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MERCURY METABOLISM AND SELENIUM PHYSIOLOGY STUDIES

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Project Description

This work employs a multiperspective approach to examine the influence of dietary mercury on selenium physiology and the influence of dietary selenium on mercury metabolism.^b The coordinated studies of this project compare the effects of dietary exposure to methylmercury consumed in the presence of graduated concentrations of dietary selenium at levels that reflect the range of human consumption. The effects of dietary selenium and methylmercury at these varied molar ratios were examined in rats, and the signs and symptoms of mercury toxicity were compared to the molar ratios of mercury and selenium occurring in pertinent tissues. Defining the influences of molar ratios of mercury and selenium on toxic effects of methylmercury was a central objective of this research. Particular emphasis has been given to examining selenium's effects on the reliability of hair and blood as indicators of methylmercury exposure and closely examining the suitability of using these indicators of exposure as indicators of risk from methylmercury exposure.

The results of this animal study are being considered and interpreted in concert with the results of the human study component of this project. Our collaboration with the Seychelles Child Development Study led by Dr. Thomas Clarkson and Dr. Gary Myers is examining selenium's role in the relationship between methylmercury exposure from maternal fish consumption and potential development of neurodevelopmental toxicity in children. Whole blood samples collected from Seychellois mother and child pairs at delivery were analyzed for their selenium contents and will be evaluated in relation to mercury contents, maternal fish consumption, and the children's development.

Characterization of relative affinities of mercury and methylmercury conjugates with selenium-vs. sulfur-containing molecular species has also been undertaken in this project. In addition to in vitro analyses using chromatographic methods to examine inorganic mercury and methylmercury binding with chemically defined sulfur and selenium species, this study has increased its scope to include quantitation

^b *Since selenium and mercury both occur in a multitude of molecular forms that continually and, in some cases, repeatedly interconvert, it is often inappropriate and inaccurate to attempt to use terms other than "mercury" and "selenium" to designate their occurrence. When specific molecular forms are discussed, they will be named; otherwise, it should be understood that the comprehensive presence of these numerous chemical forms are all-inclusively designated by the use of the name of the element.*

of relative amounts of selenium-vs.-sulfur conjugates with mercury in vivo. Since toothed whales are long-lived mammals with high seafood consumption rates, their tissue trace element distributions are appropriate models for examining potential interactions between mercury and selenium and their resulting distribution in tissues of humans with high seafood consumption. Whale liver tissues were collected from stranded beluga and pygmy sperm whales provided courtesy of the National Oceanic and Atmospheric Administration. Mercury binding to sulfur and selenium molecules in these tissues was determined in collaboration with Dr. Frank Huggins of the University of Kentucky.

This project is collaborating with Dr. Leslie Cooper of the Mayo Clinic in examining trace element accumulation in heart tissues of patients with dilated cardiomyopathy (DCM). An earlier Italian study found abnormally high mercury and antimony concentrations in myocardial tissue from these patients. While the earlier study used extremely small needle biopsy samples of heart tissue, this study has collected substantially larger samples. This study examines mercury, antimony, and selenium concentrations in tissue samples collected from human hearts removed from DCM patients during transplantation procedures. Since these explanted hearts are otherwise discarded, availability of the entire heart enables sampling of substantially more sample mass for analysis. This will minimize potential for analytical defects such as contamination and signal artifacts that might have affected results of the earlier study.

