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PHYSIOLOGICALLY BASED PHARMACOKINETIC MODEL OF MERCURY–SELENIUM INTERACTIONS

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Project Description

This project examines data generated from a separate but complementary animal research study entitled “Investigating the Importance of Mercury–Selenium Interactions.” The goal of that project was to examine the role of selenium in protecting against and treating of mercury toxicity. The goal of this CATM-funded project is to develop a calculation model to interpret and evaluate the research data regarding mercury–selenium interactions observed in the animal study and examine how mercury and selenium accumulation in the tissues of the exposed rats is associated with their risks of developing signs and symptoms of neurotoxicity. Previous work on developing physiologically based pharmacokinetic (PBPK) models has been applied to animal and human data to reflect the associations between methylmercury exposure and risks of toxic outcomes.

This project extends the PBPK model of methylmercury distributions and effects to include consideration of methylmercury effects on the distribution and potential bioavailability of the physiological essential trace element selenium. Since a significant database already exists that describes selenium distribution and retention in tissues, integrating the PBPK models with the existing database and the results from the complementary animal study should provide a more comprehensive understanding of the interactions between mercury and selenium.

The result of these combined approaches has been named the Physiologically Oriented Integration of Nutrients and Toxins (POINT) model. This POINT model will be used to evaluate and interpret the results of complementary animal studies, and its interpretations will be extended to evaluate the expected effects of mercury–selenium interactions in epidemiological studies of human exposure to methylmercury. The findings of the POINT model will be used to evaluate the conventional hypothesis that “maternal methylmercury exposures are directly associated with risks of adverse child development outcomes.” The conventional hypothesis will be compared to the updated hypothesis that “maternal methylmercury exposures in excess of dietary selenium intakes are directly associated with risks of adverse child development outcomes.” The high methylmercury concentrations and relatively low selenium contents characteristic of certain shark and pilot whale meats pose health risks that have been associated with harm to developing children. However, recent research from the Faroes has shown that consumption of ocean fish counteracts harmful effects of methylmercury exposure instead of causing harm. Other studies have shown that increasing maternal consumption of ocean fish has beneficial effects

on child development outcomes. These effects appear to occur because the relatively low methylmercury contents of ocean fish are not substantial enough to compromise the beneficial effects of the selenium that is abundantly present.

Goals and Objectives

The overall goal of this project was to develop a computational method of interpreting and evaluating research data to assess mercury and selenium interactions and accumulation in tissues. This single-compartment POINT model will be used to evaluate and interpret the individual organ tissues and its consequent effects on selenium physiology. Since this work provides an added tool for the evaluation of selenium's protective effects in this seafood safety issue, research data will be added to the database and applied to this POINT model for a more comprehensive analysis to help in future risk assessments.

Rationale

The biological availability of dietary selenium for participation in selenium physiology can vary dramatically depending on the molecular forms of the selenium. Selenium accumulation in plants occurs because of the non specific uptake of selenium and sulfur from soil and subsequent incorporation of selenium into selenomethionine that is employed as a methionine analogue. Dietary selenomethionine is readily incorporated into proteins because selenomethionine is esterified to methioninyl-tRNA (ribonucleic acid) synthetase (K_m 7 μ M) at rates only slightly less favorable than for methionine itself (K_m 11 μ M). However, in contrast to the rapid release and selenoenzyme utilization of selenium from other dietary sources, selenomethionine can be reused through many cycles of protein synthesis. Therefore, selenium from selenomethionine is a "slow release" form that can eventually be degraded to supply selenium for selenocysteine synthesis. Because of the slow release of selenium from selenomethionine, it is often used in dietary supplements for safety reasons. However, a slow release form is obviously not ideal for rapidly restoring metabolic pools of biochemically active selenium for selenocysteine synthesis. Figure 1 presents a simplified depiction of selenomethionine, selenocysteine, and inorganic selenium metabolism.

Selenium is required for the normal function of 25–30 proteins that exhibit tissue-dependent occurrence and distribution, but preferentially maintain in-brain and neuroendocrine tissues. Selenium is specifically incorporated into selenocysteine, a rare and unique amino acid that is structurally analogous to cysteine but genetically and functionally distinct. Selenocysteine is the 21st proteinogenic amino acid, but it was not initially recognized in classical genetics because it employs complex mechanisms during the decoding of the UGA "STOP" codon. For selenocysteine insertion at UGA codons, a specific RNA stem loop structure in the 3'-untranslated region of the mRNA is required. The catalytic activities of Se-dependent enzymes (selenoenzymes) depend upon the superior redox activity of Se present in the amino acid selenocysteine present at their active sites. In proteins, selenocysteine exerts its prominent and specific functions because of its high redox potential and the low pKa value (5.7) of its selenol (-SeH) group compared to that of most of the sulfhydryl (-SH) groups of cysteine residues (pKa ~ 8.5). The Se of selenocysteine is the primary chemical participant that performs the actual biochemical function of these enzymes and is incorporated into the polypeptide chain by using UGA as the encoding codon. During each cycle of selenoprotein formation, selenocysteine must be degraded to release inorganic selenide that can be reused in the next cycle of selenocysteine synthesis. Selenide is the transitional substrate for selenophosphate synthetase, and tRNA-mediated formation of selenocysteine and is formed during each cycle of selenoprotein synthesis.

