JV TASK 96 – PHASE 2 – INVESTIGATING THE IMPORTANCE OF THE MERCURY–SELENIUM INTERACTION

Final Report

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ABSTRACT

In order to improve the understanding of the mercury issue, it is vital to study mercury's effects on selenium physiology. While mercury present in the environment or food sources may pose health risks, the protective effects of selenium have not been adequately considered in establishing regulatory policy. Numerous studies report that vulnerability to mercury toxicity is inversely proportional to selenium status or level. However, selenium status has not been considered in the development of the reference dosage levels for mercury exposure. Experimental animals fed low-selenium diets are far more vulnerable to mercury toxicity than animals fed normal selenium, and animals fed selenium-rich diets are even more resistant. Selenium-dependent enzymes in brain and endocrine tissues can be impaired by excessive mercury exposure, apparently because mercury has an extremely high binding affinity for selenium. When selenium becomes bound to mercury, it is unable to participate in the metabolic cycling of selenoprotein synthesis. Because of mercury-dependent impairments of selenoprotein synthesis, various antioxidant and regulatory functions in brain biochemistry are compromised.

This report details a 2-year multiclient-funded research program designed to examine the interactions between mercury and selenium in animal models. The studies explored the effects of dietary intakes of toxic amounts of methylmercury and the protective effects of the normal dietary range of selenium in counteracting mercury toxicity. This study finds that the amounts of selenium present in ocean fish are sufficient to protect against far larger quantities of methylmercury than those present in typical seafoods. Toxic effects of methylmercury exposure were not directly proportional to mercury concentrations in blood, brain, or any other tissues. Instead, mercury toxicity was proportional to molar ratios of mercury relative to selenium. In order to accurately assess risk associated with methylmercury or mercury exposures, mercury–selenium ratios appear to be far more accurate and effective in identifying risk and protecting human and environmental health. This study also finds that methylmercury toxicity can be effectively treated by dietary selenium, preventing the death and progressive disabilities that otherwise occur in methylmercury-treated subjects. Remarkably, the positive response to selenium therapy was essentially equivalent regardless of whether or not toxic amounts of methylmercury were still administered. The findings of the Physiologically Oriented Integration of Nutrients and Toxins (POINT) models of the effects of mercury and selenium developed in this project are consistent with the hypothesis that mercury toxicity arises because of mercury-dependent inhibition of selenium availability in brain and endocrine tissues. This appears to occur through synergistic effects of mercury-dependent inhibition of selenium transport to these tissues and selective sequestration of the selenium present in the tissues. Compromised transport of selenium to the brain and endocrine tissues would be particularly hazardous to the developing fetus because the rapidly growing tissues of the child have no selenium reserves. Therefore, maternal consumption of foods with high mercury–selenium ratios is hazardous. In summation, methylmercury exposure is unlikely to cause harm in populations that eat selenium-rich diets but may cause harm among populations that consume certain foods that have methylmercury present in excess of selenium.

TABLE OF CONTENTS

LIST OF FIGURES

LIST OF TABLES

JV TASK 96 – PHASE 2 – INVESTIGATING THE IMPORTANCE OF THE MERCURY–SELENIUM INTERACTION

EXECUTIVE SUMMARY

Mercury (Hg) contamination is widespread, resulting from its use in many products and its emission from combustion systems. The current level of understanding of the complex interplay of Hg emission, transport, deposition, and recycling of different forms of Hg does not allow modelers to correlate particular atmospheric emission sources with human exposure. Therefore, regulatory policies assume sensitivity to Hg is essentially equivalent across the globe, but research indicates this is not true. Selenium (Se), an essential nutrient for many physiological processes, is beneficial in protecting against Hg exposure. Studies using animal models have established that low-Se status increases vulnerability to Hg toxicity while enhanced dietary Se status is protective. However, the biochemical mechanism for Se's protective effect against Hg remains unclear. The influences Hg and Se have upon one another may share a common basis in the exceedingly high binding affinity between these elements. Although Se influences Hg's effects in the body, it is imperative to consider the opposing effect of Hg on Se physiology. This vital aspect is key to understanding Hg toxicity, yet it has been seriously overlooked. Alarmingly, Hg not only has the ability to cross the placental and blood–brain barrier, but its high affinity for Se enables it to specifically sequester Se in the brain and endocrine tissues, consequently impairing neurobehavioral development, brain neurotransmitter activities, thyroid hormone homeostasis, antioxidant mechanisms, immunity, growth, and development.

Therefore, in order to understand how Hg fundamentally alters human biology and fetal development and assess its true exposure risk, our long-term goal is to determine the biochemical mechanism of MeHg toxicity and the extent of Se's involvement. It is our central hypothesis that Hg toxicity is the result of Hg's ability to sequester intracellular inorganic Se and inhibit the formation of essential selenoenzymes in the central nervous system, whereas toxicity will not occur when Se status extends this threshold.

The objectives of this project were to assess the biochemical interactions between Hg and Se through studies uniquely designed to reflect human patterns of MeHg exposure accompanied by various dietary levels of Se. To meet this objective, the following three tasks were performed: 1) examination of the influences of Hg and Se on one another's tissue distribution and the protective and potential therapeutic effects of selenium against mercury's toxic effects as determined by neurofunctional analyses, 2) examination of Se in maternal and fetal blood samples collected in the current Seychelles study, and 3) expansion of the development of a computational method of interpreting and evaluating research data by using the Physiologically Oriented Integration of Nutrients and Toxins (POINT) model reflecting the influence of MeHg on Se availability in various tissue compartments.

The overall conclusion of this project indicates that Hg toxicity is only evident in situations resulting in Hg exposures in molar excess of Se. Additionally, the Se concentrations used in this study, which were less than those typically found in ocean fish and other selenium-rich foods, protected against the toxic effects of MeHg present at much higher concentrations than those

occurring in normal seafood diets. Likewise, administering dietary Se to MeHg-intoxicated rats reversed weight loss, stopped the progressive loss of motor function that otherwise occurs, and prevented death of exposed animals, regardless of whether MeHg exposure was continued at high levels or removed from the diets.

JV TASK 96 – PHASE 2 – INVESTIGATING THE IMPORTANCE OF THE MERCURY–SELENIUM INTERACTION

INTRODUCTION

Selenium (Se)-dependent effects that counteract toxicity otherwise associated with exposure to methylmercury (MeHg) is a major issue in seafood safety. However, there is a critical gap in the knowledge base regarding the magnitude of these effects. The data generated from this project support the premise that simply defining the amount of Hg present in food provides an inaccurate indication of the risk associated with its consumption. This project was designed to assess the significance of Se's protective effect from dietary Hg exposure at realistic and human consumption levels. This issue is extremely important because the health risks from dietary MeHg consumption are dependent on the relative amount of Hg *and* Se present in foods. Likewise, the Se present in many foods, including fish, counteracts the toxic effects of high Hg exposures, thus minimizing or eliminating the potential risk from low-level Hg exposure from ocean fish consumption.

BACKGROUND

Exposure to Hg commonly results from eating fish containing MeHg that has accumulated in the food chain. Yet, conflicting observations and conclusions have arisen from the ongoing studies of children neonatally exposed to Hg from maternal fish and whale consumption. Major studies in the New Zealand and Faroe Islands populations report neurological defects accompany MeHg exposure, but no adverse effects were indicated in the Seychelle Islands population, even though their average MeHg exposure is greater. In the United Kingdom study, no harm was associated with increasing fish consumption, but avoiding fish consumption was associated with impaired neurodevelopment. However, Se, an important nutrient present in many foods including fish, has a potent protective effect against Hg toxicity. Although Se influences Hg's effects in the body, it is imperative to consider the opposing effect of Hg on Se physiology. This vital aspect is key to understanding Hg toxicity and may explain the differences noted in these studies, yet it has been seriously overlooked.

Se's protective effects against MeHg is attributed to the high binding affinities between inorganic Hg (Hg^{2+}) and the inorganic forms of Se that are continuously formed within all living animal cells. Although MeHg is the form that is initially consumed, Hg^{2+} is released from MeHg inside cells following demethylation (by lyase enzymes). When the toxicity of MeHg is considered, it is important to understand the unrivaled high binding affinities of MeHg and Hg^{2+} with inorganic forms of Se that arise within cells during every cycle of selenoenzyme synthesis. The affinity constant for Hg and the Se in selenocysteine (SeCys), the amino acid present at the active sites of all Se-dependent enzymes, is $\sim 10^{22}$, and the free selenides that form during each cycle of SeCys synthesis have an exceptionally high affinity constant for Hg: 10^{45} (Dyrssen and Wedborg, 1991). Since total Se is usually in great excess of Hg, cells can maintain their normal rates of selenoenzyme synthesis. However, if the amount of MeHg incorporated in a cell exceeds

the available Se, normal selenoenzyme synthesis is vulnerable. Once Se is sequestered by Hg, it is unavailable for selenoenzyme synthesis. Therefore, the protective effect of Se may be because adequate levels of Se are available to offset the amount sequestered by Hg (Figure 1).

Ironically, until approximately 45 years ago, Se itself was only known as a poison. It is now recognized that Se is essential for the normal function of 20–30 enzymes in the body and that two of the 22 primary amino acids normally present in all cells of all animals (selenomethionine and SeCys) are distinguished by the presence of Se (Behne et al., 2000). Selenomethionine is biochemically equivalent to methionine and is chiefly regarded as an unregulated storage compartment for Se. In contrast, SeCys is tightly regulated and specifically incorporated into proteins (selenoproteins) that perform numerous biological functions. The Se of SeCys 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 (Burk and Hill, 1993). Remarkably, SeCys is the only amino acid that must be degraded and reformed during each cycle of selenoprotein synthesis. This process releases selenide from SeCys during the degradation step, rendering it vulnerable to Hg sequestration (see Figure 1). The normal cycle of selenoprotein synthesis is depicted in Figure 1A, while disruption of this cycle by toxic quantities of Hg is depicted in Figure 1B. Selenide, the mercury binding partner with the highest known affinity for Hg, is freed during selenoprotein breakdown. This form and all of the other unmethylated forms of selenium are available to bind with Hg. Formation of insoluble Hg selenides (HgSe) makes Se unavailable for continuing participation in future cycles of selenoprotein synthesis.

Se is essential for the normal physiological function of many systems of the body, and poor Se status can have adverse effects on these systems. Se can act as a growth factor (Ramauge et al., 1996), has powerful antioxidant (Behne et al., 2000) and anticancer (Combs and Lu, 2006)

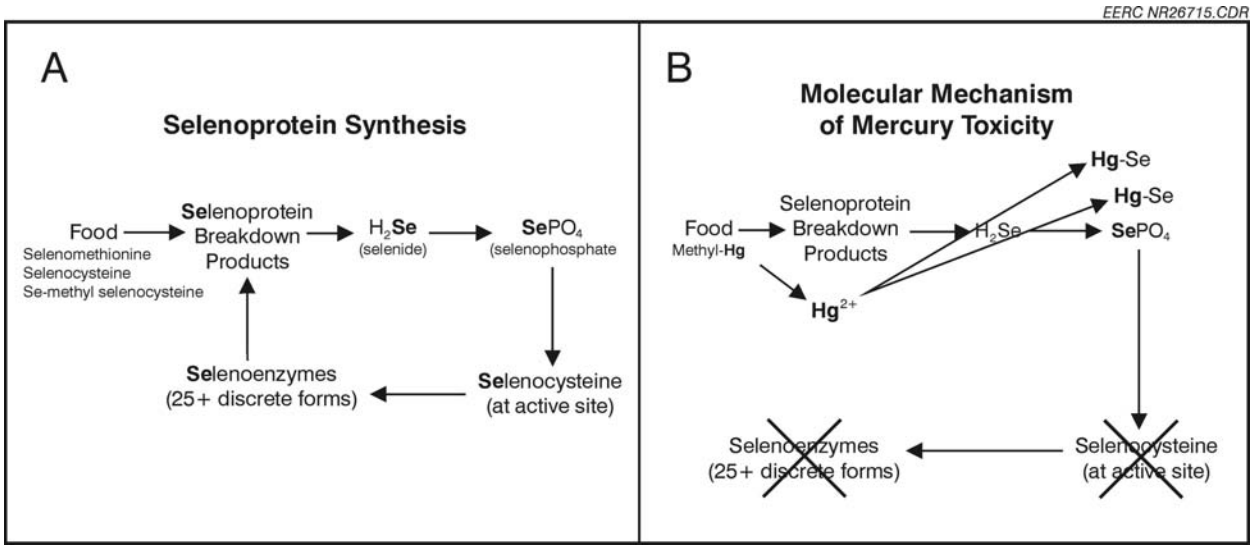

Figure 1. Overview of selenoprotein synthesis (A) and the hypothesized mechanism of Hg toxicity (B).

properties, and is essential for normal thyroid hormone homeostasis (Ramauge et al., 1996) and immunity (Roy et al., 1995). Studies indicate Se has important roles in development, reproduction, cardiovascular disease, and mood disorders. The potential role of defects in Se physiology in disease is becoming increasingly apparent. Compromised Se-dependent metabolic processes have been linked to congenital muscular dystrophy, autism, Alzheimer's and Down syndromes, brain tumors, diabetes, liver diseases, and any condition associated with increased oxidative stress or inflammation such as rheumatoid arthritis, pancreatitis, asthma, and obesity (for reviews see Whanger, 2001; Rayman, 2000; Kohrle et al., 2000).