Goal

These studies are intended to improve the understanding of selenium-dependent prevention of methylmercury toxicity and to characterize the pathological effects of mercury toxicity on selenium physiology. The goal of the in vitro study was to evaluate binding behavior between mercury and selenium and characterize the relative affinities of mercury–sulfur binding in comparison to mercury binding with selenium. The goals of the in vivo study were to examine the influence of dietary methylmercury intake on selenium distribution in tissues and explore the effects of dietary selenium intake on mercury distribution and the relationship between Hg:Se molar ratios and prevention or development of mercury toxicity. Mercury concentrations in hair and blood provide an index of mercury exposure, but the influence of dietary selenium on mercury distribution in hair and blood had never been evaluated prior to this project. Since hair and blood are the primary indices of methylmercury exposure and potential risk, characterizing the effects of selenium status on mercury distribution into these compartments and evaluating the suitability of these measures of risk of potential harm from methylmercury exposure were further goals. The goal of the study of blood selenium contents in Seychellois mothers and children was to evaluate the effects of fish consumption on their selenium status in relation to their mercury exposure. The goal of the study of trace element contents of hearts of cardiomyopathy patients is to quantitatively assess mercury, antimony, and selenium in diseased heart tissues in comparison to normal hearts.

Rationale

Although the physiological consequences and manifestations of methylmercury toxicity have been well described (1–5), the direct molecular mechanisms responsible for the various physiological perturbations reported have not been well defined. It has been recognized for decades that physiological consequences of MeHg exposure may relate to impairments of selenoenzyme synthesis or activities (6). The mechanism of selenium-dependent protection against methylmercury toxicity and the mechanism of mercury toxicity itself converge in the selenium sequestration hypothesis (Figure 1). Evidence in support of this hypothesis is growing. For instance, although lipid peroxidation is known to accompany Hg

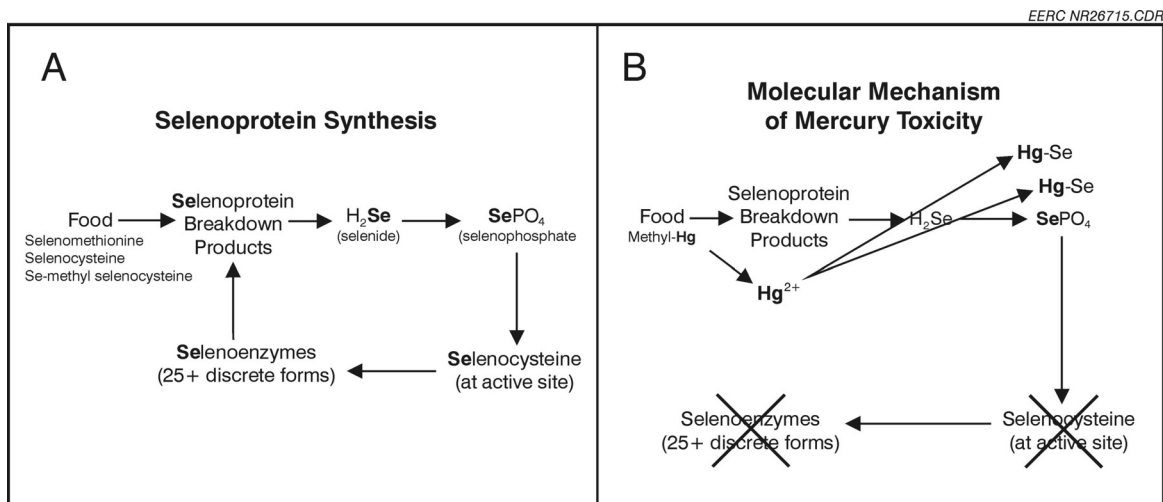


Figure 1. Selenoenzyme synthesis (A) and hypothesized mechanism of mercury toxicity (B).

toxicity, it has only recently been recognized that Hg does not directly promote lipid peroxidation as copper and iron do (7). Instead, inorganic mercury (Hg^{2+}), but not methylmercury, has been shown to inhibit selenium-dependent glutathione peroxidase activity. Since methylmercury is eventually demethylated to release Hg^{2+} that can subsequently sequester selenium, the selenium sequestration hypothesis has the additional virtue of explaining the latency period that accompanies exposure to toxic amounts of methylmercury (8). This would also explain why methylmercury exposure compromised selenoenzyme activities in tissues of neonatal experimental animals whose dams were fed low dietary Se, but not in tissues of neonates whose dams were fed selenium-enriched diets (9, 10).