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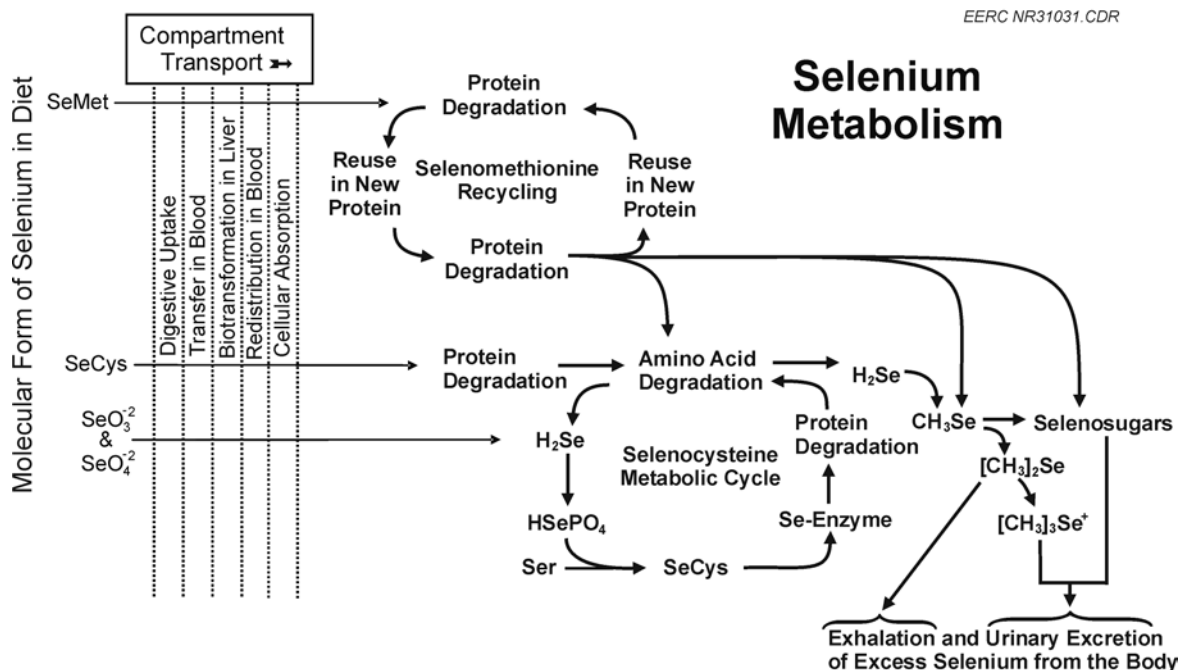


Figure 1. Comparison of metabolic activities of selenomethionine, selenocysteine, and inorganic selenate and selenite.

Sodium selenite is widely used in nutrition studies to examine physiological effects of Se supplementation because it is well absorbed and rapidly forms selenide that is readily incorporated into selenoproteins (Figure 1). Upon absorption, selenite is reduced by glutathione to elemental Se⁰ in the form of selenodiglutathione (GS-Se-SG) which is further reduced by glutathione reductase to selenide, the substrate required for selenocysteine production.

Methylmercury not only has the ability to cross the placental and blood–brain barriers, but its high Se affinity enables it to specifically sequester cellular Se by forming insoluble Hg selenides (HgSe), essentially abolishing future bioavailability of the bound Se and thereby impairing its participation in Se-dependent enzyme activities required for normal motor function, brain neurotransmitter activities, antioxidant mechanisms, growth, and development. A simplified depiction of the normal cycle of selenoprotein synthesis is shown in Figure 2A, and the proposed “Se sequestration” mechanism of disruption of the Se metabolic cycle by exposure to toxic quantities of Hg is shown in Figure 2B.