The importance of Se in the endocrine system is emphasized by the fact that mechanisms have evolved to maintain normal concentrations of Se in these tissues even when other tissues have little or no Se. Severely low Se status can be made to occur in experimental animals continuously fed diets that contain negligible amounts of Se. After a period of months, the Se concentrations in peripheral tissues such as liver, skeletal muscle, and blood of these animals become drastically reduced to levels below 1% of normal. However, their brain tissues retain 60% of the Se concentration found in control animal brains (Behne et al., 2000). However, Behne and others report their offspring have similarly low Se levels in their peripheral tissues but maintain ~60% of normal Se contents in their brains through six generations of continuous feeding of low-Se diets.

Although brain Se concentrations in animals fed low-Se diets have not been reduced to less than 60% of normal, Hill showed that feeding diets containing less than 0.1 ppm Se to selenoprotein P knockout mice reduced their brain Se concentrations to 43% of normal, the lowest brain Se concentration achieved in any experimental animal model (Hill et al., 2003). While rats with brain Se at 60% of normal appear asymptomatic, the knockout mice demonstrated pronounced loss of motor coordination. Motor coordination could be restored and brain Se replenished by feeding them diets containing 2 mg Se/kg. As further evidence demonstrating the importance of Se-dependent physiology, the total disruption of selenoprotein synthesis in mice, achieved by knocking out the selenocysteinyl-tRNA gene, resulted in early embryonic lethality (Bosl et al., 1997).

Consequently, any substance that can enter the brain and disrupt selenoprotein synthesis in these tissues will accomplish what multigenerational Se deprivation cannot. Hg not only has the ability to cross the placental and blood–brain barrier, but its high Se affinity enables it to specifically sequester the brain's Se by forming insoluble Hg selenides, consequently impairing Se-dependent enzyme activities required for normal motor function, brain neurotransmitter activities, antioxidant mechanisms, and development.

Since selenium physiology is severely impacted by MeHg toxicity, it is important to delineate the ways MeHg affects Se distribution and bioavailability. All life processes that are observed at the individual level arise because of interactive functions of various physiological systems. These systems are composed of individual organs that perform their functions through cooperation of their individual cell components. The selenium physiology of the cell is reflected in the functioning of organ tissues, and the health of the individual is dependent upon the well-regulated functioning of these organ systems. Therefore, in order to define the ways in which MeHg adversely affects Se bioavailability and how this affects an individual's health, it is

important to define the MeHg–Se interactions at the molecular, cellular, and organ tissue levels of that individual. This project will collect the necessary data to define these interactions and provide insights on the Se balance of the cell, the Se budget of the organ tissues, and the Se economy of the individual subject.

Physiologically based pharmacokinetic (PBPK) models have been used as a method of creating mathematical reflections of the toxicodynamics of MeHg and its metabolites (Byczkowski, 2005; Smith and Farris, 1996; Young et al., 2001; Shipp et al., 2000; Kirman et al., 2003; Clewell et al., 1999; Gray, 1995). However, these models have not considered the influence of Se physiology in assessing MeHg toxicity risk. Therefore, in order to assess the influence of MeHg on Se availability in various tissues, this project is being used to develop a Physiologically Oriented Integration of Nutrients and Toxins (POINT) model. Since Se physiology and its distributions in tissue compartments are fairly well understood (Zachara et al., 2001; Kaneko et al., 1999; Westermarck, 1977; Behne et al., 1996), POINT models of Hg–Se interaction studies can readily be integrated with the established PBPK models.

Therefore, this project extends the PBPK model of MeHg exposure into a calculated 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 Se and MeHg. 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, their absorption and excretion rates, and their calculated demethylation and complexation rates. This POINT model is currently 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.

In order to correctly evaluate risk of MeHg exposure, it is imperative to determine the threshold of MeHg-dependent toxicity on inhibition of Se physiology. Since the health risk from MeHg varies in relation to Se intake, clarifying the significance of these Hg–Se interactions is essential. Ocean fish is one of the richest sources of dietary Se and is also the main source of MeHg exposure; therefore, these interactions are especially important concerning fish consumption risks and advisories. Elucidating the extent of Se's protection against Hg toxicity is vital because research is proving that significant nutritional and developmental benefits are lost when fish consumption is needlessly avoided.

GOALS AND OBJECTIVES

The goal of this EERC program was to improve the understanding of Hg–Se binding interaction and its involvement in Se-dependent prevention of MeHg toxicity. Our research tasks are designed to identify, quantify, evaluate, and report the effects of selenium in protecting against potential consequences of mercury exposure. As the expansion of a multiclient-funded research program, this project explored the biochemical interactions between mercury and selenium through studies uniquely designed to reflect human patterns of MeHg exposure with various dietary levels of selenium. Since the major issue of public concern regarding mercury

exposure is dietary MeHg present in fish consumed by humans, it is important to replicate this route of exposure in models that employ dietary selenium and mercury present at meaningful concentrations for determination of their interactions and effects. A tremendous amount of work has been done with the intent of examining MeHg exposure and the protective influence of selenium. However, few studies have attempted to replicate the normal dietary exposure route of these elements or closely examine the effects of these elements on the distributions of one another in the tissues. The specific objectives of this project were to 1) determine the influences of Hg and Se on one another's tissue distribution and neurobehavioral effects and examining the protective and potential therapeutic effects of selenium against mercury's toxic effects as determined by neurofunctional analyses, 2) refine and report results from the current Seychelles study, and 3) augment the development of a computational method of interpreting and evaluating research data by using the POINT model reflecting the influence of MeHg on Se availability in various tissue compartments.

In order to meet the goals and objectives of the project, the research activities were divided into three tasks:

- Task 1 – Examining Se's Protective and Therapeutic Effect Against MeHg Neurotoxicity
- Task 2 – Examining Se's Role in Seychelles Mothers and Children
- Task 3 – Computational Model of MeHg–Selenium Interaction Physiology

EXPERIMENTAL

Task 1.1 – Examining Se's Protective Effect Against MeHg Neurotoxicity

Diet Preparation

Diets used in this study were based on the AIN-93G formula for laboratory rodents customized through use of low-Se torula yeast as the protein source (Teklad, Madison, Wisconsin). See Tables 1 and 2 for composition details. This diet provides a low but nutritionally adequate level of Se for rodents (Reeves et al., 2005). The basal diets were augmented with Na_2SeO_4 to adjust Se concentrations to levels that reflect the nutritionally relevant range of dietary Se concentrations. Diets for this study were low-Se, adequate-Se, or rich-Se prepared to containing ~0.1, 1.0, or 10.0 μmol Se/kg (~0.01, 0.08, or 0.8 ppm Se, respectively). The 10.0-μmol Se/kg concentration in the rich-Se diet is well below the 25-μmol Se/kg (~2 ppm Se) level that is accepted as a high but beneficial concentration in nutrition studies and approximates the average amount of Se present in typical varieties of ocean fish (~10 μmol Se/kg).

Each of the Se diets was prepared with either the low (0.5 μmol of MeHg/kg; 0.1 ppm Hg) basal level of MeHg or supplemented to contain 50 μmol of MeHg/kg (10 ppm MeHg) added in the following manner. Diets provided by Teklad were prepared with only 60 g/kg of oil/kg, −1%

Table 1. Modified Mineral Mix and Composition of AIN-93G Torula Yeast-Based Diets*

Mineral Mix Composition	g/kg	Diet Ingredients	g/kg
Calcium Carbonate, anhydrous, 40.04% Ca	555.26	Torula yeast	300
Sodium Chloride, 39.34% Na	73.5	DL-methionine	6.7
Copper Carbonate, 57.47% Cu	0.143	Arginine	0.1
Potassium Iodate, 59.3% I	0.01	Tryptophan	0.3
Ammonium Paramolybdate, 4 Hydrate, 54.34% Mo	0.008	Soybean oil	70
Sodium Metasilicate, 9 Hydrate, 9.88% Si	1.45	Mineral mix	35
Chromium Potassium Sulfate, 12 Hydrate, 10.42% Cr	0.275	Cellulose	50
Lithium Chloride, 16.38%Li	0.017	Vitamin Mix	10
Boric Acid, 17.5% B	0.082	Choline bitartrate	1
Sodium Fluoride, 45.24% F	0.064		
Nickel Carbonate, 45% Ni	0.032		
Ammonium Vanadate, 43.55% V	0.007		
Powdered Sucrose	369.15		
Total	1000	Total	1000

* Sodium selenite additions replaced sucrose in Se-supplemented diets. Mineral mix was added at 35 g/kg (3.5%) of total diet prior to mixing.

Table 2. Hg:Se Molar Ratios in Diets

Dietary MeHg Content	Dietary Se Content, 0.1 µmol Se/kg	Dietary Se Content, 1.0 µmol Se/kg	Dietary Se Content, 10.0 µmol Se/kg
0.5 µmol MeHg/kg	5.00	0.50	0.05
50 µmol MeHg/kg	500.00	50.00	5.00

less than the AIN-93 recommended levels. This purposeful omission allowed MeHg to be added in safflower oil at 1% wt/wt basis to complete the 70 g/kg recommended for this diet. Required amounts of MeHgCl (Sigma-Aldrich, St. Louis, Missouri) were dissolved in safflower oil and mixed for 30 minutes to ensure homogeneous distribution.

Diets were prepared in 1.5 kg batches using 15 g of oil distributed evenly over 1485 g and completely mixed together for 5 minutes to obtain an even distribution of MeHg in the diet. After mixing, representative fractions of these diets were set aside for total Hg analysis. Diets that had been prepared with no MeHg added were found to contain 0.51 ± 0.35 µmol Hg/kg (0.10 ± 0.07 ppm Hg), apparently arising in conjunction with component materials comprising the basal diet. This level of Hg contamination is similar to what has previously been observed in laboratory animal diets (Weiss et al., 2005) Therefore, the actual Hg concentrations in the diet after the MeHg addition were ~0.5 and 50 µmol MeHg/kg (0.1 and 10 ppm Hg).

The Hg:Se molar ratios for these diets are shown in Table 2. As can be observed in the table, the Hg:Se ratios run from highly toxic (500) to relatively low (0.05). It is significant to note that the Hg:Se ratio in ocean fish approximately corresponds to the lowest Hg:Se ratio used in this study (0.05). The mass quantities of Hg exposure would be greater than occur in this diet, but the Hg:Se molar ratio would be very similar.

Animal Study

The 90 weanling male Long Evans rats were distributed into nine weight-matched groups (ten rats/group) with equivalent mean body weights (128.2 ± 1.5 g) that were randomly assigned to one of the nine dietary treatments (three levels of Se, two levels of MeHg, in a 3 × 2 feeding study design). Rats from each treatment group were individually maintained in polyethylene plastic cages provided with deionized water, and their designated diets constantly provided *ab libitum*. Treatment groups were housed in an animal facility with room temperature maintained at 28°C, humidity at 53%, and a 12-h light–12-h dark cycle. Food consumption, body weight, and motor function of the rats were monitored twice weekly to recognize and quantify onset and development of anorexia, growth inhibition, impaired growth efficiency, and neurological defect end points as signs and symptoms of MeHg toxicity that might develop during the 18-week study.

Diet-dependent differences in food consumption, growth, and growth efficiency were analyzed using ANOVA to assess Hg–Se interactions and compared using t-tests (Microsoft Office Excel, http://office.microsoft.com). On Day 126 of the study, rats were intraperitoneally injected with ketamine-rompun (mixed 1:1.37) at a constant 1 μL/g body weight dosage. Syringes prepared with K_2EDTA were used to collect 10–15 mL of blood via cardiac exsanguination. Blood samples were mixed by repeated inversion and stored in an ice bath prior to centrifugation for 15 minutes at 1600 × g and separation into plasma and packed cells. Tissues were removed, cleared of exogenous materials, rinsed in normal saline, patted dry, wrapped in prelabeled aluminum foils, and flash-frozen in liquid nitrogen. Samples were stored at −85°C until ready for elemental analysis.

In parallel with Study 1, when rats fed low-Se, high-MeHg/kg diets began showing neurological signs and weight loss in Week 11, parallel groups (ten rats per group) of rats were either maintained on this diet or were switched to Se-rich diets containing 10 μmol Se/kg with or without 50 μmol MeHg/kg for the duration of the study. The motor function of these sets of rats continued to be monitored, and the restorative effects of supplemental dietary selenium was assessed.

Sample Analysis

Diets and blood samples of ~0.2 g were weighed into single-use, trace element-free 50-mL digestion tubes (Environmental Express, Mt. Pleasant, South Carolina 29464), with every tenth sample being prepared in duplicate and with elemental spike recovery samples accompanying each batch. Each digestion batch included analysis blanks and certified reference materials (dogfish muscle certified reference material DORM-2, National Research Council of Canada, Ottawa, Ontario, Canada).

