



Nicholas V.C. Ralston  
Principal Investigator

## PHYSIOLOGICALLY BASED PHARMACOKINETIC MODEL OF MERCURY–SELENIUM INTERACTIONS

**Key Personnel:** Laura Raymond (EERC), Steven Benson (EERC)

### ***Project Description***

This project is being conducted using the data generated from a separate but complementary research project entitled “Investigating the Importance of Hg–Se Interactions.” This project is developing a computational method of interpreting and evaluating research data originating from that research effort as a synergistic activity. A previously developed physiologically based pharmacokinetic (PBPK) model was applied to reflect mercury interactions. The previous PBPK model is being extended into a more comprehensive Physiologically oriented integration of nutrients and toxins (POINT) model that reflects the influence of methylmercury and its metabolites on selenium availability in various tissue compartments. The POINT model will be used to evaluate and interpret the results of the complementary rat study, but its results can readily be extended to evaluation of studies of human exposure to methylmercury and consequent effects on selenium physiology. Although the disproportionately high methylmercury contents characteristic of certain seafoods appear likely to pose pronounced health risks to humans that consume them, the protective effects of the selenium that are otherwise abundantly present in ocean fish must also be considered. This work provides an evaluation of selenium’s protective effects in the seafood safety issue.

### ***Goals***

The objective of this project is to improve the understanding of selenium’s role in seafood safety issues through a quantitative thermodynamic and kinetic analysis of its distribution and interactions with methylmercury in vulnerable tissues of the body such as brain. The biochemical mechanism of methylmercury’s pathological disruptions of cell physiology responsible for its toxic effects appears to arise as a result of mercury-dependent sequestration of selenium. The goal of this study is to examine the effects of dietary exposures to graduated amounts of methylmercury and selenium and relate molar ratios of mercury and selenium in tissues to the effects of methylmercury toxicity.

### ***Rationale***

Since disruption of Se metabolism is a notable aspect of methylmercury toxicity and selenium status is known to be inversely related to vulnerability to mercury toxicity (1–5), it is important to

investigate the kinetics and thermodynamics of methylmercury and its metabolites with respect to selenium physiology. Although PBPK models have developed mathematical reflections of the toxicodynamics of methylmercury and its metabolites (6–14), these models have all failed to consider selenium physiology.

Fortunately, the current state of development of the understanding of selenium physiology and its distribution in tissue compartments is substantial (e.g., 15–18), and POINT models of mercury–selenium interaction studies can readily be integrated with current PBPK models of methylmercury and its metabolites. The resulting POINT models will integrate data reflecting elemental distributions and molar ratios of molecular forms of these elements occurring in tissues as a result of methylmercury exposure and employ thermodynamic models to establish selenium sequestration effects. PBPK models have not previously been used in risk assessments (19); however, a fully developed POINT model will provide a perspective that would not only assist in evaluation of existing epidemiological data, but may also help to identify populations at risk from methylmercury exposure and delineate them from those that are not.

Recent CATM experiments indicate that vulnerability to methylmercury toxicity is inversely proportional to nutritional selenium status. Since ocean fish are among the richest dietary sources of selenium, it is important to develop a broader understanding of the interactions between mercury and selenium and how this relationship might influence fish advisories based on EPA's reference dose level for methylmercury exposure. Methylmercury toxicity appears to be related to the high binding affinities (20) between inorganic mercury ( $\text{Hg}^{2+}$ ) and the inorganic forms of selenium that are continuously formed within all living animal cells (21).

Although methylmercury is a highly bioavailable form that is absorbed by the digestive system, rapidly distributed through the blood supply, and readily accumulated in tissues of the central nervous system, it may not be the form responsible for the toxic intracellular effects associated with high methylmercury exposures. It is known that  $\text{Hg}^{2+}$  is gradually released from intracellular methylmercury, apparently through enzyme-catalyzed demethylation. Inorganic mercury's affinity for the inorganic selenium forms that are continuously and repeatedly created within cells is approximately a millionfold greater than its affinity for sulfur.