The term used for sulfur-containing molecules is “mercaptan,” meaning Hg-capturing, and sulfur does have a high affinity for Hg. However, selenide’s affinity for Hg is approximately a million times higher than sulfide, the analogous sulfur molecule. As shown in Figure 3, the abundance of sulfur is approximately 100,000 times greater than selenium, but the much higher affinity of Hg for Se more than compensates for this difference in their relative abundance. Furthermore, the metabolic pathways leading to sulfide formation are relatively rare, but selenide is formed during each cycle of selenocysteine synthesis and is, therefore, uniquely vulnerable to Hg binding. Formation of insoluble Hg selenides (HgSe) renders the bound Se unavailable for continuing participation in future cycles of selenoprotein

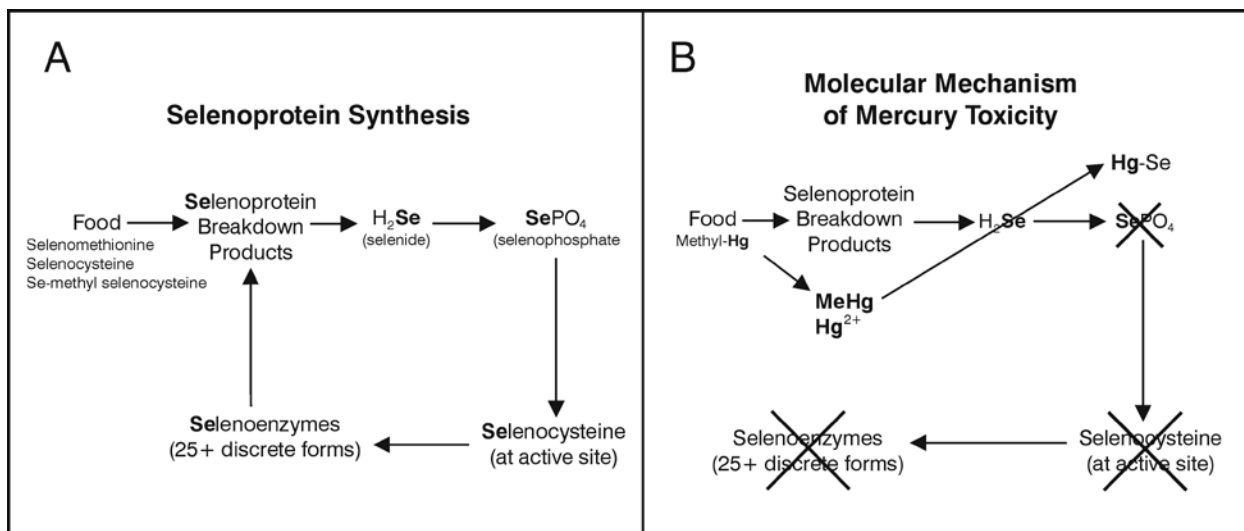


Figure 2. Simplified overview of normal selenoprotein synthesis (A) and Se sequestration effects of high MeHg exposure on selenoprotein synthesis (B).