Samples were treated with 5 mL of HNO_3 (Fisher Trace Metal Grade, Fisher Scientific, www.fishersci.com) and heated at 85°C in deep cell hot blocks (Environmental Express) for 24 hours in capped tubes to preserve samples from trace element contamination. Samples were cooled, 1.5 mL of 30% H_2O_2 (Fisher Certified A.C.S., Fisher Scientific) was added, and samples

were recapped and returned to heating in the dry block at 85°C for 8 hours more. Samples were cooled, and 15 mL of 12 N HCl (Fisher Trace Metal Grade, Fisher Scientific) were added. Samples were heated at 90°C for 90 minutes to reduce SeVI to SeIV. Samples were cooled and diluted to 50 mL with double-distilled water. Samples were further diluted into instrumental calibration ranges and analyzed for Hg content by cold-vapor atomic absorption spectrophotometry using a CETAC M-6000A (CETAC Technologies, Omaha, Nebraska), and Se was analyzed by hydride generation atomic absorption spectroscopy using a PS Analytical Dual Millenium Excalibur (PS Analytical, Deerfield Beach, Florida). Before data from sample analysis runs were entered into the database, MeHg and Se concentrations in sample digestion blanks and elemental recoveries in samples of certified reference materials were evaluated to qualify the analysis batch data for inclusion.

Total Hg and Se mass concentrations (parts per million) for each sample were converted to molar concentrations (micromole per kilogram). Means and standard deviations of molar concentrations of Hg and Se were calculated and graphed for each tissue. Elemental concentrations of Hg and Se in tissues of different dietary treatment groups were analyzed using ANOVA to assess Hg–Se interactions and compared using t-tests (Microsoft Office Excel, http://office.microsoft.com).

Task 1.2 – Examining the Therapeutic Effect of Se Against MeHg Neurotoxicity

Diet Preparation

Diets used in this study were a selection of the same diets as used in Task 1. To initiate MeHg toxicity in the animals to be studied, all 40 animals were fed the same low-Se, high-MeHg (~0.1 μmol Se/kg; 50 μmol of MeHg/kg) diet that was fed in the Protection Study. When signs of neurotoxicity (hind limb crossing, difficulty walking) were first observed in Week 11 of the Protection Study, the 40 animals on this diet were randomized by weight and neurological status into four matched groups of ten animals each. One group was maintained on the low-Se, high-MeHg diets. Since the diet of this group was unchanged, it also continued to participate in the Protection Study, but served as the unrescued control group in the Therapy Study. The other three experimental treatment groups were switched to diets that contained either high or low MeHg and high or low Se (a 2 x 2 design).

Animal Study

In parallel with the Protection Study performed in Task 1, when rats fed low-Se, high-MeHg/kg diets began showing neurological signs and weight loss in Week 11, parallel groups (ten rats per group) of rats were either maintained on this diet or were switched to Se-rich diets containing 10 μmol Se/kg with or without 50 μmol MeHg/kg for the duration of the study. The motor functions of these rats were evaluated twice a week in order to assess the effects of dietary treatment.

Control Group: No Dietary Se Rescue, Maintained on High-MeHg/Low-Se Diets

This group was not rescued by dietary intervention and thus continued participating in the Protection Study while simultaneously serving as the unrescued control for the Therapy Study. Therefore, the results of this group reflect the toxic effects from sustained exposure to catastrophically high levels of dietary MeHg. Although the amounts of MeHg exposure are many times higher than any typical exposure concentrations, poor dietary Se availability is a common occurrence in many areas of the world and, therefore, a viable dietary concern. All rats from the Therapy Study were fed this diet during the first 11 weeks of the study. This control group was maintained on this diet throughout the entire study period.

Experimental Group Rescued with Low-MeHg/Low-Se Diets

This group was no longer exposed to dietary MeHg, but remained on the low-Se diet. Therefore, the source of toxicity was removed, but treatment with Se supplementation was not administered. This treatment group reflects the current medical treatment regime in cases of accidental Hg or MeHg poisoning. Although the source of toxicity would be removed, not supplying supplemental Se as part of the treatment slows down recovery from MeHg-dependent Se depletion.

Experimental Group Rescued with High-MeHg/High-Se Diets

Some cases of human exposure to high levels of dietary MeHg have been accompanied by other foods that are a rich source of dietary Se, for example, populations that consume large amounts of ocean fish along with seafoods such as whale meats that contain large amounts of MeHg. Although the Hg levels used in this study are many times higher than any seafood known to be consumed by humans, the Se levels in the rescue diets approximate the average amount present in ocean fish. Therefore, this experimental group reflects the protective effect of consuming Se-rich ocean fish in counteracting toxic effects developed from prior and concurrent exposures to diets with high MeHg contents.

Experimental Group Rescued with Low-MeHg/High-Se Diets

We hypothesize that therapeutic interventions in cases of accidental Hg or MeHg poisoning should involve not only eliminating the source of the exposure, but also should include providing dietary Se to replace the Se lost to Hg-dependent sequestration. This experimental group was switched to rich-Se diets without any added MeHg, thereby reflecting the effects that would be expected in treatment of patients that had been exposed to toxic amounts of Hg.

For Task 1.2, rats from each treatment group were individually maintained in polyethylene plastic cages as in Task 1.1. On Day 126 of the study, rats were terminated using identical methods to those used in Task 1.1 Sample preparation, digestion, and analysis for tissues from the Therapy Study were conducted identically to sample handling for the Protection Study.

Task 2 – Examining Se's Role in Seychellois Mothers and Children

The dominant source of dietary MeHg exposure and Se intake in the Seychelles is the ocean fish they regularly consume in large quantities. As is true for most varieties of ocean fish, the Se content of these fish tends to be present in great excess of their MeHg contents. Since the ocean fish the Seychellois eat are rich in Se, their blood Se levels are enriched. Previous work had established the Se contents of maternal and umbilical cord blood collected after delivery. When maternal blood Se concentrations were low, fetal blood Se tended to be in excess of maternal Se. When maternal blood Se concentrations were rich, fetal blood Se tended to lower. These results indicate that maternal delivery of Se across the placenta to the fetus is homeostatically controlled. The aim of this task was to prepare presentations of these results for regional, national, and international meetings.

Task 3 – Computational Model of MeHg–Se Interaction Physiology

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 rat is growing rapidly, the concentrations of this accumulated Se and MeHg are constantly affected. 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.

The single compartment MeHg and Se molar concentrations are calculated on a daily basis as follows:

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

Where:
A = total moles of element accumulated
I = dietary intake
a = % absorbed)
E = % 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 has 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 of the multicompartmental POINT model.

RESULTS AND DISCUSSION

Task 1.1 – Accumulation and Distribution of Se and MeHg

Effects of Dietary MeHg and Se on Growth

One of the more prominent signs of Hg toxicity in experimental animals is depressed growth. To monitor MeHg toxicity and the effects of dietary Se status, individual body weights were measured weekly and plotted to reveal dose- and time-dependent effects. The rats fed diets containing selenium in a range of concentrations from low (0.1 µmol Se/kg) to normal (1.0 µmol Se/kg) or selenium rich (10 µmol Se/kg) all grow at optimal rates (Figure 2).

Development of signs and symptoms of toxicity such as depressed growth was dependent on the selenium status of the exposed animals (Figure 3). After 3 weeks of methylmercury exposure (~10 ppm; 50 µmol MeHg/kg), the growth of rats fed low-selenium diets displayed diminished growth relative to the control group fed low-selenium diets without methylmercury. Growth of the methylmercury-exposed rats fed adequate dietary selenium was only marginally affected, and the growth rates of the rats fed selenium-rich diets did not diminish and tended to show slightly greater weight gains than their non-mercury-exposed control group.

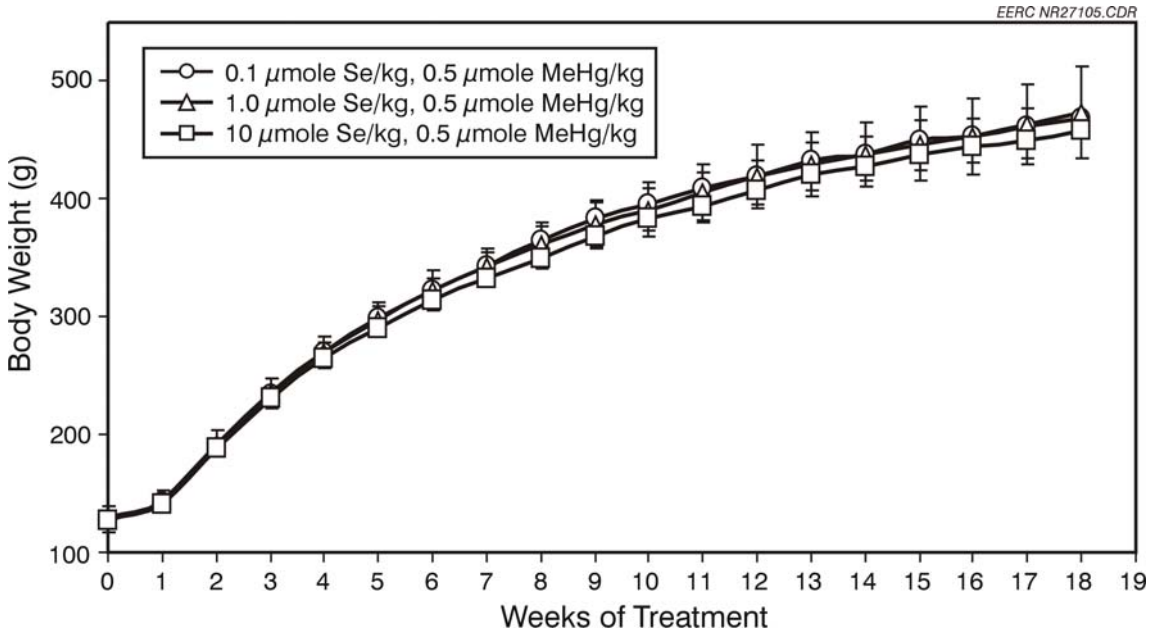

Figure 2. Protection study: growth of groups of rats fed low, normal, or enriched dietary selenium. Data depict means ± standard deviations for group body weights in grams at the times indicated. By itself, dietary selenium did not influence the weight gain of the rats.

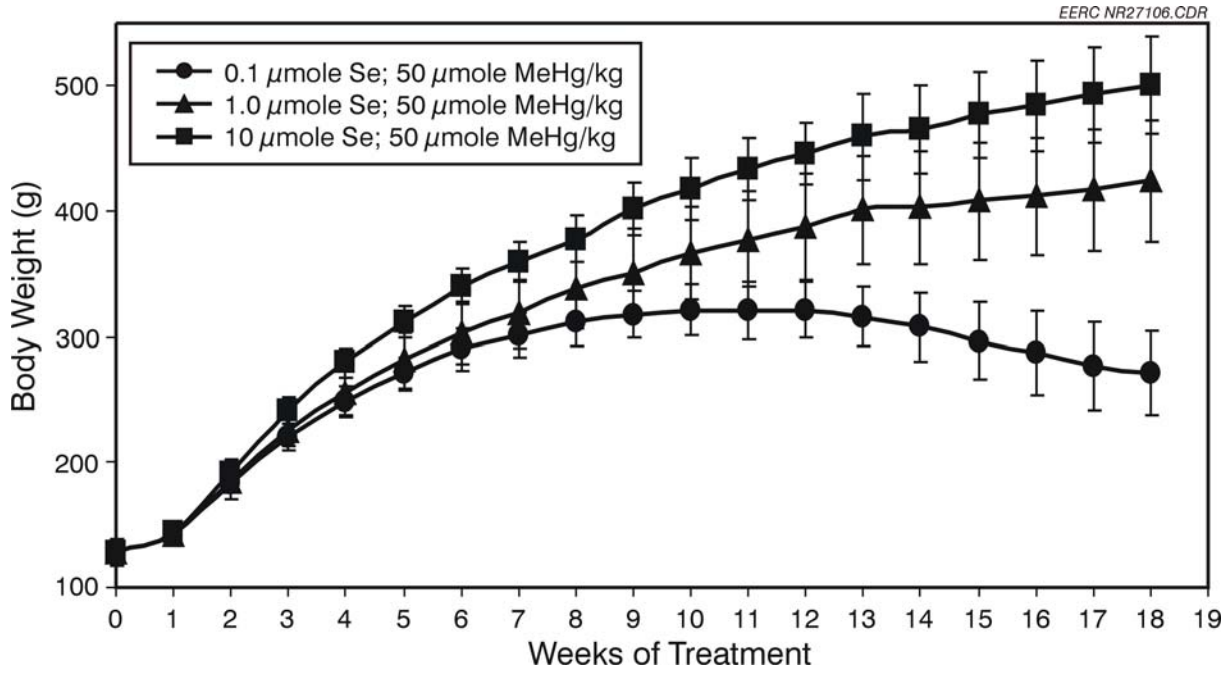

Figure 3. Protection study: growth of rat treatment groups fed low-, normal-, or selenium-rich diets supplemented with 10 ppm (~50 µmol MeHg/kg) methylmercury. Data depicted are means ± standard deviations for group body weights (in grams) at the times indicated.

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 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 showed severe motor disabilities and deaths began to occur at the start of Week 18.