It appears that sufficient selenium must be available to offset the quantity lost to mercury-binding and support synthesis of selenoenzymes in order to prevent the onset of physiological consequences of methylmercury exposure. Since selenoenzymes are present in all cells of all animals, it is possible that the reason selenium-dependent protection against mercury toxicity has been observed in all species studied is due to the apparent necessity of sustaining selenoenzyme synthesis in brain and neuroendocrine tissues (11–15).

Proper understanding of how various molecular forms of mercury interact with cellular biomolecules is essential for understanding its toxicological behavior and health ramifications of human and environmental exposures. Mercury accumulates in the liver over the lifespan of the animal. However, despite high accumulations of heavy metals in vital organs, the animal often shows no adverse signs of toxicity. Mercury accumulates in the liver in minimally hazardous inorganic forms, predominantly bound with selenium.

A 1999 report on trace elements in tissues obtained from patients with idiopathic dilated cardiomyopathy (IDCM) found mean mercury concentrations in heart tissues were 22,000 times higher in IDCM patients than in control subjects (16). Antimony concentrations were also found to be remarkably higher in cardiac tissues from these same patients. Of 30 elements measured, no other element showed unusually high trace element accumulations. No unusual exposures to these elements were noted among the patients, but accumulation of these elements coincided with the severity of symptoms.

The present study applies a multiperspective approach to examining the hypothesis that the molecular mechanism of methylmercury toxicity is selenium sequestration. Formation of highly insoluble

complexes of mercury selenides obstructs selenium bioavailability and prevents it from participating in the synthesis of selenium-dependent enzymes. This postulate is linked to the hypothesis that the mechanism of selenium's protective effect against methylmercury toxicity is the result of supplying sufficient selenium to offset losses to mercury sequestration and sustain normal rates of selenoenzyme synthesis. The studies of this project are intended to test these hypotheses and establish the effects of selenium on indices used to evaluate mercury exposure.

Approach

Objective 1

Chromatographic analysis is being used to compare the direct binding interactions of methylmercury and inorganic mercury with selenocysteine and cysteine. Additional assessments of HgSe formation in a mammalian model are being performed by x-ray absorption fine structure (XAFS) analysis on liver tissues collected from stranded beluga and pygmy sperm whales, provided courtesy of the National Oceanic and Atmospheric Administration.

Objective 2

The objective of this work was to investigate the effect of dietary mercury on selenium distributions and selenoenzyme activities in rats fed varying concentrations of methylmercury and selenium and simultaneously characterize selenium's protective effects against mercury toxicity. Since measurements of mercury in hair and blood did not accurately predict risk of mercury toxicity, there was clearly an urgent need to discover a more accurate index of risk from methylmercury exposure. Because the molar ratios of dietary methylmercury and selenium were found to be the most important aspects regarding initiation of toxicity, the predictive value of mercury:selenium molar ratios in blood were assessed as a more reliable index of risk.

Objective 3

Conflicting observations and conclusions have arisen from the ongoing studies of mercury-dependent health effects in the Faroe Islands and in the Seychelles Islands. While researchers in the Faroe Islands reported neurological defects in children exposed to low levels of mercury in the womb, the Seychelles study has found no adverse effects from prenatal methylmercury exposure. The differences between the observations and conclusions reported in these studies appear likely to be due to dietary distinctions in molar ratios of mercury and selenium in the foods consumed by the study populations. The objective of this study is to perform selenium analysis in the ongoing Seychelles Study. Selenium is being included as a concomitant variable in the neurodevelopmental assessment of children born to mothers with known exposures to methylmercury from fish. We measured selenium in whole blood samples collected from ~500 blood samples collected from cord blood and mothers participating in the current Seychelles study. These values will be assessed in concert with dietary records from the individual subjects and analytical data reflecting blood mercury contents.