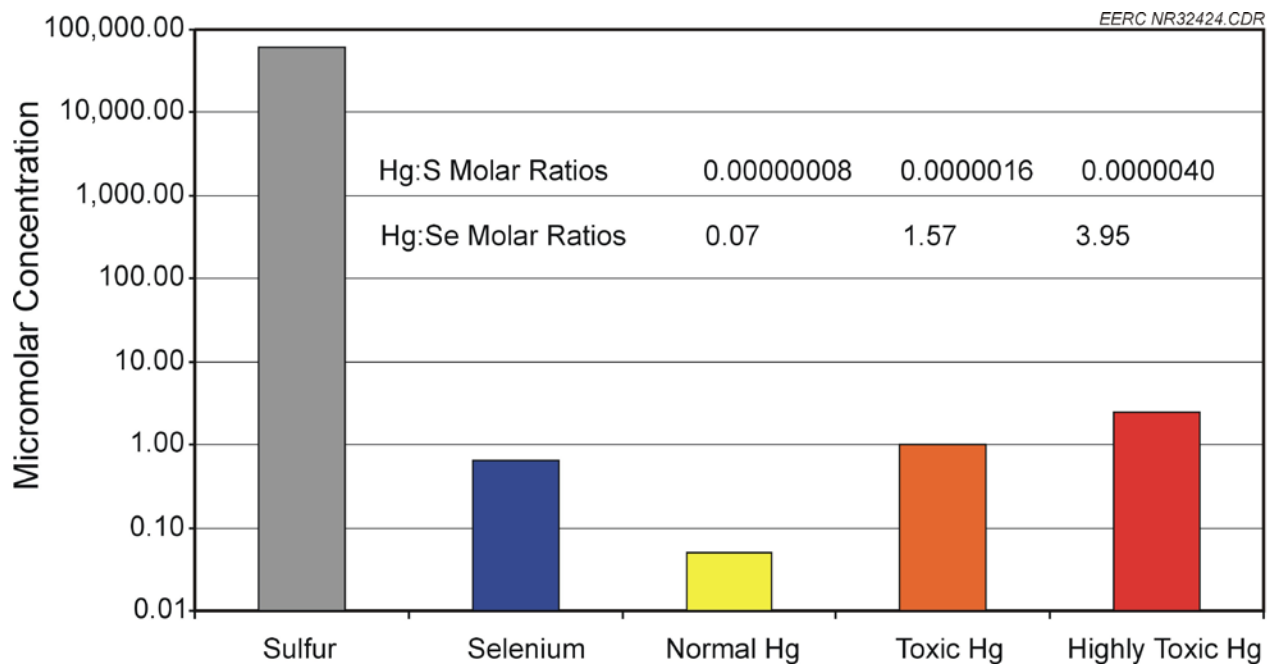


Figure 3. Molar relationships between sulfur, selenium, and mercury in the body.

synthesis. Since intracellular Se is usually present in great excess of Hg, cells can normally maintain optimal rates of selenoenzyme synthesis. However, if the concentration of MeHg incorporated in a cell exceeds that of Se, the Se that is biologically available for normal selenoenzyme synthesis becomes diminished. Once Se is sequestered by Hg, it is unavailable for selenoenzyme synthesis. Therefore, the protective effect of supplemental Se may occur because adequate levels of Se supplied by dietary intake can offset the amount sequestered by Hg.

As seen in Figure 3, the concentrations of Hg known to be toxic are in sufficient molar excess of Se to potentially impair Se bioavailability, but the comparable molar fractions relative to sulfur are infinitesimal. Therefore, toxic Hg exposures appear far more likely to impair Se physiology than sulfur physiology.

Disruption of Se metabolism is a notable aspect of methylmercury toxicity and selenium status is inversely related to vulnerability to mercury toxicity (1–5). Therefore, it is important to investigate the relationship between methylmercury pathology and selenium physiology. Prior PBPK models have developed mathematical reflections of the toxicodynamics of methylmercury and its metabolites (6–14), but these models fail to consider selenium. Fortunately, information pertaining to selenium physiology and its tissue distribution and kinetics is substantial (e.g., 15–18) so POINT models of mercury–selenium interaction studies can be created. The POINT model will integrate data reflecting elemental distributions and molar ratios of molecular forms of these elements in tissues and model estimated selenium sequestration effects. Although PBPK models have not been used in risk assessments (19), a fully developed POINT model will intend to provide a perspective that can assist in evaluating existing epidemiological data and help to identify populations that are protected or at greater risk from methylmercury exposure.

Methylmercury toxicity appears to be related to the high binding affinities (20) between inorganic mercury (Hg^{2+}) and the inorganic forms of selenium that are continuously formed within all living animal cells (21). 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 an understanding of the interactions between mercury and selenium. The presence of rich amounts of selenium may influence fish advisories based on EPA's reference dose level for methylmercury exposure.

Approach

The symptoms of MeHg toxicity were assessed in relation to Se dietary intakes and bioavailability by using POINT model computations of MeHg and Se intakes, absorption and excretion rates, and their calculated demethylation and complexation rates. This POINT model is currently being used to evaluate and interpret the animal study results, including results from complementary rat studies but, ultimately, this model can be extended for evaluations of human exposures to MeHg and the consequent effects on Se physiology.

Progress/Status

The complementary animal study providing data for this project has been completed, and elemental analysis of blood, brain, liver, kidney, and testes from the animals has been finished.

When MeHg exposure is relatively low (0.1 ppm; 0.5 μmol MeHg/kg), the growth of animals fed low, adequate, or rich levels of dietary Se (0.1, 1.0, or 10 μmol Se/kg) does not vary, but the addition of high MeHg (10 ppm; 50 μmol MeHg/kg) results in significant Se-dependent effects on health and growth of the animals.

Development of signs and symptoms of toxicity such as depressed growth was dependent on the Se status of the exposed animals (Figure 4). After 3 weeks of MeHg exposure (~10 ppm; 50 μmol MeHg/kg), rats fed low-Se diets displayed diminished growth relative to the control group fed low-Se diets without MeHg. Growth of rats fed adequate dietary Se was diminished by high MeHg exposure, but the growth of rats fed Se-rich diets was unaffected or slightly greater than the low MeHg control group.