Effects of Dietary MeHg and Se on Elemental Distributions in Tissues

Blood

In human studies of MeHg exposure, Hg concentrations in blood are often measured as an indicator of Hg exposure. Although blood is an easily available sample source with immediate implications regarding current exposures and has proved to be somewhat adequate for quantifying MeHg exposure status, the utility of this measurement as an index of harm is less certain. The results from this study show direct relationships between dietary Se intakes and blood Se concentrations and between MeHg exposure and blood Hg concentrations (Tables 3 and 4, respectively). The elemental concentrations in blood are on a wet-weight basis, while those shown for all other tissues are currently in a dry-weight format. The normal blood Se concentration was 5.87 ± 0.42 µmol Se/kg, but it diminished ~85% when dietary Se was low and increased ~25% when dietary Se intakes were rich. In rats fed adequate Se, increasing MeHg exposure diminished blood Se contents by ~30%, while in rats fed rich-Se diets, increasing

Table 3. Protection Study Tissue Selenium

MeHg, µmol/kg	Se, µmol/kg	Dietary, Hg:Se	Blood, µmol Se/kg	Kidney, µmol Se/kg	Liver, µmol Se/kg	Testes, µmol Se/kg	Brain, µmol Se/kg
0.5	0.1	5	0.83 ± 0.35[a]	14.04 ± 4.07[a]	1.15 ± 0.45[a]	61.16 ± 6.32[a]	5.40 ± 0.52[a]
0.5	1.0	.5	5.87 ± 0.42[b]	65.74 ± 8.50[b]	37.05 ± 10.81[b]	81.79 ± 9.27[b]	6.04 ± 0.95[a,b]
0.5	10	0.05	7.25 ± 0.31[c]	90.87 ± 6.70[c]	45.08 ± 4.09[b]	80.89 ± 7.02[b]	6.34 ± 1.10[b]
50	0.1	500	0.85 ± 0.31[a]	19.54 ± 5.53[d]	1.77 ± 0.36[a]	49.15 ± 8.98[c]	2.77 ± 0.99[c]
50	1.0	50	4.22 ± 0.25[b]	189.96 ± 46.64[e]	30.40 ± 4.84[b]	52.15 ± 18.84[a,c]	5.80 ± 1.06[a,b]
50	10	5	8.25 ± 0.70[d]	920.00 ± 149.64[f]	96.53 ± 13.56[c]	64.79 ± 12.59[a]	24.48 ± 5.23[d]
Compared to Normalization Group			14.2%	21.4%	3.1%	74.8%	89.3%
			100.0%	100.0%	100.0%	100.0%	100.0%
			123.4%	138.2%	121.7%	98.9%	105.0%
			14.4%	29.7%	4.8%	60.1%	45.8%
			71.9%	288.9%	82.1%	63.8%	96.0%
			140.4%	1399.4%	260.5%	79.2%	405.2%

Values shown in the table reflect means ± SD (dry-weight basis) of tissues collected from animals fed the indicated levels of Se and MeHg for 63 days (n = 8 per group). Values with different superscripts are statistically different from one another ($p < 0.05$).

Table 4. Protection Study Tissue Mercury

MeHg, µmol/kg	Se, µmol/kg	Dietary, Hg:Se	Blood, µmol Hg/kg	Kidney, µmol Hg/kg	Liver, µmol Hg/kg	Testes, µmol Hg/kg	Brain, µmol Hg/kg
0.5	0.1	5	0.03 ± 0.01[a]	1.02 ± 0.36[a]	0.17 ± 0.18[a]	0.11 ± 0.09[a]	0.16 ± 0.17[a]
0.5	1.0	.5	0.04 ± 0.01[a]	1.10 ± 0.28[a]	0.09 ± 0.04[a]	0.12 ± 0.13[a]	0.11 ± 0.07[a]
0.5	10	0.05	0.03 ± 0.02[a]	0.90 ± 0.30[a]	0.11 ± 0.10[a]	0.09 ± 0.05[a]	0.09 ± 0.09[a]
50	0.1	500	354.15 ± 40.40[b]	1061.67 ± 169.26[b]	478.83 ± 77.42[b]	264.05 ± 57.01[b]	130.80 ± 20.33[b]
50	1.0	50	356.15 ± 33.54[b]	1552.54 ± 212.27[c]	317.31 ± 75.61[c]	181.09 ± 28.24[c]	101.66 ± 22.69[c]
50	10	5	348.77 ± 42.95[b]	2429.24 ± 208.77[d]	439.23 ± 87.93[b]	214.98 ± 20.55[b]	125.16 ± 18.74[b]
Compared to Normalization Group			77.8%	93.0%	201.6%	92.9%	149.8%
			100.0%	100.0%	100.0%	100.0%	100.0%
			64.9%	82.1%	130.9%	73.9%	85.9%
			99.4%	68.4%	150.9%	145.8%	128.7%
			100.0%	100.0%	100.0%	100.0%	100.0%
			97.9%	156.5%	138.4%	118.7%	123.1%

Values shown in the table reflect means ± SD (dry-weight basis) of tissues collected from animals fed the indicated levels of Se and MeHg for 63 days (n = 8 per group). Values with different superscripts are statistically different from one another ($p < 0.05$).

MeHg exposure resulted in an increase of Se retention by ~40%. Hg:Se molar ratios in the blood of rats fed high-MeHg diets were 561.85 in rats fed low-Se diets and 84.67 and 42.64 in blood of rats fed adequate and rich dietary Se, respectively.

Kidney

Consistent with previous work, this study finds high levels of Se and Hg accumulate in kidneys. After 18 weeks of dietary treatment, Se contents in kidneys of rats fed adequate Se with low MeHg (0.5 μmol Se/kg; 0.5 μmol MeHg/kg; hereafter referred to as normal diets) were 65.74 ± 8.50 μmol Se/kg. Dietary Se levels were ~80% lower in kidneys of rats fed low-Se diets and ~40% higher in rats fed rich-Se diets in the absence of MeHg exposure (Table 3). When MeHg exposure was low the dietary Se exposure was reflected in kidney Se levels however high MeHg exposure increased Se retention in kidneys. High MeHg exposure (50.0 μmol MeHg/kg) resulted in a 3-fold increase of Se retention when dietary Se was at adequate and a 14-fold increase in Se retention when Se was rich. When rats were fed high MeHg, kidney Hg:Se ratios were 52.89 in low-Se rats, 8.59 in adequate-Se rats, and 2.26 in rats fed rich dietary Se (Table 5).

Liver

The contents of Se observed in liver tissues of rats fed low-, adequate-, or rich-Se diets were consistent with observations reported in previous studies. Livers from rats fed normal diets contained 37.05 ± 10.81 μmol Se/kg (Table 3). In rats fed low-Se diets, liver Se diminished to less than 5% of normal. Dietary Se exposure was consistently reflected in liver Se levels when MeHg exposure was low, but compared to normal diets, high MeHg exposure resulted in

Table 5. Protection Study Tissue Hg:Se

MeHg, μmol/kg	Se, μmol/kg	Diet, Hg:Se	Blood, Hg:Se	Kidney, Hg:Se	Liver, Hg:Se	Testes, Hg:Se	Brain, Hg:Se
0.5	0.1	5	0.051 ± 0.036	0.079 ± 0.036	0.188 ± 0.231	0.012 ± 0.032	0.035 ± 0.033
0.5	1.0	0.5	0.007 ± 0.002	0.016 ± 0.005	0.002 ± 0.001	0.002 ± 0.002	0.016 ± 0.012
0.5	10	0.05	0.004 ± 0.002	0.010 ± 0.003	0.002 ± 0.002	0.001 ± 0.001	0.015 ± 0.012
50	0.1	500	561.85 ± 166.04	52.89 ± 13.13	277.00 ± 55.57	5.68 ± 2.33	40.82 ± 7.84
50	1.0	50	84.67 ± 10.30	8.59 ± 2.25	10.83 ± 3.79	4.00 ± 1.69	17.72 ± 3.45
50	10	5	42.64 ± 6.80	2.26 ± 0.91	4.58 ± 0.95	3.45 ± 0.90	5.30 ± 1.27
Compared to Normalization Group			125.2%	7.2%	220.4%	9.6%	32.6%
			100.0%	100.0%	100.0%	100.0%	100.0%
			52.6%	61.7%	109.7%	52.2%	90.2%
			663.6%	616.0%	2556.9%	141.9%	230.3%
			100.0%	100.0%	100.0%	100.0%	100.0%
			50.4%	26.3%	42.3%	86.1%	29.9%

Values shown in the table reflect means ± SD of Hg:Se ratios in tissues collected from animals fed the indicated levels of Se and MeHg for 63 days (n = 8 per group).

~1.5-fold increase in Se in livers of rats fed rich-Se diets. High MeHg exposures resulted in slightly increased Se retention in livers of rats fed low dietary Se but did not affect Se retention in rats fed adequate dietary Se. When MeHg was low, Se levels did not influence accumulation of Hg in liver, but when MeHg exposures were high, Hg accumulation was similar in rats fed low or rich dietary Se, but much lower in rats fed adequate Se diets (Table 4). High MeHg resulted in Hg:Se ratios of 277.0 in rats fed low-Se diets, 10.83 in rats fed adequate-Se diets, and 4.58 in rats fed rich-Se diets (Table 5).

Testes

The effects of MeHg on testes Se contents has not been previously studied. The contents of Se observed in testes of rats fed low-, adequate-, or rich-Se diets were less sensitive to dietary Se and MeHg than any other tissue (see Table 3). Testes from rats fed normal diets contained 81.79 $\pm$ 9.27 μmol Se/kg, higher than any other tissue analyzed in this study. In rats fed low-Se diets, testes Se diminished only slightly. These results support the premise that Se is highly conserved in this tissue when dietary Se intakes are low. In contrast to liver and kidney where Se contents tended to increase in rats fed rich-Se diets that were exposed to high dietary MeHg, testes Se contents significantly diminished with increasing MeHg exposure. The reason for this occurrence is unknown but is likely to involve MeHg inhibition of Se availability. The proportional distribution of Hg and Se in testes of rats fed high-MeHg diets resulted in Hg:Se ratios of 5.68, 4.0, and 3.45 in rats fed low-, adequate-, and rich-Se diets, respectively (Table 5). Therefore, MeHg was less dramatically in excess of Se in testes than in any of the other tissues, possible due to the testes' Se-conserving mechanisms.

Brain

In contrast to Se distributions in somatic tissues that varied greatly depending on dietary Se intakes, brain Se contents were similar to Se in testes, in that only modest changes occurred in response to dietary manipulations (Table 3). Brain Se diminished only 11% when dietary Se diminished 50% and increased only 10% when dietary Se increased more than tenfold. Dietary Se had little effect on brain Se levels when Hg exposure was low, but with high Hg exposure, Se concentrations increased ~ 4-fold in rats fed rich-Se diets compared to control animals. As with other tissues, dietary MeHg exposure was reflected in brain Hg levels (Table 4). High-MeHg diets resulted in brain Hg:Se ratios that were 40.82 in rats fed low-Se diets, 17.72 in rats fed adequate-Se diets, and 5.30 in rats fed rich-Se diets. In rats fed low-Se diets, high MeHg exposure resulted in dramatic decreases in brain Se contents to levels less than 50% of normal. In multigenerational Se deprivation studies, it has taken several generations to diminish brain Se to this degree. In this study as well as our previous studies, rats exposed to high-MeHg–low-Se diets showed severe diminishments in brain Se in only 18 weeks. This rapid deprivation of brain Se appears to occur because of the combined synergistic effects of limited Se dietary supply and impaired Se redistribution from somatic tissues to the rapidly growing brain.

The only group showing symptoms of neurotoxicity during the 18-week study were the rats that were maintained on high-mercury, low-selenium diets. Hind limb-crossing symptoms were apparent by Week 11 in this group, but high mercury groups on normal or rich-selenium diets did not show any signs of impairment during this 18-week study. From Week 11 on, the

number of individuals in this group that displayed hind limb crossing in this treatment progressively increased until Week 15 when it became uniformly present in all animals in this group. Motor impairments in this group increased in severity during Weeks 15–18, until declining health of the individuals in this treatment group required their termination, and the Feeding Study was concluded.

Brain tissues of rats in the other mercury-exposed groups contained similar amounts of mercury, but the Hg:Se molar ratios were quite different. In brains of individuals fed high-mercury diets, the molar ratios were ~41:1 in rats fed low-selenium diets, ~18:1 in rats fed normal-selenium diets, and ~5:1 in rats fed rich-selenium diets. Part of the reason for these substantial differences in molar Hg:Se ratios is due to mercury-dependent impairments in delivery of Se to the brain. Brain Se levels in rats on low-selenium–high-methylmercury diets contained only ~46% of normal levels. Depletion of selenium delivered to the brain compartment and mercury-dependent sequestration of the selenium that was present would have left very little selenium available to perform its normal functions in the brain. Brain Se levels in methylmercury-treated rats on normal-selenium diets were unaffected, whereas brain Se in methylmercury-treated rats on rich-selenium diets displayed a 4-fold increase.

Task 1.2 – Examining the Therapeutic Effect of Se Against MeHg Neurotoxicity

When rats fed low-Se, high-MeHg/kg diets began showing neurological signs and weight loss in week 11 of the Protection Study, parallel groups (ten rats per group) of rats were either maintained on this diet or were switched to Se-rich diets containing 10 μmol Se/kg with or without 50 μmol MeHg/kg for the duration of the study (Note: Day 77 of the Protection Study = Day 1 of the Therapy Study). As shown in Figure 4 the MeHg-exposed rats that did not receive dietary Se continued to lose weight, and their health and motor function ability progressively declined. Animal deaths necessitated termination of this group after a total of 18 weeks of exposure. The rats fed Se-rich diets started gaining weight and improving in health almost immediately. There were only slight differences in recovery rates between the groups treated with Se-rich diets in the presence or absence of high dietary MeHg. Since this dietary Se level is slightly less than the average Se concentration present in typical varieties of ocean fish (see data presented later in this report), feeding ocean fish to supplement Se levels in diets is expected to be similarly effective in preventing signs and symptoms of MeHg toxicity, even when MeHg is present at these high (~10 ppm Hg) levels.