Selenide is a transitory intermediate in the formation of selenocysteine, a rare but important selenium-containing amino acid. This amino acid is present at the active sites of all of the known selenium-dependent enzymes (selenoenzymes) and is present in 25–35 proteins (21). Like all proteins, selenoproteins wear out as a result of performing their tasks in the body and must be regularly replaced. Individual proteins have half lives that range from a few hours to a few days. When senescent proteins are broken down, their component amino acids can generally be reused to make new proteins. However, the selenium amino acid selenocysteine is unique in that it must be completely degraded to release its selenium before it can be reused. A completely new selenocysteine must be created for each cycle of protein synthesis. In the reducing environment that is natural inside cells, the selenium released during each cycle is converted into selenide. This form of selenium has extremely high affinity for mercury. In the presence of mercury, the eventual result is formation of mercury selenide ( $\text{HgSe}$ ) aggregates which are the dominant form of mercury present in tissues. Once sequestered in the insoluble  $\text{HgSe}$  form, selenium is no longer available for participation in selenoenzyme synthesis, and all the activities it once supported cease. In the absence of these essential activities, pathologic consequences accrue. This appears to be the molecular mechanism and major proximal cause of mercury toxicity.

Intracellular availability of selenium is usually in great excess of the small amounts of intracellular  $\text{Hg}^{2+}$  that arise from the low levels of methylmercury exposure from consuming common types of ocean fish. As a result, cells easily maintain their normal level of freely cycling selenium, and their selenoenzyme synthesis proceeds unchallenged. However, when foods containing toxic amounts of

dietary methylmercury are consumed and HgSe formation occurs at too great a rate, rapid and irreversible loss of bioavailable selenium prevents synthesis of selenocysteine, eliminating selenoenzymes and their normal biochemical functions. The brain and neuroendocrine tissues appear to be particularly vulnerable upon loss of these functions since no treatment other than mercury toxicity has ever diminished their selenoenzyme activities. Since selenoenzymes are active in all cells of all animal life, it is clear that mercury-dependent obliteration of selenoenzyme activities is a unifying aspect of mercury toxicity in both human and environmental exposure. Improving the understanding of mercury toxicity from the perspective of selenium physiology will provide important insights regarding the means of assessing, maintaining, and restoring human and environmental health following mercury exposure.

### *Approach*

Because the consortium-sponsored animal research project and this CATM-sponsored modeling project are separate but complementary and because the data will be shared between these two projects, the task structure of both projects is presented.

Task 1 of the consortium study (selenium protection study) examined dietary exposure to toxic concentrations of methylmercury and graduated concentrations reflecting the physiologic range of selenium intakes, low (0.1- $\mu\text{mol Se/kg diet}$ ), normal (1.0- $\mu\text{mol Se/kg diet}$ ), and rich (10- $\mu\text{mol Se/kg diet}$ ). The rich dietary selenium level is slightly less than the average selenium concentration present in ocean fish. This study was designed to monitor easily observed symptoms characteristic of methylmercury toxicity such as reduced food consumption, growth failure, and impaired motor coordination. These symptoms of methylmercury toxicity were assessed in relation to selenium dietary intakes and are considered in relation to selenium bioavailability calculated using POINT model computations of methylmercury and selenium intakes, absorption and excretion rates, and their demethylation and complexation rates.

Task 2 of the consortium study (selenium therapy study) characterized the nature and extent of recovery from methylmercury toxicity using dietary selenium as a rescue therapy. Treatment with selenium-rich diets, either with or without continued methylmercury exposure, was assessed in regard to effects on the characteristic symptoms of methylmercury toxicity. POINT models were applied as described in Task 1.

This project extends the PBPK model of methylmercury exposure into a POINT model that incorporates a balanced toxicological and physiological perspective of the observed food consumptions, weight gains, and motor function abilities of experimental animals fed diets containing graduated concentrations of selenium and methylmercury. Selenium and mercury concentrations in the various tissues of the animals participating in the consortium study are still being assessed, so the present model is currently oriented on the basis of trace element concentrations observed in tissues of animals from two similar previous studies performed by EERC researchers. As trace element results from the current study become available, they will be incorporated into the evolving POINT model. By computational extension of these results, a human POINT model is being created. This model is based on information arising from the methylmercury-poisoning incidents in Japan and Iraq as well as human studies performed in New Zealand, the Faroes, the Seychelles, and the United Kingdom. This POINT model will be used to assist in interpretation of the results of these studies.