Objective 4

This task's objective was to establish mercury, antimony, and selenium concentrations in heart ventricular myocardial tissues collected from explanted hearts obtained from transplant patients suffering from DCM. The trace element concentrations in these tissues will be compared to those

observed in similarly collected tissues from patients diagnosed with ischemic cardiomyopathy and control samples collected at autopsy from hearts of patients dying of causes unrelated to cardiomyopathy.

Progress/Status

Task 1

The equipment required for chromatographic analysis of mercury and selenium was initially unavailable, prompting us to perform preliminary examinations of their binding interactions through an alternate means. Since the planned examination of HgSe formation in vitro was intended to establish the potential for HgSe formation in vivo, we increased the scope of this task to include examination of HgSe in tissues of mammals with high seafood consumption. In collaboration with Dr. Frank Huggins of the University of Kentucky, a no-cost XAFS study at the Stanford synchrotron identified HgSe molecular forms occurring as significant proportions of total Hg in whale liver tissues. This has encouraged further cooperation on analysis of mercury and selenium species in other tissues. Predatory whales are suitable models for examining biochemical and physiological effects of fish consumption on distribution of mercury and selenium in other long-lived mammals such as humans.

Task 2

The rat study has been completed, and tissue samples collected at the end of the study have been analyzed for mercury and selenium (17). The study's results have been presented at a series of regional, national, and international meetings. A manuscript describing the results of this study is in preparation for submission for publication.

Task 3

The maternal and cord blood samples collected as part of the current Seychelles study has been analyzed for selenium and has been entered into the database at the University of Rochester. These results are being used to assess beneficial effects of fish consumption upon neurodevelopmental end points measured in growing children. The results from this study have been presented at regional, national, and international meetings. Two manuscripts detailing the results of this study are currently in preparation for publication.

Task 4

The measurement of mercury and selenium in heart tissues was delayed because, contrary to expectations of even internationally prominent heart researchers, no heart tissue repository of fresh frozen samples appears to exist in the United States or Europe. As a result of this project's urging in collaboration with Dr. Leslie Cooper of the Mayo Clinic, heart tissues from transplant patients are being collected and analyzed at the University of Missouri. A review article entitled "The Roles of Selenium and Mercury in the Pathogenesis of Viral Cardiomyopathy" was invited by the editor of the journal *Congestive Heart Failure* and has been submitted. The article describes the potential relationship between mercury and selenium in the viral etiology of this disease and discusses the mercury hyperaccumulation issue.

Results

In Vitro Study: Examination of Direct Interactions between Mercury and Selenium

The XAFS data indicate that HgSe is the major form of mercury in beluga whale liver samples. Similarly, the data for mercury in the pygmy sperm whale liver indicate that much of the mercury is present as HgSe. The molar ratio of Hg:Se is less than 1.0 for both samples. Whereas HgSe constitutes about 66% of the total selenium in beluga whale liver and about 17% of the total selenium in pygmy sperm whale liver.

In Vivo Study: Interactive Effects of Dietary Methylmercury and Selenium in Rats

One of the most readily observed consequences of methylmercury toxicity in animal models is impaired growth. Rats develop a substantial proportion of their adult brain and body mass during the weeks after weaning. During this study, rats fed mercury-free diets grew rapidly, more than tripling their body weight as they gain nearly 300 grams. In the absence of mercury, weight gains between rats fed low, normal, or rich dietary selenium were equivalent. However rats fed high levels of methylmercury showed distinct differences in their growth rates that were directly proportional to their selenium intakes. Rats on low-selenium diets and fed high-methylmercury diets gained $\sim 24 \pm 2\%$ less than the control group fed low-selenium diets without added methylmercury. Exposure to this same level of methylmercury impaired growth among rats fed diets with normal levels of dietary selenium by $\sim 14 \pm 4\%$, but no growth impairment occurred among rats fed selenium-rich diets. Using this growth impairment as an indication of relative toxicity, relationships between dietary treatments and observed distributions of mercury and selenium in the analyzed tissues reveal unexpected discrepancies between expectations of risk that would be inferred from hair and blood mercury concentrations and the observed toxic effects.