The tissue concentrations of the four groups in the therapy study (high Hg/low Se, high Hg/high Se, low Hg/low Se, and low Hg/high Se) are listed in Tables 6 and 7 with the molar ratios listed in Table 8.

Control Group Without Dietary Rescue

The Hg:Se molar ratios in the diets of this group were 500:1, but the molar ratios in brain tissues were ~40:1. The health of this group steadily declined, eventually resulting in uniformly severe motor disabilities and death. Because of the poor health of this group, they were terminated during Week 7 of the study.

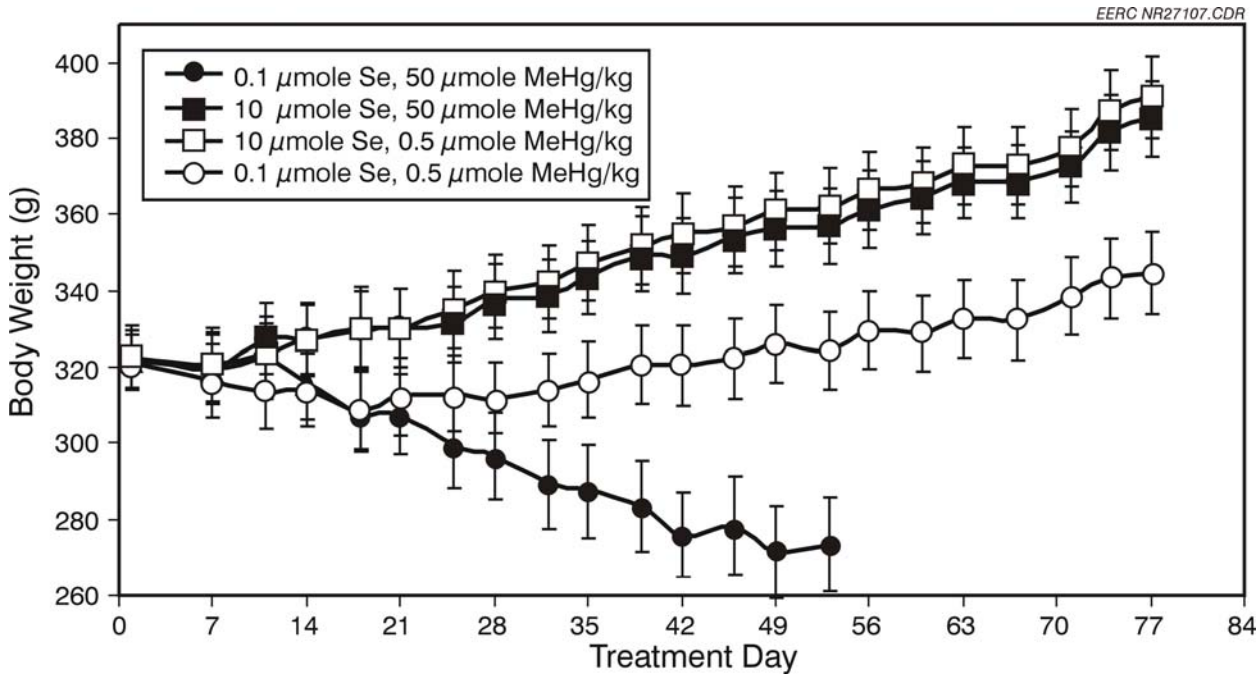

Figure 4. Therapy study: dietary treatment-dependent effects on growth.

Table 6. Therapy Study Tissue Selenium

MeHg, μmol/kg	Se, μmol/kg	Dietary, Hg:Se	Blood, μmol Se/kg	Kidney, μmol Se/kg	Liver, μmol Se/kg	Testes, μmol Se/kg	Brain, μmol Se/kg
50	0.1	500	0.85 ± 0.31[a]	19.54 ± 5.53[a]	1.71 ± 0.36[a]	49.15 ± 8.98[a]	2.96 ± 1.07[a]
50	10	5	8.57 ± 1.23[b]	1198.67 ± 170.69[b]	79.53 ± 16.02[b]	78.50 ± 5.04[b]	22.86 ± 4.22[b]
0.5	10	0.05	7.92 ± 0.63[b]	207.61 ± 47.98[c]	46.58 ± 4.37[c]	86.04 ± 3.57[c]	7.04 ± 1.15[c]
0.5	0.1	5	0.35 ± 0.14[c]	10.57 ± 1.84[d]	1.04 ± 0.19[d]	60.28 ± 4.49[d]	4.02 ± 0.62[d]
Compared to Control Group			100%	100%	100%	100%	100%
			1013%	6133%	4641%	160%	772%
			936%	1062%	2719%	175%	238%
			41%	54%	61%	123%	136%

Table 7. Therapy Study Tissue Mercury

MeHg, µmol/kg	Se, µmol/kg	Dietary, Hg:Se	Blood, µmol Hg/kg	Kidney, µmol Hg/kg	Liver, µmol Hg/kg	Testes, µmol Hg/kg	Brain, µmol Hg/kg
50	0.1	500	354.15 ± 40.40[a]	1061.67 ± 169.26[a]	478.83 ± 77.42[a]	264.05 ± 57.01[a]	130.80 ± 20.33[a]
50	10	5	368.81 ± 40.38[a]	2311.27 ± 280.41[b]	367.10 ± 96.63[a]	202.70 ± 31.43[b]	121.06 ± 11.69[a]
0.5	10	0.05	16.76 ± 2.82[b]	237.24 ± 62.91[c]	18.58 ± 2.53[b]	9.68 ± 1.58[c]	7.61 ± 1.80[b]
0.5	0.1	5	17.62 ± 3.12[b]	97.76 ± 25.33[d]	17.25 ± 2.68[b]	10.34 ± 2.00[c]	5.70 ± 1.98[c]
Compared to Control Group			100%	100%	100%	100%	100%
			104%	218%	77%	77%	93%
			5%	22%	4%	4%	6%
			5%	9%	4%	4%	4%

Table 8. Hg:Se Ratios in Therapy Study Tissues

MeHg, µmol/kg	Se, µmol/kg	Diet, Hg:Se	Blood, Hg:Se	Kidney, Hg:Se	Liver, Hg:Se	Testes, Hg:Se	Brain, Hg:Se
50	0.1	500	561.85 ± 166.04	52.89 ± 13.13	277.00 ± 55.57	5.68 ± 2.33	40.82 ± 7.84
50	10	5	43.65 ± 6.91	1.94 ± 0.18	4.64 ± 1.17	3.15 ± 0.98	5.39 ± 0.68
0.5	10	0.05	2.12 ± 0.37	1.14 ± 0.16	0.40 ± 0.07	0.11 ± 0.02	1.02 ± 0.28
0.5	0.1	5	57.89 ± 24.73	9.25 ± 1.91	17.09 ± 4.22	0.17 ± 0.02	1.36 ± 0.62
Compared to Control Group			100.00%	100.00%	100.00%	100.00%	100.00%
			12.33%	0.18%	0.97%	1.19%	4.12%
			0.60%	0.11%	0.08%	0.04%	0.78%
			16.35%	0.87%	3.57%	0.06%	1.04%

Experimental Group Rescued with Low-MeHg/Low-Se Diets

When MeHg was removed from the diets of the rats receiving low Se, the Hg levels dropped dramatically, indicting the Hg's clearance from the body. The Hg:Se molar ratios in the diets of this group was 5:1, and the brain molar ratio decreased to ~1.3:1. This group gained weight and progression of toxicity symptoms ceased.

Experimental Group Rescued with High-MeHg/High-Se Diets

When Se was added to the diets of the animals receiving high MeHg, Se status was regained in all tissues analyzed. Other than the kidney, there were notable differences in the tissue concentrations of Hg or Se compared to the corresponding group receiving this diet in the Protection Study. However, in the Therapy Group, kidney Se retention was increased by ~30%

compared to the corresponding group receiving this diet in the Protection Study (1200 vs. 920 μmol Se/kg, respectively). The Hg:Se molar ratios in the diets of this group was 5, but the brain molar ratios was decreased to ~5.4. This corresponded to the same molar ratios seen in the Protection Study group receiving this diet. In the Protection Study, this group displayed no negative effects. In the Therapy Study, the rats switched to this diet gained weight, and progression of toxicity symptoms ceased.

Experimental Group Rescued with Low-MeHg/High-Se Diets

When MeHg was removed and Se was added to the diets, the Se levels in all tissues increased to similar levels noted in the Protection Study. However, the kidney Se levels increased ~130% compared to the Protection Study group. Hg levels also decreased to levels noted in the low-MeHg/low-Se groups, but kidney Hg retention also increased to similar molar levels as Se (207.61 ± 47.98 μmol Se/kg vs. 237.24 ± 62.91 μmol Hg/kg). The Hg:Se molar ratio in the diets of this group was 0.05, but the brain molar ratio also decreased to ~1. This group rapidly regained weight and neurological symptoms stopped getting worse and showed tendencies toward improvement.

Neurofunctional Assessments

Hind limb crossing is the result of severe disturbances in the portions of the brain that control motor function. As a result of extensive damage and death of neurons in this brain region, several motor defects arise in methylmercury-exposed rats. In comparison to normal rats, which allow their hind legs to splay out in a relaxed fashion when picked up by the tail, rats with hind limb crossing display a convulsive, and apparently uncontrollable, spastic pulling in and crossing of their hind limbs. As the severity of this symptom intensifies, other defects in motor control and coordination rapidly become apparent, such as inability to walk normally or maintain balance.

In Figures 5 and 6, a score of zero indicates a healthy animal with no perceptible signs of compromised motor control. A score of "−1" indicates a noticeable spasticity of hind limb movement with one leg pulled in, a "−2" indicates both legs pulled in, but not crossed, and a "−3" indicates the rat displayed fully crossed hind limbs, a sign of severe neurotoxicity. The mean values for each group of ten animals are displayed in the figures along with the standard errors of the means (SEM). As described above, at the start of the experiment, rats were pseudo-randomly distributed into groups of equivalent mean weight. These groups displayed similar degrees of slightly impaired motor function as are apparent in Figures 5 and 6 and the effects of the various dietary feeding treatments were subsequently followed. Regardless of selenium levels, rats that had methylmercury removed from their diets continued to display worsening neurologic health for the first two weeks (Figures 5). Thereafter, the rats fed enriched dietary selenium displayed a tendency toward gradual recovery. Rats on low-selenium diets that were no longer exposed to methylmercury in their diets apparently stabilized after 2 weeks but did not show a tendency toward recovery (Figure 5).

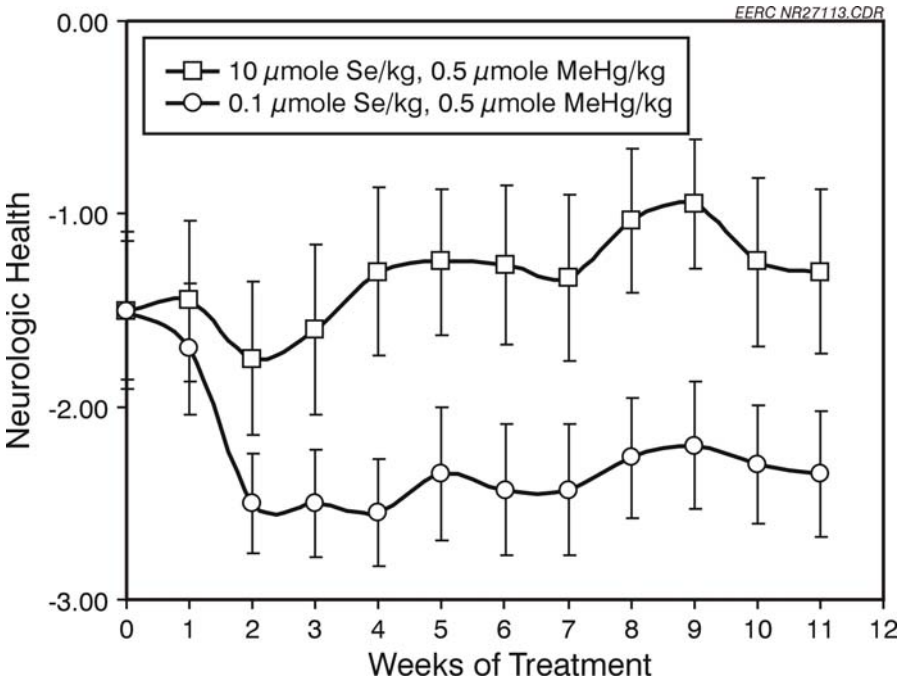

Figure 5. Effects of dietary treatment on development of methylmercury toxicity in rats. Data depict means ± SEM for negative signs of neuromotor health at the times indicated.