### Progress

The selenium protection study investigating selenium's protective effects against methylmercury toxicity has completed the animal exposure task. Its results complement and strongly confirm the findings of our earlier studies of this subject. Rats fed diets containing selenium in a range of concentrations from low ( $0.1 \mu\text{mol Se/kg}$ ) to normal ( $1.0 \mu\text{mol Se/kg}$ ) or selenium-rich ( $10 \mu\text{mol Se/kg}$ ) all grow at optimal rates (Figure 1).

However, when rats are exposed to toxic dietary concentrations of methylmercury ( $\sim 10 \text{ ppm}$ ;  $50 \mu\text{mol MeHg/kg}$ ), their resistance to development of signs and symptoms of toxicity such as depressed growth are quite different. Figure 2 clearly shows that sensitivity to methylmercury toxicity is dependent on the selenium status of the exposed animals. After 3 weeks of methylmercury exposure, the rats fed low-selenium diets began to show signs of diminished growth relative to their control group fed low-selenium diets that did not contain methylmercury. While the growth of methylmercury-exposed rats fed diets containing normal dietary selenium was only marginally affected, rats fed selenium-rich diets did not show any sign of methylmercury causing a diminished growth rate and actually tended to show slightly greater weight gains than their non-mercury-exposed control group.

By the tenth week on the diet, rats fed low-selenium diets supplemented with methylmercury started to lose weight and showed hind limb crossing. The weight gains of rats fed normal selenium diets were slightly diminished relative to their no-mercury control group, but the growth of rats fed selenium-rich diets remained optimal. No signs of neurotoxicity were observed among rats fed normal or selenium-rich diets during the 18-week study. The selenium protection study was terminated when methylmercury-exposed rats in the low-selenium group started to show severe disabilities and deaths.