These results confirm that hair and blood indices provide a reasonable reflection of mercury exposure, but that simply measuring mercury distributions in these indices can be misleading. Dietary methylmercury intakes are proportionately represented by hair and blood mercury contents, but it is important to note that these indices are also somewhat sensitive to dietary selenium intakes. Increasing dietary selenium resulted in increased mercury distributions into these compartments, a feature that was particularly evident in blood (see Figure 2).

Since ocean fish are rich in Se, these results suggest fish-eating populations will have accentuated hair and blood Hg in relation to their actual Hg exposure. More importantly, our results suggest the consequences that might otherwise be expected to attend MeHg exposure from ocean fish would be greatly offset by their rich Se contents. In contrast, it is important to note that freshwater fish are not uniformly rich in Se; therefore, the protective effects we noted will not necessarily occur in populations that eat freshwater fish. Based on our findings, it is further possible that examinations of hair and blood Hg in Se-deprived areas will result in an underestimation of Hg exposure, but the exposure would be expected to be accompanied by far worse toxic consequences.

Since simply measuring mercury in hair or blood provides an inaccurate indication of risk, a more sophisticated approach to mercury risk assessment is needed. Methylmercury toxicity is highly dependent on the selenium status of the exposed individual. Thus it is obvious that assessments of selenium should be integrated into future methylmercury risk assessments. The most straightforward way to accomplish this goal is to establish a new index that incorporates molar ratios of mercury and selenium. Elemental analysis of blood provides a reasonably accurate reflection of selenium status and methylmercury

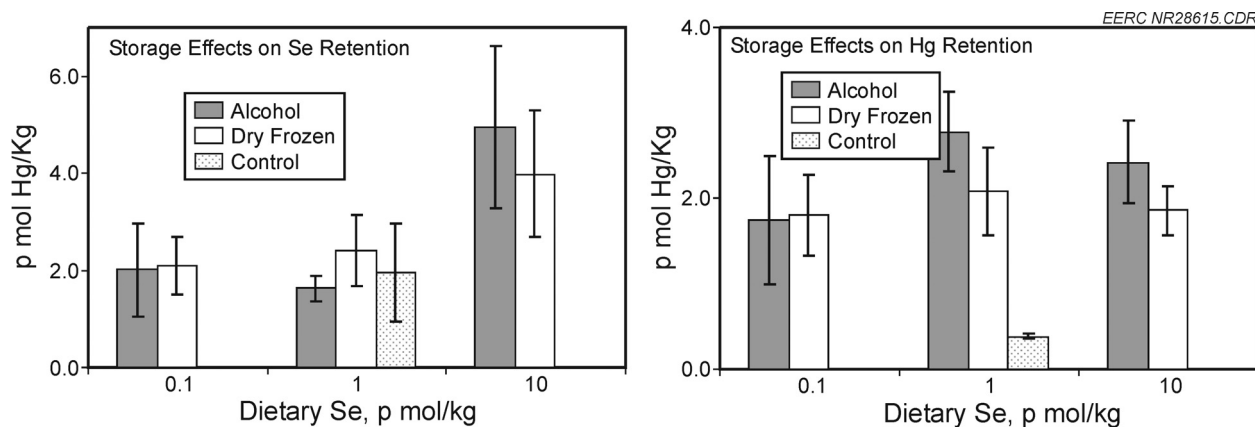


Figure 2. Relative toxicity of methylmercury vs. Hg in hair (A) and blood (B).

exposure. Therefore, it is not surprising that integrating these blood measures in a molar ratio format provides a highly reliable and consistent reflection of relative toxicity risk, as shown in Panel A of Figure 3.