By the tenth week on the diet, rats fed low-Se diets supplemented with 50 μmol MeHg/kg started to lose weight and individual group members showed hind limb crossing and other signs of impaired motor control. The weight gains of rats fed high-MeHg diets accompanying normal Se diets were also diminished relative to their control group, but growth of MeHg exposed rats fed Se-rich diets remained optimal. No signs of neurotoxicity were observed among rats fed normal or Se-rich diets during the 18-week study. The study was terminated when rats fed high MeHg low-Se diets began to show severe motor disabilities and deaths began to occur at the start of Week 18.

Dietary intakes of the individual animals in the treatment groups were continuously monitored throughout the course of the Protection and Therapy Studies. From these data, it is possible to calculate the total MeHg exposures and dietary Se intakes of each individual animal. Applying the absorption and excretion rates for these materials, it is possible to create a single compartment model that reflects the overall selenium economy of the individual subjects and the total amount of MeHg they have accumulated and retained.

Throughout this project, the Hg:Se molar relationships are critically important; therefore, all calculations were performed using the molar basis. Since the weanling rats are growing rapidly, the concentrations of this accumulated Se and MeHg are constantly affected by its growing mass and volume. Therefore, the molar concentrations of the accumulated Se and Hg within the total compartment are partially diminished by this dilution effect, but this is a relatively minor effect.

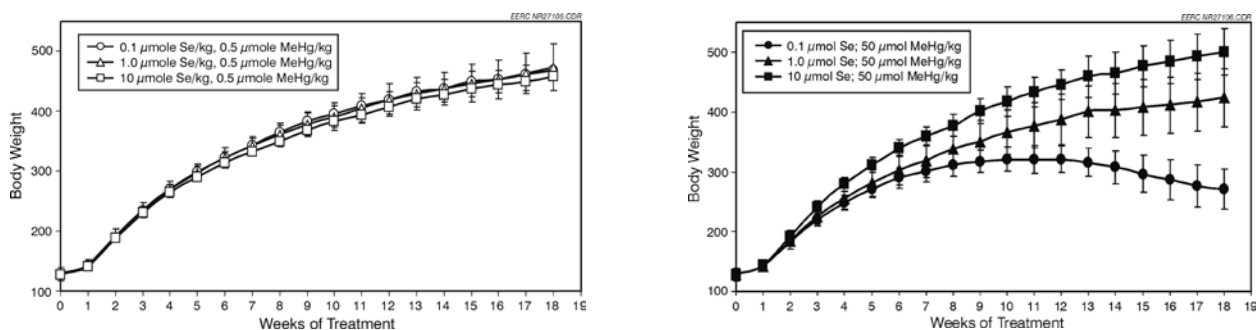


Figure 4. Growth of groups of rats fed low, normal, or enriched dietary Se in the absence (left panel) or presence (right panel) of high dietary MeHg. The data shown depict group means \pm standard deviations for animal body weights (in grams) at the times indicated.

Although the actual processes of accumulation and excretion are discontinuous, for simplicity, the daily flux into and out of the single compartment model and resulting amounts of MeHg and Se (in molar concentrations) are individually calculated on a daily basis as follows:

$$\text{Molar concentration} = \frac{[A + (I \times a)] - E}{M} \quad [\text{Eq. 1}]$$

Where: A = total moles of element accumulated.
 I = dietary intake.
 A = moles absorbed.
 E = moles excreted.
 M = total mass of individual.

The distribution of Se in various rat tissues has previously been determined in numerous studies, but interactive influences of MeHg on Se distribution and Se on MeHg distribution have only been studied in the present projects. Similarly, PBPK modeling of tissue MeHg distributions has been expertly performed on results of various animal studies. Unfortunately, none of the prior PBPK model studies have recognized or incorporated the influence of dietary Se on Hg distributions. Therefore, results of the current study are being used as the basis for the multicompartmental POINT model.

Since issues of concern regarding MeHg exposure arise from dietary intake of seafoods and Se, it is important to study the physiologically relevant routes of Hg and Se uptake. The results of the current study coincide with observations from other studies of Se-Hg interactions in demonstrating that Hg risk assessments must also consider Se levels in food. The data support the hypothesis that dietary Se provides a potent protective effect against dietary Hg exposure. This study also indicates that levels of dietary Se that are slightly less than the average amounts present in commercial ocean fish are sufficient to prevent toxic effects from chronic exposure to MeHg from fish consumption. In fact, the study diets contained ~100 times more MeHg than is present in typical human diets that include seafood. Using data regarding food intakes and growth from a parallel study of Hg:Se interactions in rats, the single-compartment model shown in Figure 5 was generated.