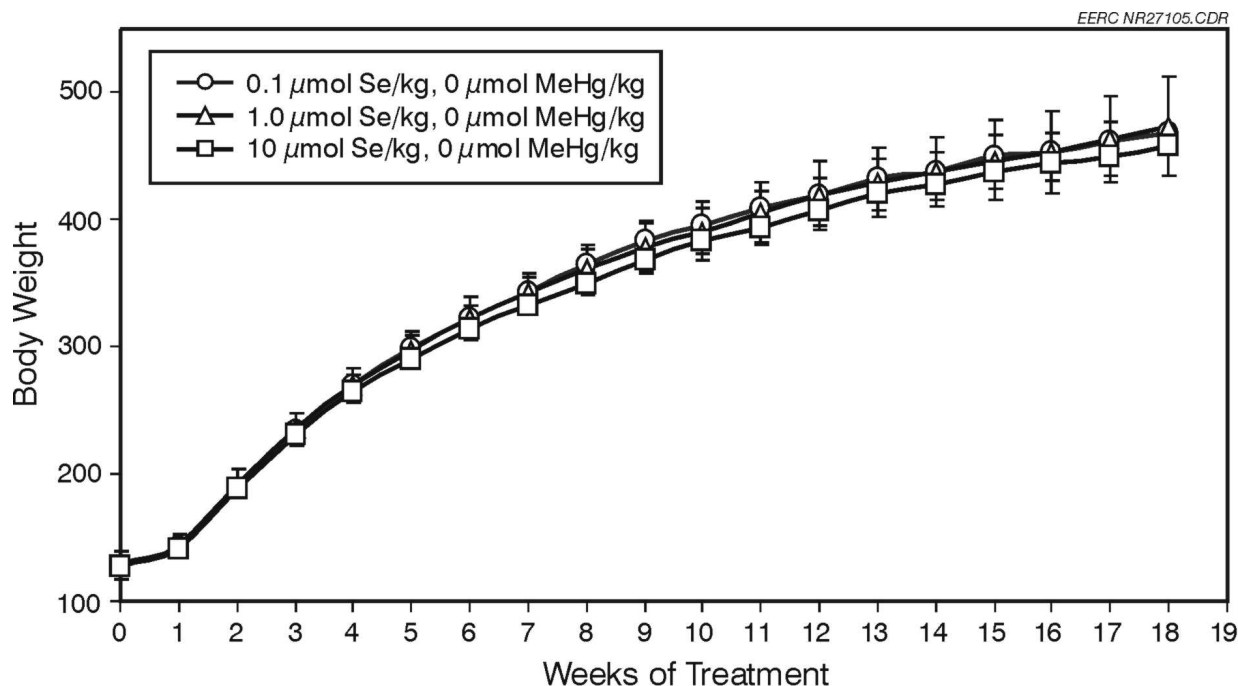


Figure 1. Growth of groups of rats fed low, normal, or enriched dietary selenium. Data depict means  $\pm$  standard deviations for group body weights in grams at the times indicated. By itself, dietary selenium did not influence the weight gain of the rats.

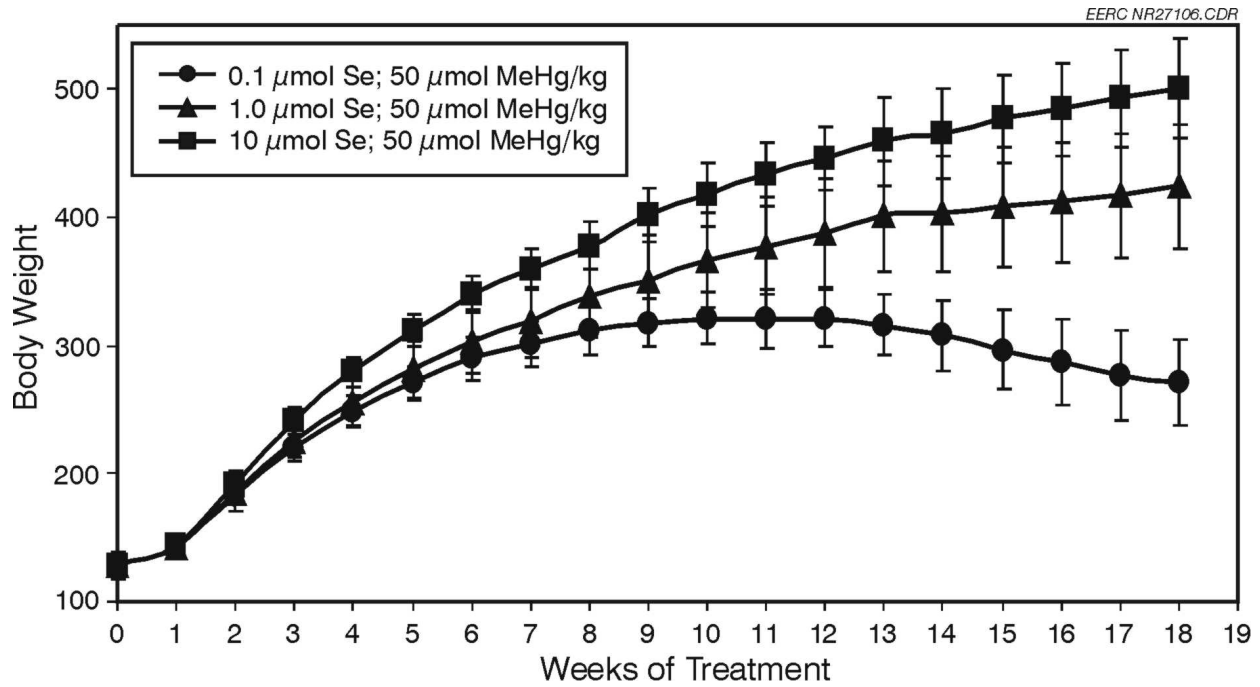


Figure 2. Growth of rat treatment groups fed low, normal, or selenium-rich diets supplemented with 10 ppm (~50 μmol MeHg/kg) methylmercury. Data depicted are means ± standard deviations for group body weights (in grams) at the times indicated.