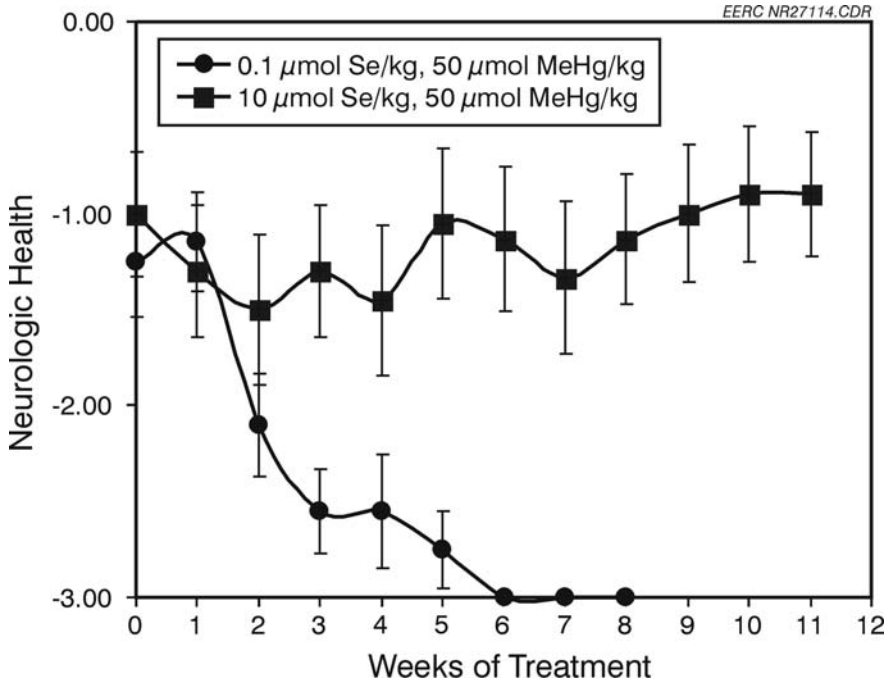

Figure 6. Effects of dietary treatment on development of methylmercury toxicity in rats. Data depict means ± SEM for signs of neuromotor health at the times indicated.

Rats on low-selenium diets that continued their high methylmercury exposure showed a rapid decline in health and neuromotor control (Figure 6). Six weeks into the treatment study, all animals in this group were displaying severe signs (−3) of neurologic damage, and shortly thereafter, individuals in this treatment group started to die. At this point (18 weeks of continuous exposure as part of the selenium Protection Study, the last 8 weeks of which serving as the untreated controls in the selenium Therapy Study), this treatment group was terminated along with the rest of the groups in the selenium Protection Study. Rats maintained on high-methylmercury diets that were switched to enriched dietary selenium at the start of the selenium therapy study were far healthier, but those that were maintained on low-selenium diets became severely disabled, and individuals died. Rats that were not continued on methylmercury but were maintained on low-selenium diets continued to decline in health for 2 weeks but began to gradually gain weight (Figure 5). However, by this time, their motor functions had already become greatly compromised but did not decline further.

When Se was present in the diet, brain Hg:Se molar ratios remained below 1, and no toxicity symptoms were displayed. When rats receiving high-MeHg/low-Se diets were rescued with diets containing rich Se, the brain Hg:Se molar ratios decreased to ~1 and the progressive worsening of Hg toxicity symptoms ceased.

Task 2 – Examining Se's Role in Seychellois Mothers and Children

The results from this task and other tasks from this project were presented at numerous regional, national, and international meetings including the following:

November 2007 – Society for Environmental Toxicology and Chemistry, Milwaukee, Wisconsin
October 2007 – International Society for Trace Element Research in Humans, Heraklion, Crete
June 2007 – Maternal Nutrition Initiative Working Group in Chicago, Illinois
July 2007 – EPA Fish Forum, Portland, Maine
November 2006 – Seafood Science and Technology Conference, San Antonio, Texas
November 2006 – Society for Environmental Toxicology and Chemistry, Montreal, Canada
November 2006 – Selenium Working Group, Bromont, Canada
September 2006 – Neurotoxicity in Aging and Development. Little Rock, Arkansas
August 2006 – Seafood Safety Workshop, Honolulu, Hawaii
August 2006 – International Conference on Mercury as a Global Pollutant. Madison, Wisconsin
September 2006 – Mercury Task Force, Bismarck, North Dakota
May 2006 – 14th International Bioindicators Meeting 2006, Baltimore, Maryland
December 2005 – Seafood & Health 2005, Washington, D.C.
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Task 3 – Computational Model of MeHg–Se Interaction Physiology

Although Hg concentrations in blood and body tissues are proportional to MeHg exposure, it is clear that exposure does not coincide with toxicity. Among rats exposed to high dietary MeHg and either low, normal, or rich amounts of dietary Se, there is actually more Hg in tissues of animals on Se-rich diets that showed no signs of toxicity than among animals that did (Table 9). This is an extreme divergence from what might have been expected by those that do not understand interactions between Hg and Se. These results do not imply that increasing tissue levels of Hg is healthy, just that tissue Hg levels alone do not provide a straightforward basis for evaluating risk. Because of the necessity of maintaining Se physiology, tissue Se contents are inversely related to MeHg toxicity. The Hg:Se molar relationship is directly correlated with MeHg toxicity in all tissues studied other than testes.

When the observed incidence of toxicity in relation to tissue Se is examined, a negative slope is also observed, indicating that increasing Se was associated with diminishing toxicity. Aside from testes, these relationships were highly significant ($p < 0.0001$). The adjusted correlation coefficients (r^2) for the inverse relationship between tissue Se and toxicity were also 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.

In Table 9, the slope of the relationship between Hg:Se ratios and toxicity are all positive, indicating that the more moles of Hg that were present relative to moles of Se in a tissue, the greater the risk of toxicity. The correlation coefficients for tissue Hg:Se are far stronger than for either of the other indices. Testes was an exception in that this tissue appeared to be far better protected against Se loss than any of the other tissues and, as a result, displayed far less dramatic shifts in Hg:Se ratios than the other tissues.

Analysis using the single-compartment POINT model during the course of the Protection Study confirm the expectation that dietary Se intakes will proportionally determine Se status (Figure 7). Regardless of dietary Se intake level, MeHg exposure diminishes Se-status, but it is clear that the usable amount of biologically available Se is very dependent on intake. Consuming diets with low Se concentrations results in a steady diminishment of tissue Se concentrations. As growth rates gradually diminish, the Se contents in these rats gradually begin to increase. Rats fed low-Se, high-MeHg diets experience rapidly diminishing Se availability within the first 2 weeks of exposure, which plateaus at a Se-deficient 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 eventually achieves a steady state, with Se bioavailability

Table 9. Correlations Between Indices and Observed Toxicity

Tissue	Slope + Intercept	Adjusted r^2	p Value
	Tissue Hg Relationship to Relative Toxicity		
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	non significant	non significant	0.34
Blood	non significant	non significant	0.20
	Tissue Se Relationship to Relative Toxicity		
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	non significant	non significant	0.25
Brain	y = -0.0145x + 0.2982	0.51	0.00003
Blood	y = -0.0186x + 0.2568	0.81	< 0.000001
	Tissue Hg:Se Relationship to Relative Toxicity		
Tissue	Slope + Intercept	Adjusted r^2	p Value
Kidney	y = 0.0079x + 0.0101	0.53	0.00001
Liver	y = 0.0015x + 0.0244	0.58	0.00001
Testes	non significant	non significant	0.09
Brain	y = 0.0021x + 0.0637	0.21	0.01
Blood	y = 0.0005x + 0.0411	0.48	0.00003

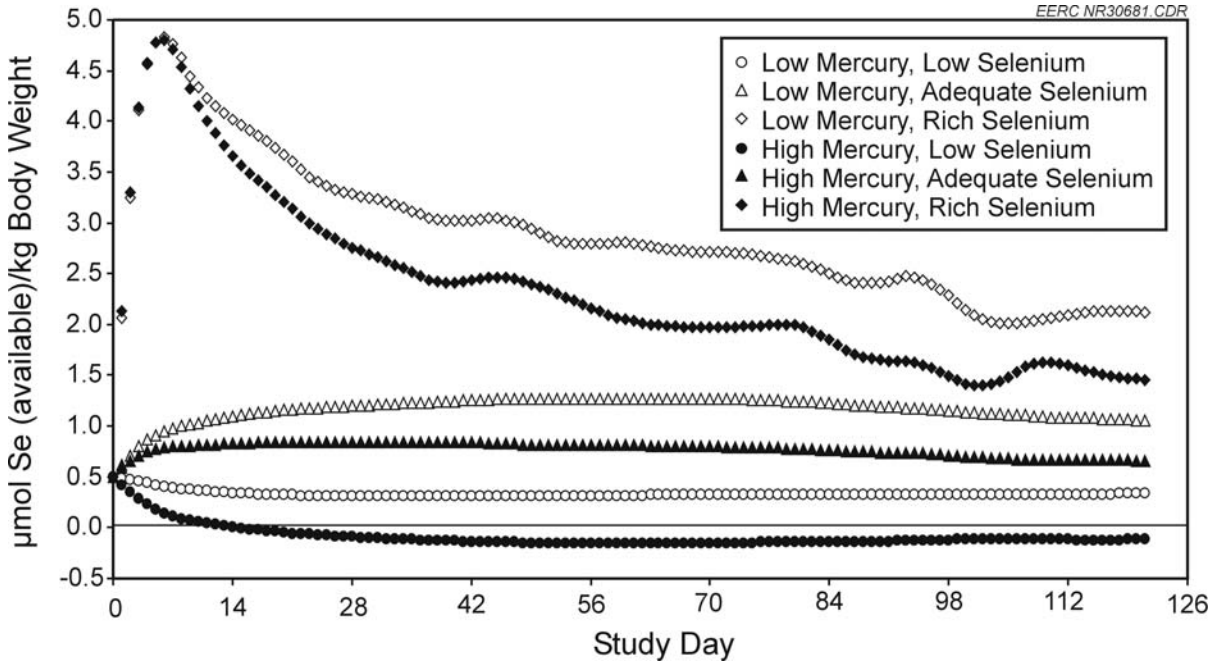

Figure 7. POINT models of dietary treatment effects on bioavailability of Se.

maintained at a greater abundance than is required for maintaining optimal Se status. This is also true in the absence of MeHg exposure, but the steady state of bioavailable Se would be greater.

The POINT model depicted in Figure 8 shows the Se status of animals in the Therapy Study. During the week before rescue, all groups have the same depleted Se status, but during the course of treatment, their computed Se status is distinctly dependent on dietary Se intake. Upon initiation of rescue with rich-Se diets, the Se status of the animals immediately improves, becoming positive from Day 1 of treatment onward. Rats that are fed diets without high MeHg have slightly better Se bioavailability than rats maintained on low-MeHg diets, but because the rate of demethylation and Se sequestration is expected to be low, the recovery rates are largely dependent upon dietary Se.

Although the POINT model indicates there should be a rapid recovery of Se available for supporting growth among rats rescued with Se-rich diets, Figure 4 clearly shows that growth did not resume until after at least 7 days on the repletion diets. The lag period between repletion of the Se status of the single compartment model and the resumption of growth of the Se-rescued rats appears likely to reflect the need to sequentially restore Se status of blood, then liver, then the vital tissues of the neuroendocrine and nervous systems. The relationships between Se and Hg in these tissue compartments will be examined more closely in a follow-up project.

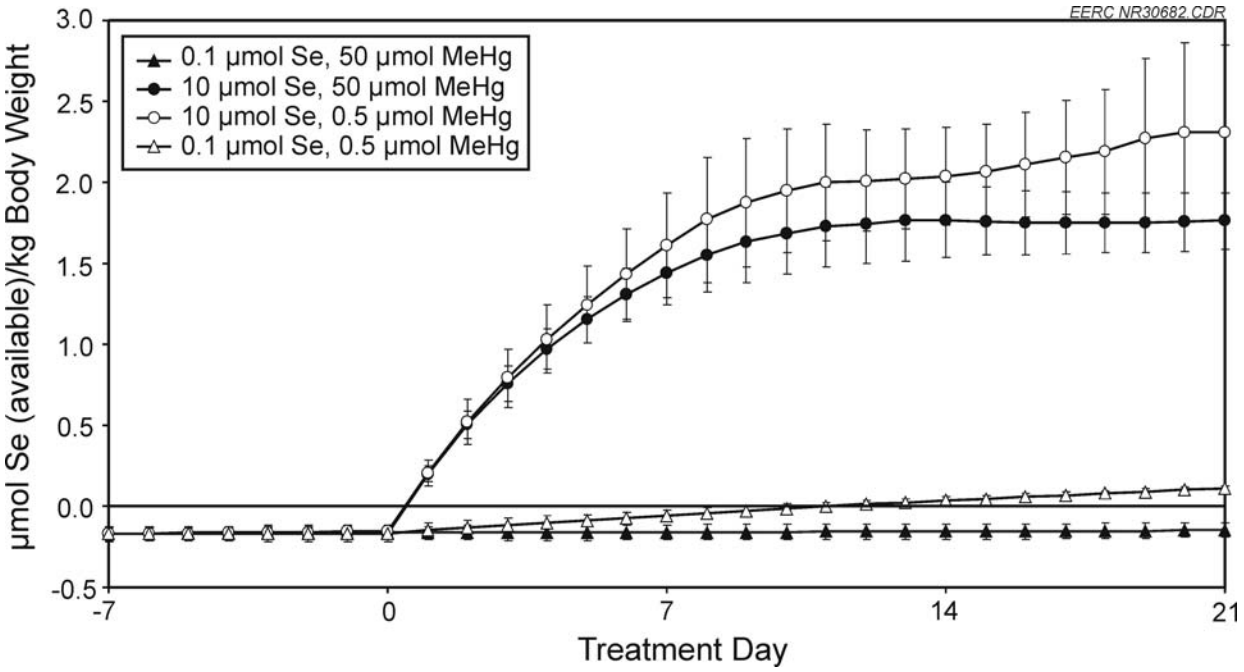

Figure 8. POINT model of effects of dietary treatment on Se bioavailability in rats. The graph depicts means ± standard deviations of group data at the times indicated.