Establishing a single-compartment POINT model revealed that consuming diets containing low Se results in a steady diminishment of tissue Se concentrations. Once growth stabilizes, the Se contents in tissues of these rats gradually begin to increase. The rapid brain growth in the weanling rat provides a reasonable reflection of both the rate and magnitude of human brain growth that occurs during gestation. Rats fed low-Se, high-MeHg diets experience rapidly diminishing Se availability within the first 2 weeks of exposure, then plateau at a Se-deficit level. The impaired weight gains of rats fed low dietary Se would be expected to soon follow. This POINT model indicates the adequate Se group that was fed high MeHg retains near normal levels of Se bioavailability after 18 weeks on this diet. A trend line based on declining bioavailability of Se during the last 16 weeks of the study indicates that after 40 weeks, Se bioavailability in this group will have declined to ~5% of normal. This corresponds to the time when groups fed these approximate amounts of Se and MeHg have been observed to show motor function defects. Among rats fed rich-Se diets, the influx of dietary Se is accompanied by a rapid rise in tissue Se that would be expected to be followed by a steady decline as excretion of Se and MeHg increase. The balance of dietary influx and excretory elimination appears to eventually achieve a steady state, with Se bioavailability maintained at a greater abundance than is required for maintaining optimal Se status. However, this expectation will need to be directly assessed in animal studies with longer exposure periods.

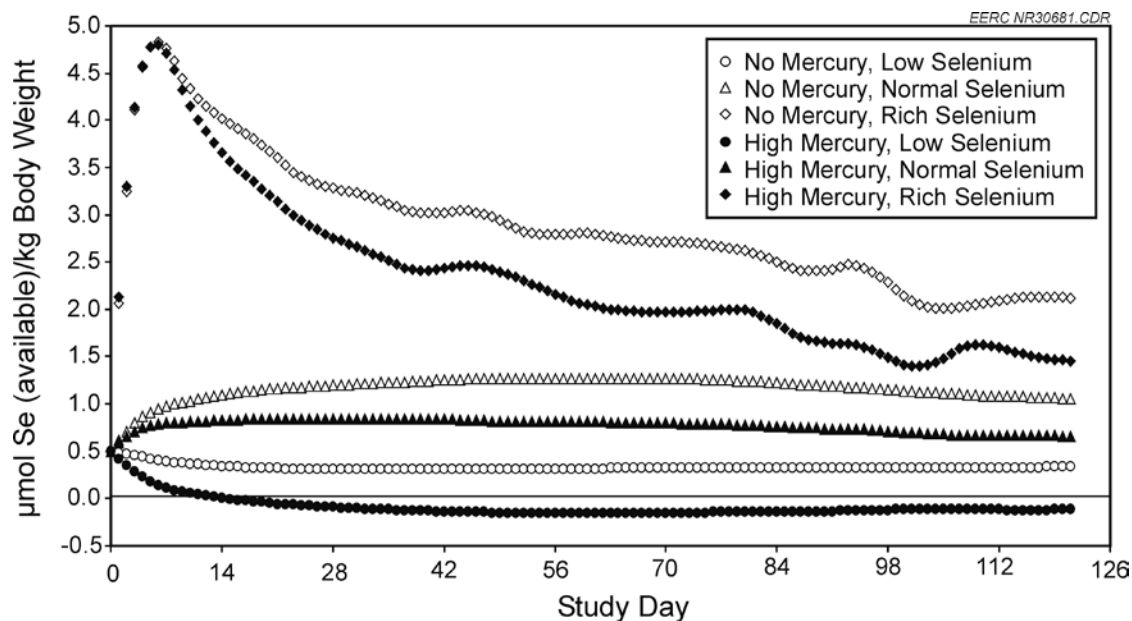


Figure 5. POINT models of dietary treatment effects on bioavailability of selenium.

The results of this study indicate dietary Se has a very potent influence on toxic effects of MeHg exposure. The molar ratios used in this study were selected to enable a direct comparison of the conventional “Hg exposure hypothesis” to the currently proposed “Se sequestration hypothesis.” If the Hg exposure hypothesis were correct, toxic effects should be exclusively or largely related to the amount of dietary MeHg consumed and the amounts of Hg accumulated in tissues. Since the dietary MeHg exposures were equivalent in the animals fed low-, adequate- and rich-Se diets but toxic effects of MeHg exposure were markedly different, the hypothesis that MeHg toxicity is directly related to MeHg exposure is not supported. Instead, the best prediction of toxic effects of dietary treatment was the Hg:Se ratios in the diet.