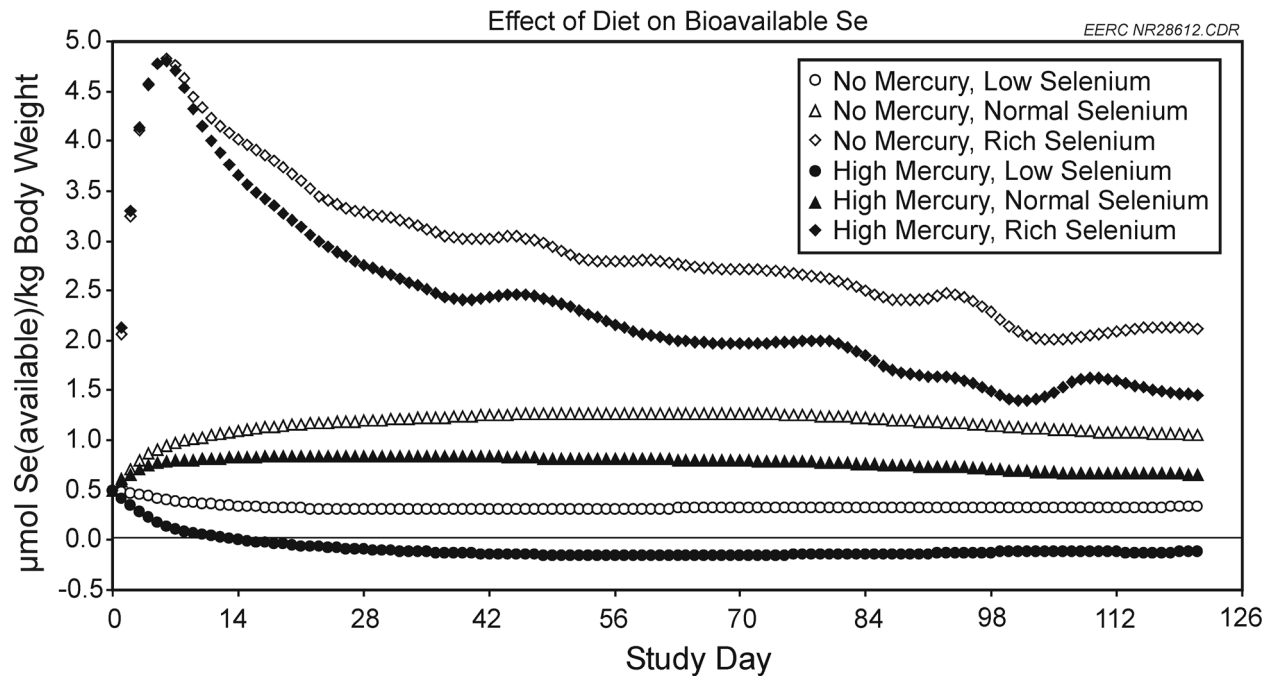


Figure 3. POINT models of dietary treatment effects on bioavailability of selenium.

Based on previous studies of dietary selenium supplementation, weanling rats fed normal levels of dietary selenium grow so fast that their tissue selenium contents do not attain optimal concentrations until they reach 6 weeks of age (Figure 3). From that point on, their tissue selenium concentrations gradually plateau. Rats fed selenium-rich diets experience an initially steep rise in selenium status that is moderated as homeostatic regulatory controls balance absorption and excretion pathways governing selenium status.

The present iteration of the POINT model employs a single-compartment version, but the fully realized form will use multiple discrete tissue compartments representing insensitive somatic tissues versus vulnerable tissues such as brain, pituitary, thyroid, and adrenals. However, even the present single-compartment POINT model reflects basic distinctions in selenium availability in animals with differing selenium intakes that were exposed to toxic amounts of methylmercury. Using known intakes of selenium and methylmercury and calculated retention and excretion rates, the single-compartment POINT model reveals that consuming diets containing 50  $\mu\text{mol}$  methylmercury/kg rapidly diminished selenium availability within 2 weeks. The impaired weight gains of rats in this group that became distinctly apparent some weeks later appear to suggest consequences were closely related to this diminished availability. The POINT model indicates that the normal selenium group fed methylmercury still retained near-normal levels of selenium bioavailability after 18 weeks on this diet. A trend line based on declining bioavailability of selenium during the last 16 weeks of the study indicates that after 40 weeks, selenium bioavailability in this group will have declined to  $\sim 5\%$  of normal. This corresponds to the time when groups fed these approximate amounts of selenium and methylmercury have been observed to show motor function defects.