The mercury:selenium molar ratios in blood and brain from rats fed high-methylmercury diets are shown in Figure 3. Blood mercury:selenium ratios (depicted in Panel A of Figure 3) provide reliable and consistent ($p < 0.001$) reflections of the risks that accompany methylmercury exposure than the blood mercury concentrations depicted in Figure 2. Mercury:selenium ratios in brain are highly correlated ($p < 0.001$) with relative toxicity, as shown in Panel B of Figure 3.

Population Study: Selenium in Blood Samples from Seychellois Mother–Child Pairs

As an indication of nutritional selenium status, whole blood is superior to plasma or serum. Plasma and serum selenium concentrations are approximately equivalent to one another, but can reflect recent dietary intakes and fluctuations in metabolic activities such as inflammation. Although plasma is often used as a general indication of selenium status, these difficulties can make interpretation difficult. Whole blood selenium concentrations are normally 10%–25% higher than plasma or serum concentrations, but plasma and serum concentrations are nonlinearly related to whole blood or red cells, correlating better at low levels of intake, but less accurately at higher levels of dietary intake. Thus whole blood selenium concentration is a more reflective measure of selenium status, especially at moderate to abundant nutritional intakes.

Over 200 maternal and fetal whole blood samples from the current Seychelles Children's Health and Development study were assessed for whole blood selenium. Of the total sample set, 148 paired sets of blood samples collected from mother and umbilical cord were collected at the time of delivery. The blood selenium concentrations observed in the total sample set and from the subset of matched pairs of fetal and maternal samples are shown in Table 1.

Maternal whole blood selenium concentrations were generally higher than fetal (cord blood) selenium. Maternal transport of Se to the fetus appears to be a tightly regulated process. The nominally adequate whole blood selenium level for optimal plasma GPx activity is accepted to be ~120 ng Se/mL, which is approximately the concentration cord blood Se centered around. Mercury:selenium ratios for

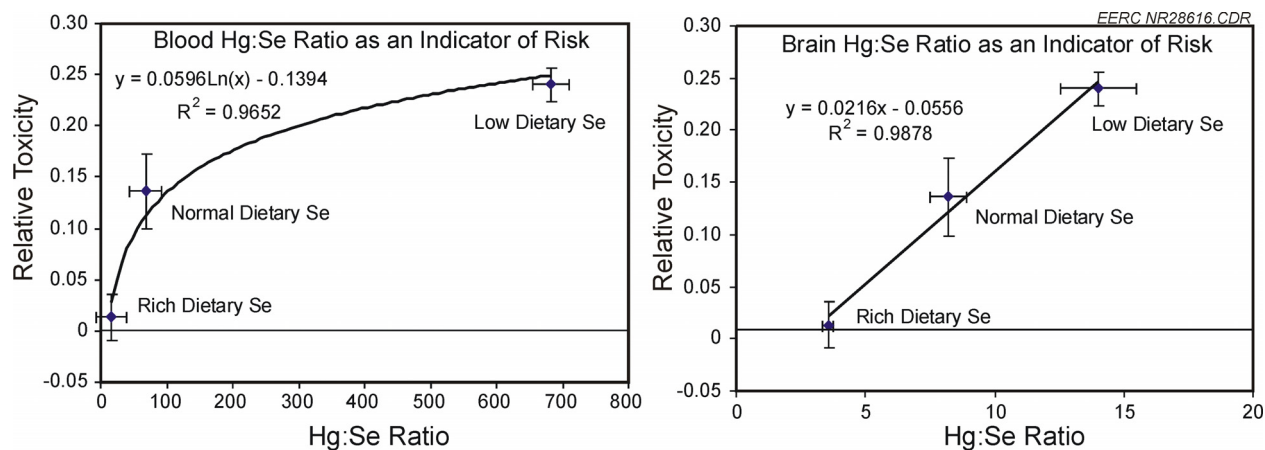


Figure 3. Relative toxicity of methylmercury vs. Hg:Se ratio in blood (A) and brain (B).