The slope equations shown in Table 1 were created using growth impairments (body weight of high-MeHg-exposed animals/body weight of low-MeHg-exposed animals) as a measure of relative toxicity. The relative toxicities observed in individual animals were plotted against the Hg, Se, or Hg:Se ratios observed in their various tissues. The data were evaluated using regression statistics to establish the adjusted correlation coefficients (r^2) and p values for the relationships. Since toxic effects were only seen in the rats exposed to the highest level of dietary MeHg, only data from those groups are shown. The most notable aspect of these analyses is that the signs for the slopes of tissue Hg concentration relationships to toxicity were negative for kidney and liver. This occurred because increasing dietary Se resulted in increased retention of Hg in these tissues but diminished toxicity in the animal. Increasing dietary Se resulted in increasing tissue Se levels that resulted in diminishing toxicity of MeHg in the animals.

When the observed incidence of toxicity in relation to tissue Se is examined, a negative slope is observed in all tissues except testes, indicating that increasing tissue Se was associated with diminishing toxicity. These relationships were highly significant ($p < 0.0001$) in kidney, liver, brain, and blood. The correlation coefficients (r^2) for the inverse relationship between tissue Se and toxicity were generally stronger than those observed for the inverse relationship between tissue Hg and toxicity. As would be expected, tissue Se levels were uniformly associated with dietary Se intake in all tissues studied.

Table 1. Correlations Between Indices and Toxicity

<u>Tissue Hg Relationship to Relative Toxicity</u>			
Tissue	Slope + Intercept	Adjusted r^2	p Value
Kidney	$y = -0.0003x + 0.7174$	0.54	0.00001
Liver	$y = -0.0049x + 0.3866$	0.27	0.01
Testes	$y = 0.002x + 0.2416$	0.15	0.03
Brain		0.34	
Blood		0.20	
<u>Tissue Se Relationship to Relative Toxicity</u>			
Tissue	Slope + Intercept	Adjusted r^2	p Value
Kidney	$y = -0.0004x + 0.3433$	0.48	0.00001
Liver	$y = -0.0049x + 0.3866$	0.63	0.000001
Testes		0.25	
Brain	$y = -0.0145 + 0.2982$	0.51	0.00003
Blood	$y = -0.0186x + 0.2568$	0.81	<0.000001
<u>Tissue Hg:Se Relationship to Relative Toxicity</u>			
Tissue	Slope + Intercept	Adjusted r^2	p Value
Kidney	$y = .0079x + 0.0101$.53	0.00001
Liver	$y = 0.0015x + 0.0244$	0.58	0.00001
Testes		0.09	
Brain	$y = 0.0021x + 0.0637$	0.21	0.01
Blood	$y = 0.0005x + 0.0411$	0.48	0.00003

The slopes of the relationships between Hg:Se ratios and toxicity are positive in all tissues assessed, indicating that the more Hg that was present relative to Se in a tissue, the greater the risk of toxicity. Testes were an exception in that they appeared to be far better-protected against Se loss than any of the other tissues and, as a result, did not reflect significant changes in Hg:Se ratios in relation to toxicity.

The next step in this project is to use the tissue Hg and Se concentrations to evaluate the partitioning of Hg and Se distributions in tissue compartments. The single-compartment model establishes the Se economy of the individual animal, but the tissue compartment model will calculate the Se budget of the individual organs. But it is important to recognize that the true physiological effects of Se occur at the cellular level, so the Se balance of the individual cells of a tissue is the real index of risk. The influence of dietary MeHg on the Se balance in individual cells is reflected in the distribution or Se budget, of these elements in the organ compartments which, in turn, are the result of the Se economy of the entire organism (Figure 6). Epidemiological studies to perform maternal MeHg exposure risk assessments are performed on populations that vary not only in MeHg exposure but also in dietary Se intakes. The effects of MeHg exposures at the population level are the aggregate effects of individual responses that reflect the Hg:Se budgets of organ tissues influenced by the Hg:Se balance of their component cells. Therefore, the computational POINT model may be used to extend insights regarding Hg:Se interactions at the molecular and cellular level to effects at the population level.

A recent reassessment of the negative effects of Hg exposure in the Faroes (>90% from pilot whale consumption) and protective effects of nutrients from fish consumption (22) indicates the practical importance of considering beneficial effects of nutrients when calculating the harmful effects of toxins