The selenium therapy study was initiated when rats fed low-selenium diets that were exposed to methylmercury began to lose weight, an early sign of mercury toxicity. At this point (Day 74 of the selenium protection study is Day 0 of the therapy study), the 40 rats that were exposed to this treatment

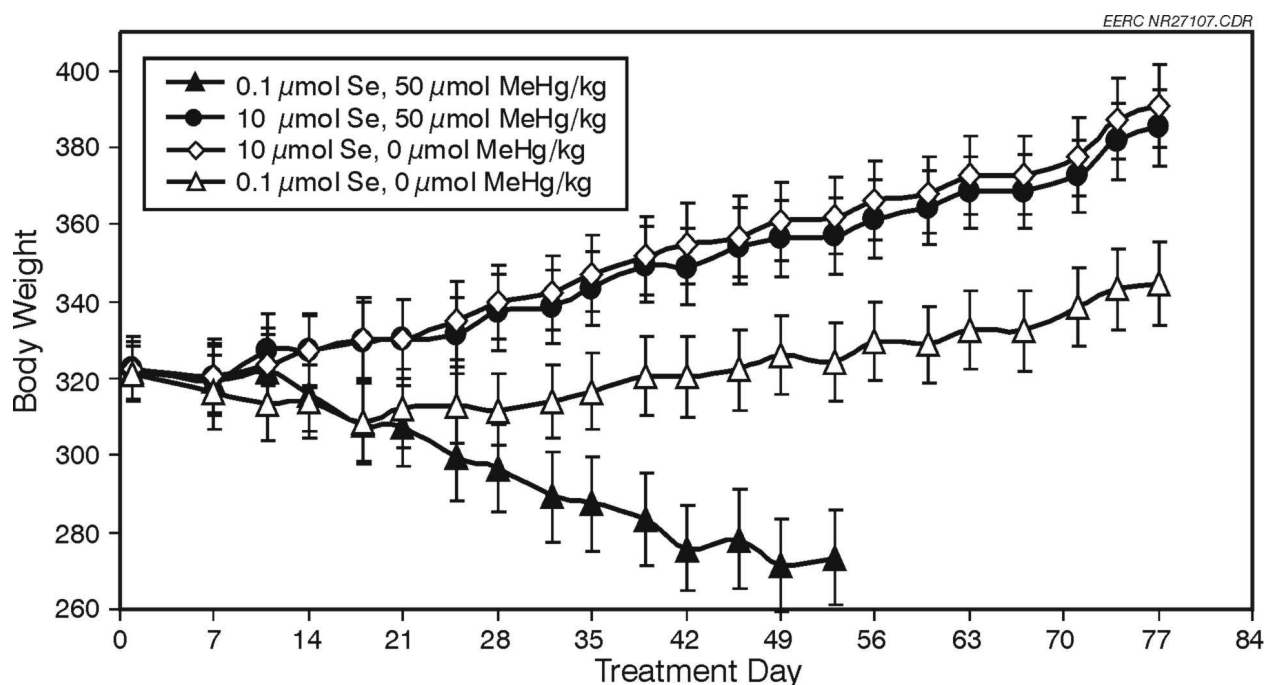


Figure 4. Effects of dietary treatment on the growth of methylmercury-intoxicated rats. Graph depicts means  $\pm$  standard deviations of body weights (g) at the times indicated.

were split into four treatment groups of ten rats each. The first group of rats continued on the same diet and thus participated in both the protection study and the therapy study. The other three groups were switched to diets that enabled comparison of the effects of dietary selenium and methylmercury on recovery. The second group was maintained on the same high mercury exposure as the first group, but with selenium-rich ( $10 \mu\text{mol Se/kg}$ ) diets instead of low ( $0.1 \mu\text{mol Se/kg}$ )-selenium diets. Groups 3 and 4 were fed diets without methylmercury but with either rich or low selenium, respectively. Weights of rats in these various dietary treatment groups are displayed in Figure 4.