Table 10 details the effects of dietary treatment on growth of rats in the Therapy Study. The group fed low Se, high MeHg only survived until Day 53, so to ensure direct comparison, only data from those days is included in the first section of the table. Rats in the unrescued control group continually lost weight, but all experimental rescue groups gained weight. The rate of weight gain was proportional to the availability of Se from their diets. The rescue group fed rich-Se diets without added MeHg grew the best, followed by the group fed rich-Se diets with added MeHg. Se-dependent restoration of growth was not significantly different between these groups, leading to the surprising finding that MeHg exposure does not matter nearly as much as Se status. Recovery of rats rescued using low-Se diets without MeHg was much slower, but steady. There was an initial 18-day trend of continuing weight loss in this group, followed by a 10-day plateau period as seen in Figure 4.

Applied to the rats in the Therapy Study, the POINT model indicates that among animals fed a low-Se diet with high MeHg exposure, even one day's consumption of the 10-µmol Se/kg diet is sufficient to bring internal bioavailability of Se back to a nearly normal range, regardless of whether or not MeHg is present (Figure 8). However, replenishing high-Se diets to low-Se animals in the actual study did not immediately restore their growth. This lag time may provide a meaningful indication of the normal rate of Se anabolic processes and the sequential replenishment of Se in blood and tissue compartments. Rats that were rescued by switching to experimental diets that eliminated high MeHg exposure but maintained on low Se did not resume a positive Se status until after 12 days on the diet. As indicated above, growth of this treatment group did not resume until several days later, suggesting that physiological recovery may required sequential restoration of a series of tissue compartments before health was restored.

DISCUSSION OF STUDY FINDINGS

The results of the mercury–selenium interaction studies indicate there is a convergence of the molecular mechanisms responsible for selenium-dependent reductions in methylmercury toxicity and the mechanism of mercury toxicity itself. Conventional thinking had been that

Table 10. Comparison of Dietary Treatment Effects on Growth

Diet Treatment					
Se	MeHg	Time Period	Slope + Intercept	Adjusted r^2	p Value
0.1	50	Days 0–53	y = -1.12 x + 328.1	0.960	<0.00001
10	50	Days 0–53	y = 0.77 x + 316.5	0.95	<0.00001
10	0.5	Days 0–53	y = 0.90 x + 315.1	0.97	<0.00001
0.1	0.5	Days 0–53	y = 0.18 x + 311.8	0.27	0.02
0.1	0.5	Days 1–18	y = -0.68 x + 321.7	0.93	0.005
10	50	Days 0–77	y = 0.77 x + 317.3	0.98	<0.00001
10	0.5	Days 0–77	y = 0.89 x + 315.8	0.99	<0.00001
0.1	0.5	Days 0–77	y = 0.47 x + 304.5	0.91	<0.00001
0.1	0.5	Days 28–77	y = 0.56 x + 297.0	0.97	<0.00001

selenium-dependent reductions in mercury toxicity occurred because "selenium acts a mercury antagonist." This meant that supplemental selenium protected against toxicity because it bound up mercury and kept it from causing harm to important cellular biomolecules. However, it is increasingly clear that the real nature of the protective relationship arises because mercury acts as a selenium antagonist, meaning the harm caused by mercury arises because it binds up selenium. In other words, selenium is the important biomolecule affected by mercury toxicity, and supplemental selenium simply ensures that enough free selenium is maintained in the tissues to support normal levels of selenium-dependent physiology. Although the distinction between these two ways of understanding the mercury–selenium interaction may seem small, the impact of the new understanding of the mechanism of mercury toxicity should have an immense influence on regulatory policies regarding environmental mercury.

If selenium acted as a mercury antagonist, it would be only moderately interesting that it could be induced to bind mercury and act to limit the ongoing toxic effects that would inevitably still accompany even low levels of mercury exposure. However, if the hypothesized selenium sequestration mechanism is correct, in order for mercury exposure to be accompanied by harmful consequences, it would require substantial quantities of mercury occurring in significant excess of dietary selenium intakes. The new selenium hypothesis predicts a completely different pattern of causation that provides a comprehensive basis for understanding the results of all the major mercury studies and provides perspective into why their observations appeared to be in conflict.

Consequences and manifestations of methylmercury toxicity have been well described as the result of intense research interest in the topic, but the molecular mechanisms responsible for these numerous physiological perturbations have previously remained undefined. Similarly undefined has been the well-recognized selenium-dependent protective effect against mercury toxicity. Although it has been known for more than 25 years that the physiological consequences of methylmercury exposure may be related to impairments of selenoenzyme synthesis or activities, previous studies have failed to closely examine the basis of this important relationship.

The postulated selenium sequestration mechanism may explain why studies of mercury exposure performed in the Faroes Islands have seen evidence of harm while similar studies performed in the Seychelles Islands have demonstrated there is no evidence of harm associated with mercury exposure from fish consumption. In the Faroes, it is calculated that 95% of the maternal mercury exposure arose from periodic consumption of pilot whale meat, while only 5% of the mercury exposure came from fish consumption. It is also important to recognize that pilot whale meat contains ~50 times higher mercury concentrations than the codfish consumed in the Faroes, resulting in a greatly magnified bolus dose effect compared to normal fish consumption.

Although the observed neurodevelopmental harms were subtle even among children that were exposed to the greatest amounts of mercury and the mercury exposure in these children was accompanied by high levels of organic toxins that were also present in the whale meat, the Faroes study has repeatedly been used as proof of harm associated with mercury exposure. One aspect of whale meat consumption that has not been previously considered is the uniquely high and disproportionate Hg:Se ratio in this unusual seafood. See Figures 9 and 10.

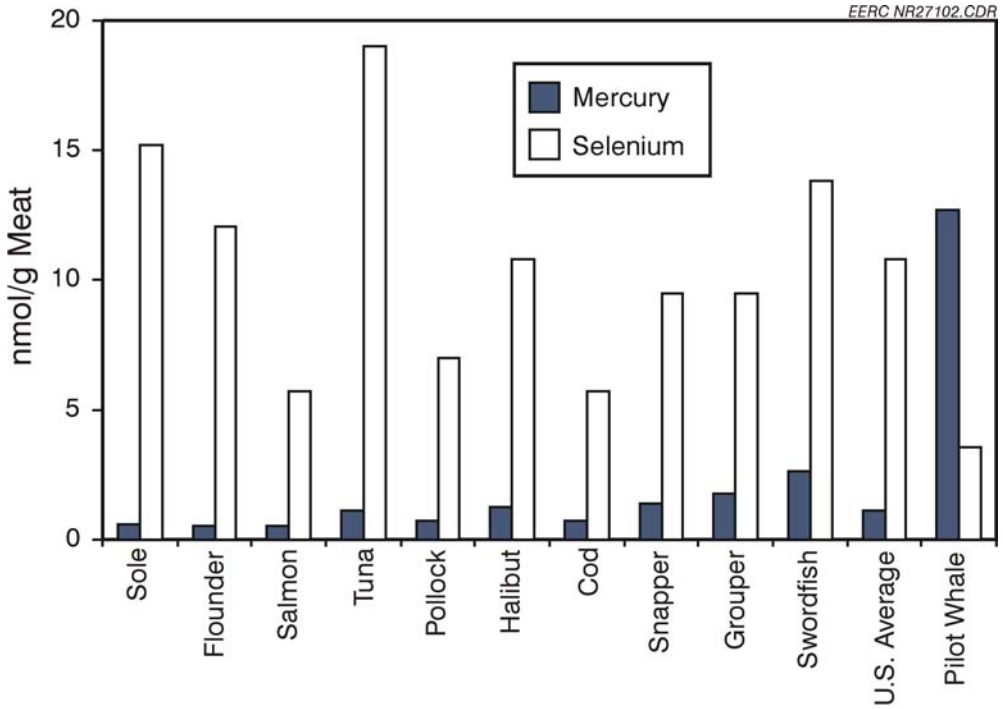

Figure 9. Molar concentrations of mercury and selenium present in seafoods. Data originate from Hall et al. (1978), other than the data for pilot whale (Julshamn et al., 1987) and swordfish (Friedman et al., 1978).

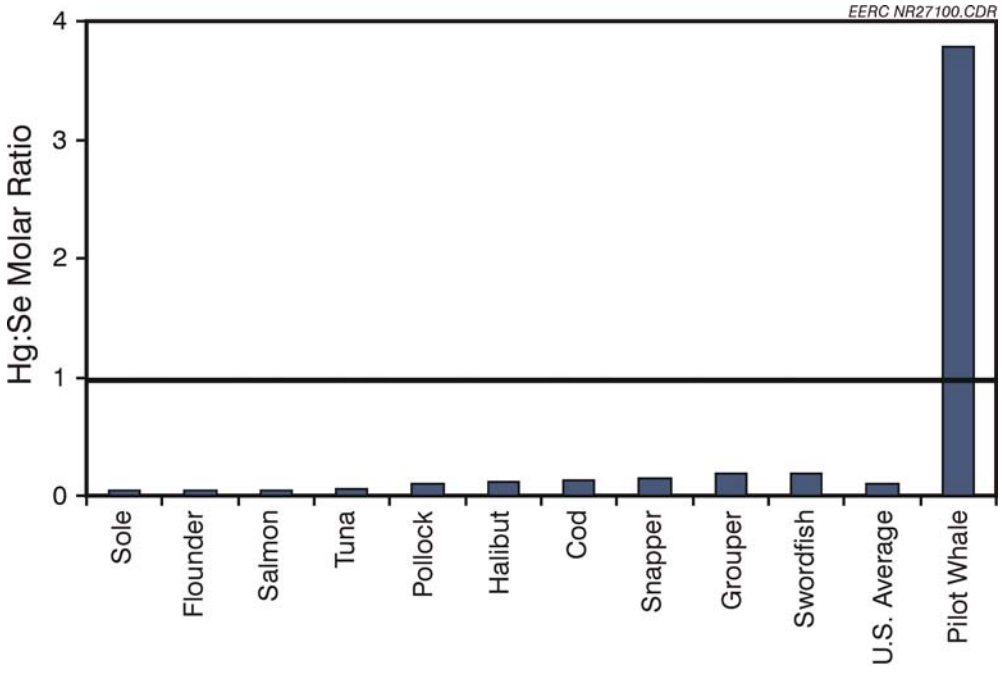

Figure 10. Molar ratios of mercury:selenium in seafoods (calculated from the data depicted in Figure 9).

In contrast to the Faroes study, the studies conducted in the Seychelles have repeatedly found no indication of harm associated with increasing mercury exposure, even though the mean maternal mercury exposure was slightly greater in the Seychelles than in the Faroes. However, in the Seychelles, virtually all maternal mercury exposure came from eating ocean fish. Based on the results of our mercury–selenium interaction studies in animals, the reason why high mercury exposures may have been associated with harm in the Faroes appears likely to be associated with the disproportionately high molar abundance of mercury in pilot whale meat. In contrast, ocean fish are characterized by a naturally high abundance of selenium that would be very protective against mercury toxicity (Figures 9 and 11).

Several other studies also show results that coincide with the selenium sequestration hypothesis, and aspects of their findings are more easily understood with this new perspective. Previous studies have found that methylmercury exposure severely compromises selenoenzyme activities in tissues of young animals whose mothers were fed low dietary selenium but not in tissues of those whose mothers were fed selenium-enriched diets. From the perspective of the new selenium hypothesis, this coincides with the expectation that sufficient selenium must be available to offset the quantity lost to mercury binding and still support the synthesis of selenoenzymes in order to prevent mercury toxicity.