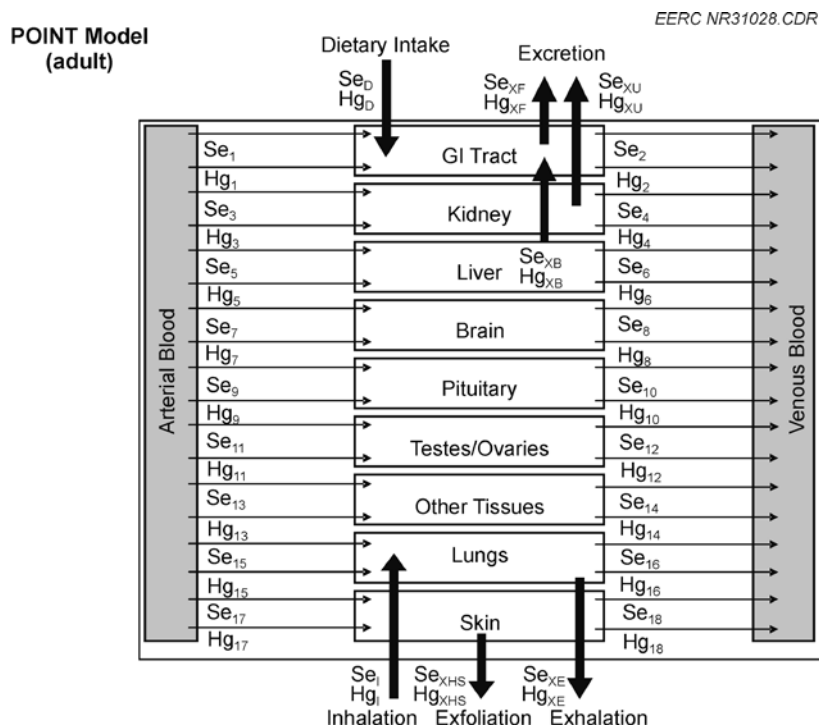


Figure 6. Multicompartment model of selenium and mercury distribution and transport.

that are also present. Interestingly, their research indicates that consumption of ocean fish had uniformly beneficial effects on all child development outcomes that were assessed. In notable cases, the benefits of fish consumption were about twice as great as the negative effects observed for MeHg exposure. The authors emphasize the importance of considering beneficial effects of nutrients when calculating the harmful effects of toxins that are also present.

A recent article from the Faroes research group (23) has considered blood Se in isolation instead of using the Hg:Se ratio that the present study finds is a superior criterion for assessing risk, their reanalysis does observe protective effects when considering Se alone. However, the results of the present study indicate that a reanalysis of their data using blood Hg:Se ratios as the dependent variable would provide far more interpretable and physiologically meaningful results. The results from their reanalysis report that the Hg:Se molar ratio was strongly correlated with Hg, $r^2 = 0.98$, $p < 0.001$, for Cohort 1, and $r^2 = 0.97$, $p < 0.001$, for Cohort 2. Therefore, the relationships that have been recognized between Hg exposure and the various outcomes they have noted in all of their previous reports would be expected to be just as strongly related to the Hg:Se ratio.

Quality Objectives – Measurement/Data Acquisition

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 assurance plan (QAP) that addresses trace metal emissions research at the EERC. The CATM QAP has been reviewed and accepted by EPA. The proposed project will follow the Quality Manual, the CATM QAP, and all revisions. An independent QA auditor reviews all aspects of QA/quality control (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 an informative 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 reflecting measures of food consumption, weight gain, and functional indices in studies where both MeHg and Se are at least partially defined will be compiled in a metaanalysis that includes quantitative and qualitative descriptors. Data sets that include both tissue concentrations of Hg and Se as well as functional indices will be given highest priority in establishing concentration-dependent relationships. POINT models of Se-deficient, Se-normal, and Se-rich animals will be developed with advice from experts in Se physiology, and functional models of effects of MeHg exposure in the Se status models will be evaluated with input from experts in Hg toxicology. Analysis will be validated by comparing positive and negative treatment controls for each independent variable assessed.

This study also helps to explain the contradictory results reported in population studies. The largest and most thorough population study, currently proceeding in the United Kingdom, has found that decreasing maternal fish consumption is associated with increased harm to the fetus and health of the children. It would be expected that the plot of their blood Hg levels would have a negative slope, similar to the results reported in this study. The added selenium supplied from increased fish consumption may, therefore, contribute to the health benefits of increased fish consumption in this population.

Assessment and Validation

Based up on the expected reproducibility of the sample analyses, the number of samples used for each task of the work plan provides statistically meaningful results using standard statistical analysis. In each of the three tasks, complete data sets will be verified, and computational model results will be compared to results from similar studies and pertinent literature. Characteristic distribution effects observed in PBPK studies of methylmercury distributions will be compared to the tissue distribution models developed as part of this project. Kinetic models of selenium flux in animals treated with diets that were not supplemented with methylmercury will be used to compare with selenium distributions among organ systems.

This project was designed with a time line and milestones that will facilitate accomplishing the goals of the project. During the course of the project, the schedule and milestones will be reviewed in order to assess progress.

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