Rats maintained on high-methylmercury, low-selenium diets gradually sickened and continued to lose weight. In contrast, rats maintained on high-methylmercury diets but switched to rich dietary selenium began to regain weight and improve in health after 1 week. Recovery rates of rats in this group were virtually indistinguishable from those of rats fed selenium-rich diets that were methylmercury-free. Regardless of whether methylmercury was present in their diets or not, robust growth was observed in rats fed selenium-enriched diets. Rats that were switched to methylmercury-free, low-selenium diets continued to lose weight at similar rates to the methylmercury-exposed group for the first  $\sim 18$  days but stabilized and began to slowly gain weight thereafter. The POINT model depicted in Figure 5 depicts the effects of diet on selenium bioavailability.

As the POINT model in Figure 5 indicates, even 1 day's consumption of a  $10\text{-}\mu\text{mol Se/kg}$  diet is sufficient to bring internal bioavailability of selenium back to a nearly normal range, regardless of whether or not methylmercury is present. However, as seen in Figure 4, no growth occurred during the first 7 days on the selenium-rich diets. This may indicate a need to sequentially replenish the blood compartment with sufficient selenium before the blood could supply the liver with selenium. This was followed by a further lag period before the liver could start to create selenoproteins for release back

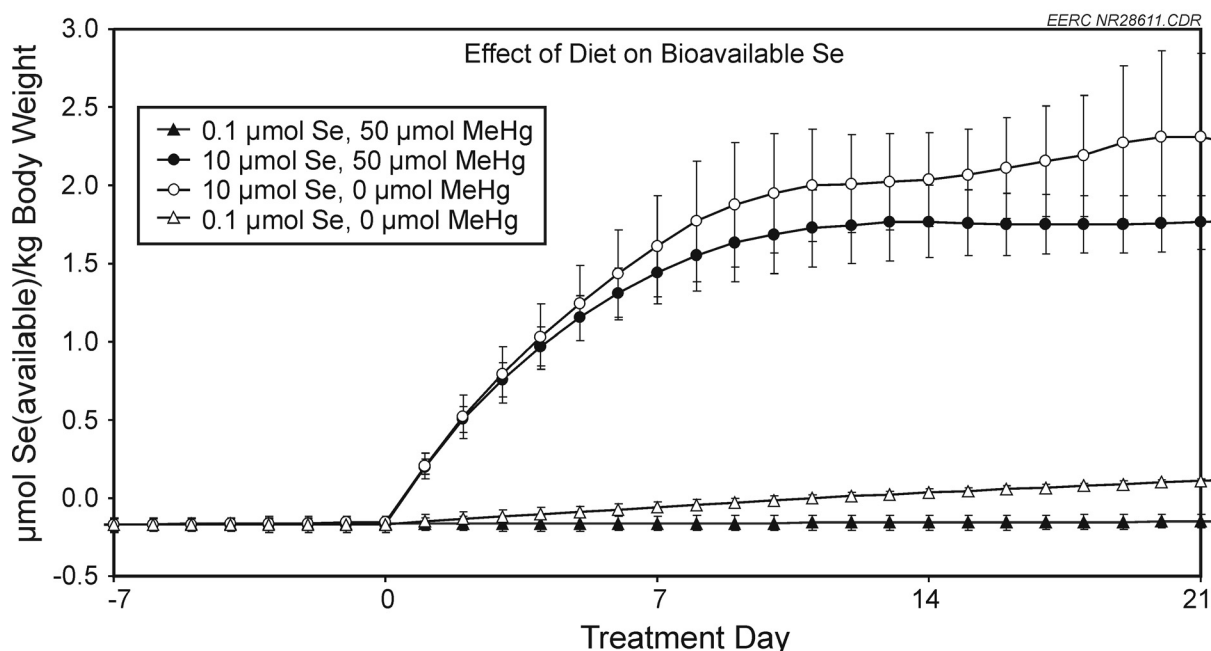


Figure 5. POINT model of effects of dietary treatment on selenium bioavailability in rats. Graph depicts means  $\pm$  standard deviations of group data at the times indicated.