Selenium contents of ocean fish are quite rich, averaging ~10 nmole Se/g, and the average Se:Hg molar ratio is approximately 12. It is currently unknown whether or not risks of methylmercury exposure are linearly related to relative Hg:Se ratios, but if they are, it would be reasonable to suggest mercury exposure from eating pilot whale meat is many times more hazardous than mercury exposure from consumption of ocean fish. Furthermore, since one of the

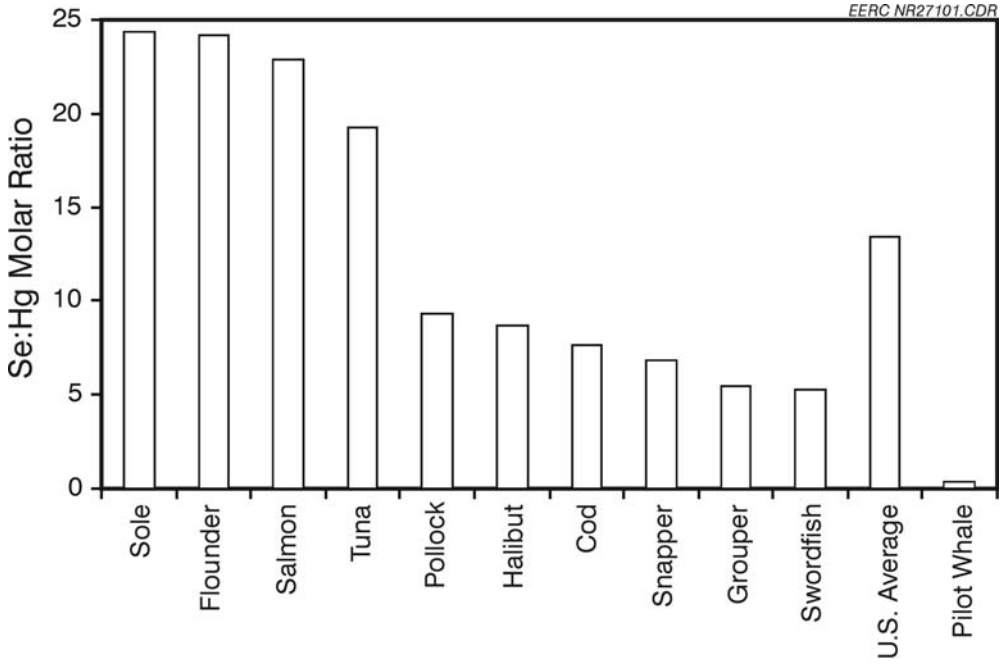

Figure 11. Molar ratios of selenium:mercury in seafoods
(calculated from the data depicted in Figure 9).

central principles of toxicology is that "the dose makes the poison," the risk of the high bolus dose of mercury from pilot whale meat is many times greater than that from eating ocean fish.

The results of the Protection and Therapy Studies offer an explanation for the contradictory results reported in human population studies. The largest and most thorough population study performed in the United Kingdom has found that decreased maternal fish consumption was associated with increased risk of adverse developmental outcomes in their children.

The plot of child performance relative to blood Hg levels would have a positive slope (higher performance accompanies high blood Hg), similar to the results reported in this study. However, the improved performance would not be the result of increased mercury exposure, but would instead be the result of improved maternal selenium and omega-3 fatty acid intakes. Since the population of the United Kingdom has generally poor selenium status, additional selenium supplied from fish consumption appears likely to have contributed multiple health benefits to developing children.

The major human studies of the effects of maternal fish consumption were designed to test the hypothesis that maternal MeHg exposure from seafood consumption is directly associated with harm to developing children. That hypothesis predicts that increasing maternal MeHg exposure will result in increasing developmental impairments of their children and these dose-dependent effects will occur in direct proportion to exposure. The results of human studies in New Zealand and the Faroes support this hypothesis. However, the Seychelles study indicates there is no dose-effect relationship, and the United Kingdom study shows an inverse relationship between exposure and harm. Since the United Kingdom study is bigger than all of the other studies combined, is the most complete and best performed study of this subject to date, and provides the best reflection of the type of MeHg exposure that is of greatest concern in the United States (consumption of normal seafoods by a first-world population consuming a typical Western diet), its results are clearly the most relevant test of the hypothesis.

The results of all of the human and animal studies are completely consistent and readily understandable from the perspective of the Se sequestration hypothesis. The series of studies we have performed in the current project were designed to evaluate the "MeHg exposure" and the "Se sequestration" hypotheses. We found dietary MeHg exposures were not a reliable indicator of risk since animals that were exposed to identical amounts of MeHg did not display identical toxic effects. Therefore, MeHg exposure is not consistently associated with methylmercury exposure alone. This confirms the MeHg exposure hypothesis is incomplete. Instead, toxic risks of MeHg exposure were consistently associated with Hg:Se ratios in the diet and in the blood and tissues of the exposed animals. This finding supports the Se sequestration hypothesis.

This study offers an explanation for the contradictory results reported in human population studies. The findings of the United Kingdom are consistent with expectations based on understanding the importance of Hg:Se ratios in the seafood safety issue. The plot of child performance relative to blood Hg levels in that study would have a positive slope (higher performance accompanies high blood Hg), similar to the results reported in this study. However, the improved performance would not be the result of increased mercury exposure, but would

instead be the result of improved maternal selenium and omega-3 fatty acid intakes. Since the population of the United Kingdom has generally poor selenium status, additional selenium supplied from fish consumption appears likely to have contributed multiple health benefits to developing children.

CONCLUSIONS

Tremendous controversy surrounds seafood safety issues regarding methylmercury exposure because study results of various studies do not coincide when considered from the perspective of methylmercury exposure alone. However, the results of the animal studies of the current project and all the major human studies of maternal mercury exposure are completely consistent when considered from the perspective of the selenium sequestration hypothesis. The series of studies performed in this project have been designed to directly test the "methylmercury exposure" vs. the "selenium sequestration" hypotheses of mercury toxicity. The levels of selenium that were used in our animal study diets are comparable to the levels normally present in ocean fish. This amount of dietary selenium was sufficient to completely counteract toxic effects of far more methylmercury than is ever found in even pilot whale meats. Dietary methylmercury exposure was shown to be an unreliable indicator of risk of toxicity since groups of animals that were exposed to identical amounts of methylmercury did not show the same levels of toxic effects. Instead, toxic risks of methylmercury exposure were consistently associated with Hg:Se ratios in the diet and in the blood and tissues of methylmercury-exposed animals. Therefore, the results of this study do not support the methylmercury exposure hypothesis, but coincide with expectations based upon the selenium sequestration hypothesis.

Selenium is very important to maintaining health because it is absolutely required by a number of essential enzymes that protect and regulate physiological processes in the brain and endocrine tissues. The selenium levels in these tissues are maintained against extraordinarily steep concentration gradients, making it almost impossible to impair selenium-dependent enzyme activities in these tissues. Other than deletion using gene knockout animal models (usually resulting in fetolethal effects), there is no way to directly eliminate these enzyme activities. The only way to significantly compromise selenium physiology in brain and endocrine tissues that has been demonstrated to date is exposure to large amounts of methylmercury exposure during fetal development. If this is done in the absence of adequate selenium resources to make up for the losses of selenium to mercury sequestration, the offspring will have diminished selenoenzyme activities (Watanabe et al., 1999). The pathology of methylmercury toxicity directly corresponds with the effects that would be expected to accompany impaired selenium physiology, and it is impossible to completely understand mercury toxicity without understanding selenium physiology. This may explain why so many seafood safety issues regarding methylmercury are still being debated after decades of research.

The following analogy is useful in developing an understanding of the biochemical interactions between mercury and selenium: Imagine that selenium is money and you are considering the health of your bank account. Eating a selenium-rich food such as ocean fish is like opening your mail and finding a check made out in your name for a sizable amount. Meanwhile, the methylmercury that is also present in the fish is analogous to getting a bill in the

same bunch of mail. A certain amount of selenium is required to support necessary activities in your brain and other tissues just like you need a certain amount of money to pay for life's necessities, such as food and housing. It is important to have enough selenium just like it is important to have more money than expenses. In this analogy, mercury toxicity would be equivalent to bankruptcy.

Using this analogy makes it easier to understand the important effects of mercury:selenium ratios in pilot whale and fish meats to illustrate their effects: When mothers in the Seychelles or in the United States eat ocean fish, they are exposed to some mercury, but their selenium intake is much greater. This is like opening your mail and finding you received a bill for $200 but also received a check for $2000. However, when the Faroese population eats whale meat, they are exposed to far more mercury than selenium. This is like receiving bills for $2000, with an income of only $500. The more mail they open (the more pilot whale meat they eat), the worse their situation becomes.

The selenium status of a developing fetus is remarkably precarious since it has no selenium reserves to call upon when there is a shortfall in transport across the placenta. Maternal selenium reserves can become depleted during pregnancy, and result in inadequate delivery to the developing child. The fetus needs to receive enough selenium to support adequate levels of selenium physiology in its brain and endocrine tissues, and these demands grow throughout pregnancy since these tissues grow very rapidly. Eating ocean fish during pregnancy will improve maternal selenium reserves and increase delivery of selenium to the growing child, but if a pregnant woman happens to eat too much pilot whale meat, the selenium she should be sending to her baby will not be delivered. This is analogous to the situation most families encounter while supporting a child at college. The child has no financial resources, but has pressing needs for the money to cover tuition, books, and room and board that must be met or his/her education will suffer. A mother that eats pilot whale meat is like a family that receives unexpectedly large bills that deplete their bank account during the time their child needs tuition money.

The subtle harms experienced by children whose mothers ate pilot whale meat in the Faroes may have occurred because the necessary amount of selenium was not delivered. Fortunately for their children, the mothers in the Faroes were also regularly eating ocean fish. The selenium from ocean fish consumption helped offset some of the shortfalls caused by eating pilot whale meat, thus preventing the methylmercury from the pilot whale meat they ate from causing far more severe harm to their children.

Although the previous human studies of maternal ash consumption were originally designed as tests of the scientific hypothesis that "maternal methylmercury exposure from seafood consumption is directly associated with harm to developing children," they are equally effective tests of the selenium sequestration hypothesis. This hypothesis explains the molecular mechanism of mercury toxicity and the molecular mechanism of dietary selenium's ability to counteract mercury toxicity. The Se sequestration hypothesis leads directly to a slight modification of MeHg exposure hypothesis, namely, that "maternal MeHg exposure in excess of Se intake from seafood consumption is directly associated with harm to developing children." This scientific hypothesis predicts that increasing maternal methylmercury exposure in

excess of dietary selenium intake will result in increasing developmental impairments of their children and these dose-dependent effects will occur in direct proportion to Hg:Se exposure.

The protective effect of eating ocean fish has also been noticed by the researchers in the Faroes. The results from a recent analysis from the Faroes Study group (Choi et al., 2008), demonstrate 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 they have recognized between Hg exposure and the various developmental harms they have noted in all of their previous reports would be expected to be just as strongly related to the Hg:Se ratio or may be even more strongly related as was observed in this study. The Faroes researchers chose to attempt considering blood selenium in isolation instead of using the Hg:Se ratio that the present study finds to be a far more superior approach for assessing risk. Although this reanalysis does observe certain protective effects when considering selenium alone, the findings of the present study strongly 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 goal of this project was to improve the understanding of Hg–Se binding interactions and their involvement in Se-dependent prevention of MeHg toxicity using physiological levels of Se. The results from this project support the premise that rather than Se offering protection from Hg toxicity through Hg sequestration, Hg toxicity may occur as the result of Se sequestration, thereby inhibiting Se-dependent physiological processes in brain and related tissues. Therefore, Se-dependent protection against Hg toxicity is the result of supplemental Se replacing the intracellular Se lost to Hg sequestration, thus maintaining normal Se-dependent physiology in brain and endocrine tissues. Likewise, the Se concentrations used in this study, which were less than typically found in ocean fish and other selenium-rich foods, protected against the toxic effects of MeHg present at much higher concentrations than those occurring in normal seafood diets.

Supporting observations and conclusions from this project are summarized as follows:

- Increasing dietary MeHg exposure accentuated retention of Se in blood and diminished Se delivery to brain.

- Compared to Hg retention in rats fed normal dietary Se, Hg retention was greater in kidney, liver, testes, and brains of animals fed rich dietary Se, possibly as a result of HgSe complex formation.

- Blood Hg levels accurately reflect MeHg *exposure* but does not accurately reflect risk.

- Among animals fed high-MeHg diets, increased Hg in brain was associated with decreased toxicity, a highly counterintuitive finding if Hg–Se interaction effects are not understood.

- Among rats fed low-Se diets, toxic effects of high MeHg exposure were apparent far earlier and with far greater severity than is typically seen in animals fed adequate-Se diets containing the same amount of MeHg.

- The Hg:Se molar ratio was a far more sensitive and accurate index of risks from Hg exposure.

- When rats displaying signs of MeHg toxicity were given Se-rich diets, they started gaining weight and improving in health almost immediately.

- Only slight differences were noted in recovery rates between "rescued" groups treated with Se-rich diets in the presence or absence of high dietary MeHg. Recovery was not dependent on MeHg concentration, but was largely dependent on dietary Se intakes.

- Typical varieties of ocean fish have a 5–20-fold molar excess of Se over MeHg content.

- The Se-rich diets used in this study contained amounts of Se approximating those present in typical varieties of ocean fish, but protected against toxic effects of MeHg present at concentrations that are log orders higher than those that naturally occur in typical seafoods.

- Low-level MeHg exposures that occur in typical varieties of ocean fish therefore appear unlikely to be harmful because Se is consistently in molar excess of MeHg. However, this is not true for pilot whales and may not be true for freshwater fish since their Se levels are highly dependent upon the Se status of their lake of origin.

- Seafood safety criteria need to incorporate reporting Hg:Se molar ratios present in the fish to provide more meaningful risk evaluations for improving public health.

- To properly evaluate risks associated with maternal MeHg exposure, future studies need to incorporate Hg:Se molar ratios in blood as a primary risk index, otherwise they are measuring MeHg exposure without a valid indication of associated risk.

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