creek

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

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.

TABLE 10
The Solubility of Mercury from Soil and Sediment*

Site	Site number	Mercury ppm in soil	Mercury ppm soluble in HNO ₃	Percent of total mercury soluble in HNO ₃
Guard-house	1 Left	1.97 ± 0.6	1.81 ± 0.19	91.88
	2 Middle	12.19 ± 1.3	2.71 ± 0.63	22.23
	3 Right	507.55 ± 40.1	326.66 ± 38	64.36
NOAA	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
K-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
Sewage Plant	13 Left	43.52 ± 5.1	16.47 ± 0.88	37.84
	14 Middle	33.18 ± 5.4	8.71 ± 1.21	26.25
	15 Right	220.85 ± 33.6	44.21 ± 3.66	20.02
East Fork Turnpike Building	16 Left	78.84 ± 7.9	37.51 ± 4.0	47.58
	17 Middle	13.33 ± 0.5	4.5 ± 0.13	33.83
	18 Right	65.43 ± 4.1	41.08 ± 4.8	62.78
Poplar Creek Perimeter Road	19 Left	22.79 ± 3.6	13.05 ± 0.69	57.26
	20 Middle	12.51 ± 1.3	8.30 ± 1.20	66.35
	21 Right	6.13 ± 0.6	2.30 ± 0.11	37.52
Bear Creek	22 Left	11.23 ± 1.1	12.12 ± 1.3	100+
	23 Middle	13.47 ± 0.6	1.98 ± 0.4	57.06
	24 Right	34.62 ± 2.1	30.59 ± 1.9	88.36
Bear Creek	25 Left	6.61 ± 1.3	3.42 ± 0.49	51.74
	26 Middle	.77 ± 0.06	.32 ± 0.11	41.56
	27 Right	2.64 ± 0.93	2.12 ± 0.35	80.30
Bear Creek	28 Left	.59 ± 0.09	.39 ± 0.14	66.10
	29 Middle	.72 ± 0.10	.52 ± 0.35	72.22
	30 Right	1.3 ± 1.7	0.81 ± 1.0	62.28

*Soil 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.

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

*96 hours post-feeding.

**Mean $\pm$ 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.

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 $\mu\text{g/g}$

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 $\mu\text{g/g}$ 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 $\mu\text{g/g}$ 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 $\mu\text{g/g}$ 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						
<u>Kidney</u>						
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	-
<u>Liver</u>						
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						
<u>Kidney</u>						
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
<u>Liver</u>						
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 1B (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						
<u>Kidney</u>						
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
<u>Liver</u>						
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 glomerulus.

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	-.702/.92
Urine creatinine					1

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.

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
<u>Controls</u>	<u>Females</u>		<u>Males</u>	
		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 $\pm$ 1
2	9 $\pm$ 3
3	5 $\pm$.6
4	6 $\pm$ 1
5	7 $\pm$ 1
6	8 $\pm$ 2
7	8 $\pm$ 1
8	8 $\pm$.5
9	7 $\pm$ 1
10	6 $\pm$.6
11	8 $\pm$ 1
12	7 $\pm$ 1
13	7 $\pm$ 2
14	6 $\pm$ 1
15	8 $\pm$ 2
16	7 $\pm$ 1
17	6 $\pm$.5
18	7 $\pm$ 1
19	5 $\pm$.5
20	6 $\pm$.6
21	10 $\pm$ 3
22	8 $\pm$ 2
23	6 $\pm$ 4
24	6 $\pm$.5
25	8 $\pm$ 1
26	5 $\pm$.5
27	7 $\pm$ 2
28	6 $\pm$ 1
29	7 $\pm$ 1
30	8 $\pm$ 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 $\pm$ 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.

Figure 3

Figure 4

Figure 5

Regression of MLOGLIVHG on MLOGSOILHG

6 MONTHS, MALES

LINEAR REGRESSION

$$r = 0.833$$

$$y = 1.34$$

$$\text{Slope} = 0.55$$

$$\text{DF} = 169$$

Figure 6

Figure 7

Figure 8

Figure 9

Figure 10

Figure 11

Figure 13

Regression of MLOGKIDHG on MLOGLIVHG

$r = 0.82$ $y = 0.95$

Slope = 0.97

DF = 208

Figure 14

Regression of FKIDHG on FLIVHG

24 MONTHS, FEMALES

LOG KIDNEY HG

Figure 15

Regression of LOG₁₀ I_{Hg} on LOG₁₀ I_{Hg}

6 MONTHS, FEMALES, NO ADD

LOG SOIL Hg

Figure 17

Regression of LOG₁₀ I_{Hg} on LOG₁₀ I_{Hg}

6 MONTHS, FEMALES, Hg ADDED

LOG SOIL Hg

Figure 18

Regression of LOGKIDNEY Hg on LOGSOIL Hg

6 MONTHS, FEMALES, NO ADD

Figure 19

Regression of LOGKIDNEY Hg on LOGSOIL Hg

6 MONTHS, FEMALES, Hg ADDED

Figure 20

Regression of MLOG LIVHG on MLOG SOIL HG

Figure 21

Regression of MLOG LIVHG on MLOG SOIL HG

Figure 22

Regression of MLOGKIDHG on MLOGSOILHG

6 MONTHS, MALES, NO ADD

Figure 23

Regression of MLOGKIDHG on MLOGSOILHG

6 MONTHS, MALES, HG ADDED

Figure 24

Regression of LOG KIDNEY HG on LOG LIVER HG

Figure 25

Regression of LOG KIDNEY HG on LOG LIVER HG

Figure 26

Figure 27

Figure 28