Table 1. Selenium Contents of Maternal and Umbilical Cord Bloods from the Seychelles

	Cord Blood, $\mu\text{g/L}$	Maternal Blood, $\mu\text{g/L}$
Total Sample Set	134.7 ± 48.7	182.0 ± 42.3
(n)	(218)	(202)
Matched Sample Set	139.1 ± 49.6	179.9 ± 41.8
(n)	(148)	(148)

Seychellois cord bloods were more uniform than cord bloods. While some cord blood samples from the Faroes approach a hazardous 1:1 molar ratio of Hg:Se, cord blood samples from the Seychelles are much lower, at around 0.2:1, or ~5 moles of selenium per mole of mercury. The whole blood selenium data obtained in this study will be used in a multivariate analysis to assess potential effects of mercury exposure and the protective effects of dietary selenium.

Pathology Study: Hg, Sb, and Se in Heart Tissues of Dilated Cardiomyopathy Patients

Samples of the explanted heart tissues removed from the patients receiving transplanted hearts are routinely preserved in formalin in blocks of paraffin for pathology studies and are stored in tissue repositories. These and frozen tissue samples collected from the hearts of patients with DCM (and control hearts at autopsy) are being collected for elemental analysis. These samples will be delivered to the University of Missouri Research Reactor Center (MURR) for neutron activation analysis of their mercury, antimony, and selenium concentrations. Statistical analysis will be applied to determine whether heart tissues from DCM patients contain significantly different quantities of trace elements than normal control heart tissues.

Quality Assurance/Quality Control

Quality Objectives

The quality objectives of this project were to obtain statistically valid and physiologically meaningful results regarding the interactions of mercury and selenium. In the in vitro study, the quality objective was to obtain meaningful analytical data regarding mercury–selenium molecular species. In the dietary treatment study performed on experimental animals, being able to measure, contrast, and compare the weight gains of rats fed diets that varied in mercury and selenium contents provides a measure of their concentration-dependent effects.

In the analysis of the Seychelles blood samples, the quality objective was to obtain analytically accurate and precise blood selenium determinations. In the study of elemental distributions in human heart samples, the quality objective was to obtain analytically accurate and precise determinations of elemental contents.

Measurement /Data Acquisition

XAFS spectra of mercury and selenium species occurring in whale liver samples were compared to authentic molecular forms used as analytical standards. Multiple spectra were collected from each sample, and mercury and selenium form analysis independently confirms HgSe occurrence and distribution in the whale liver tissues. Rat weights in the in vivo study were measured using calibrated instruments certified accurate to 0.1 g that were carefully checked using a weight standard at the start and finish of daily measurements.

Rat weights were measured weekly, and observed values for each animal were individually plotted to validate consistency. All data points for each treatment group were included in determining the mean values and standard deviations for the weights at each weighing. Mercury and selenium contents in the hair, blood, and tissue samples were digested and analyzed alongside certified reference materials and calibration standards following standard protocols. Selenium contents in the Seychelles blood samples were each determined in triplicate alongside certified human blood quality control samples and calibration standards according to established protocols. Measurements of elemental compositions of hearts from normal hearts removed at autopsy are being compared to hearts from patients with DCM alongside certified quality control samples and calibration standards according to established protocols.

Assessment and Validation

The analysis data from the standard protocols used in this project indicate acceptable analytical accuracy and precision. Certified reference materials and control sample analytical results were within the expected range. The analytical results from the animal study reflect expected trends in measured indices. The heart tissue analysis task currently under way will be performed according to standard protocols, and results will be validated using certified reference materials and control samples.

Potential Users/Technology Transfer

The findings of these studies provide important information for EPA, the U.S. Department of Energy, the U.S. Food and Drug Administration, and the World Health Organization that may assist these agencies in making regulatory policy decisions regarding mercury. Recognizing the influence of molar

relationships between mercury and selenium will help these agencies in assessing the potential risks of human mercury exposure and differentiating populations at risk from those that are not.

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