into the blood. Once selenoproteins start appearing in blood, there may be further sequential restoration of preferentially supplied tissues before growth renormalizes. One aspect of the selenium depletion that occurs as a consequence of mercury toxicity is the abolition of all selenoproteins, including selenophosphate synthetase. Selenophosphate synthetase activity is needed to create selenophosphate, a necessary precursor in the selenocysteine synthesis. Since selenophosphate synthetase is itself a selenoenzyme, a rate-limiting step in restoring selenium physiology is creating a viable selenophosphate synthetase. Since growth hormone originates from the pituitary, mercury toxicity's effects on selenium metabolism may limit growth hormone production. The restoration of normal growth may reflect the lag period from restoration of selenium availability to renewed synthesis of growth hormone. Other stages of metabolic recovery would include but not be limited to restoration of normal activities of thyroid and adrenal tissues.

### ***Quality Objectives***

The EERC quality management system (QMS), authorized and supported by EERC managers, is in effect and governs all programs within the organization. Additionally, the CATM program employs a quality management plan (QMP) that addresses trace metal emissions research at the EERC. The CATM QMP has been reviewed and accepted by EPA. The proposed project will follow the Quality Manual, the CATM QMP, and all revisions. An independent QMP auditor reviews all aspects of quality assurance/quality control (QA/QC) for this project. This section addresses quality objectives, procedures for measurement/data acquisition, and procedures for assessing and validating data and results that are specific to this CATM project.

The primary quality objective for this project is to develop a computational Se physiology model that can be used to reflect pharmacokinetics and metabolic interactions of MeHg and its products as they relate to effects of toxic exposures in animals and humans of varying Se status. The data described are being compiled for quantitative and qualitative meta-analysis. POINT models of Se-deficient, Se-normal, and Se-rich animals are being developed, and functional models of the effects of MeHg exposure within these models will be evaluated. Analysis will be validated by comparing positive and negative treatment controls for each independent variable assessed.

### ***Measurement/Data Acquisition***

The methods used in the analytical components of this project employ appropriate methods for analysis according to established protocols in place at the EERC. Sampling procedures, instrument calibrations, and QC considerations are included in the protocols. Selenium analysis is being performed using hydride-generated atomic fluorescence according to established analytical protocols of the EERC Analytical Research Laboratory (ARL). Mercury analysis is being performed using cold-vapor atomic absorption (CVAA) methods according to established analytical protocols of the ARL.

### ***Assessment and Validation***

The analysis data for the standard protocols used in this project indicate acceptable analytical accuracy and precision. The sample analysis and numbers of samples used provide statistically meaningful results, based on standard statistical analysis. Only data that are complete, repeatable, and/or can be verified by similar studies and literature references will be used for computational modeling.

The project is designed with a time line and milestones that will facilitate in determining whether or not the objectives and the goal of the project are achieved. During the course of the project, the schedule and milestones will be reviewed in order to assess the progress of the project.

### ***Status***

The protection and therapy studies have completed the animal exposure tasks. Sample analysis is under way and will be used to inform the POINT model. In order to examine dietary effects of selenium in protection from and treatment of methylmercury toxicity, a preliminary single-compartment POINT model that integrates known dietary intakes in concert with approximated incorporation and excretion rates has been developed. The current model provides results that appear consistent with observed and predicted effects of selenium–methylmercury interactions.

### ***Potential Applications and Benefits***

Perhaps the most important beneficial application of the findings from this study is the future application of dietary selenium as a means of treating individuals that have been accidentally exposed to toxic quantities of mercury. No effective treatment for mercury toxicity had been recognized prior to this study. Although mercury toxicity is rare, it is clear that selenium supplementation to prevent or reverse its consequences would be a reasonable treatment modality for clinicians working with patients that have been exposed to high doses of mercury. Understanding the effects of selenium in prevention of methylmercury toxicity will also help regulatory agencies estimate risks from fish methylmercury exposures in human populations.

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