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MERCURY METABOLISM AND SELENIUM PHYSIOLOGY STUDIES

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Project Description

This research examines the influence of mercury on selenium physiology and the influence of selenium upon mercury metabolism. In vivo studies performed in rats are being used to examine the effect of low, adequate, and selenium-rich diets on methylmercury accumulation in tissues and molecular associations between mercury and selenium. The relationship between mercury neurodevelopmental toxicity and selenium status in relation to human mercury consumption is also being quantified through a study involving maternal–fetal pairs selected from a population from the Seychelles Islands with known exposures to high quantities of methylmercury from fish.

Goal

These studies are intended to help clarify our understanding of the influence of mercury on selenium physiology as well as the influence of selenium on the effects of mercury exposure, at the molecular, cellular, animal, and human population levels. In a series of biochemical studies at the EERC as well as a population study conducted in concert with the Mercury Research Group from the University of Rochester, New York, important questions will be resolved regarding the effects of methylmercury on normal selenium physiology as well as selenium’s protective effect against methylmercury exposure.

Rationale

The research community acknowledges that the robust nutritional selenium status of U.S. citizens is an important asset in maintaining public health since dietary selenium enhances cancer resistance and supports the immune system. Dietary selenium has a further beneficial role in that it provides protection against negative consequences of mercury exposure. Research studies in animal models have shown that selenium deficiency increases vulnerability to mercury toxicity while enhanced dietary selenium status is protective. However, the biochemical mechanism for selenium’s protective effect against mercury remains unclear. Selenium clearly influences mercury metabolism, but mercury’s impact on selenium physiology appears to be equally important. Recent studies have shown that mercury exposure diminishes the activity of selenium-dependent enzymes. It is reasonable to consider that the influence mercury and

selenium have upon one another shares a common basis through the exceedingly high binding affinity between these elements.

Approach

Objective 1

Mercury has an extraordinarily high affinity for selenium; thus exposure to mercury can result in sequestration of selenium within cells of vulnerable organs. At high dietary-mercury concentrations, formation of these insoluble mercury selenides may result in diversion of selenium from selenoprotein synthesis and diminishment of selenoenzyme activities.

Our objective is to investigate the effect of dietary mercury on selenium distributions and selenoenzyme activities in rats fed varying concentrations of mercury and selenium. The EERC is currently investigating tissues that are expected to be most vulnerable to consequences of mercury-dependent selenium sequestration.

Objective 2

Numerous studies have applied hair analysis as a means of determining mercury exposure. Likewise, hair mercury concentrations are used worldwide as an indication of mercury exposure and body burden. A recent study of Midwestern residents' fish consumption in which methylmercury exposures calculated from survey data were compared to analyzed hair mercury concentrations found a ~6-fold lower hair mercury than expected. The higher levels of selenium present in the diets of the residents in the upper Midwest of the United States, and specifically North Dakota and Minnesota, may be the reason why mercury levels observed in hair samples from subjects in this study were six times lower than predicted. Additionally, studies in cattle have indicated that selenium concentrations in hair vary with hair color. Forensic science has also determined that dark hair can be used to determine ingestion of many toxic chemicals, but light colored hair is not as reliable. Therefore, not only do we question if selenium has an effect on hair mercury distribution, but also whether hair color may be a determining factor. By using Long Evans rats fed varying concentrations of mercury and selenium, this study allowed us to examine the effect of selenium on hair mercury concentration in both light and dark hair.

Objective 3

Conflicting observations and conclusions have arisen from the ongoing studies of mercury-dependent health effects in the Faroe and 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, even at levels of exposure 10–20 times higher than what is common in the United States. In further contrast, maternal fish consumption in the Seychelles correlated with an improved neurodevelopmental outcome in some indices. The discrepancies between the observations and conclusions reported in these studies may be due to dietary differences in the study populations. Selenium's protective effect against mercury toxicity may be one dietary factor but had not been assessed. The objective of this study is to include selenium analysis in the ongoing Seychelles Study. Selenium and mercury analysis is being included as concomitant variables regarding neurodevelopmental assessment end points of the children born to mothers with known exposures to methylmercury from fish. We measured blood selenium in 250 maternal–fetal pairs from the current Seychelles study.

Selenium in the blood samples was measured in triplicate by atomic absorption spectrometry according to standardized methods. Selenium contents were determined using a Perkin-Elmer model 5100 employing a GFAAS 600 with a transversely heated graphite furnace equipped with Zeeman background correction. The Zeeman system offers improved background correction over deuterium arc-corrected systems. Cord and maternal whole blood samples, along with certified human blood quality control (QC) samples and calibration standards, were diluted 1:10 with 900 μL of 0.1% Triton X-100 and a 10 μL aliquot of diluted sample, and 10- μL of 200 mg/L Pd (matrix modifier) was delivered onto the graphite platform using an AS-60 autosampler. Samples were dried, preashed at 1200°C, and followed by atomization at 2250°C for analysis. Quantification is based on the measurement of light absorbed at 196.0 nm by ground state atoms of selenium from a selenium electrodeless discharge lamp (EDL) source.

These values will be assessed in concert with dietary records from the individual subjects and analytical data reflecting blood mercury contents. The blood concentrations of omega-3 fatty acids, mercury, and selenium will be examined in relation to neurodevelopmental end points assessed in the children as they grow. The children are currently approaching or recently reached 1 year old, and their development is currently being monitored by our Seychelles collaborators.

Progress/Status

The tissue samples that were collected from the rat study have been or are in the process of being analyzed for mercury, selenium, and selenoenzyme activity. Future activities will include plotting the molar ratios for mercury and selenium in each of the tissues analyzed and calculating the fraction of each bound and free forms. Correlations will be determined between these calculated quantities and their distributions in the low molecular weight fractions isolated from each of the analyzed tissues. Further studies will be performed on these isolated fractions to characterize the nature of the molecular species present in the low molecular weight fractions and seek to ascertain the quantities of mercury and selenium present as mercury selenide complexes in this fraction. This study will be the first to directly assess this aspect of mercury's metabolic interactions with selenium.

The maternal and cord-blood samples collected from babies born in the Seychelles have been analyzed for selenium and have been entered into the study database at the University of Rochester. These selenium analyses will be used to assess beneficial effects of fish consumption upon neurodevelopmental end points being measured in the growing children.

Results

Animal Study

One of the more prominent signs of mercury toxicity in experimental animals is depressed growth. Figure 1 depicts the means \pm standard deviations for the weights of rat groups fed diets that varied in mercury and selenium contents at weekly intervals during the course of the 9-week study. The weights of rats fed diets that were selenium-deficient (1-A), selenium-adequate (1-B), or selenium-rich (1-C) are shown for the treatment groups at each time point.

Rats fed diets with high (75 $\mu\text{mole Hg/kg}$; 15 ppm) mercury concentrations gained less weight than rats fed diets containing 0 or 2.5 $\mu\text{mole Hg/kg}$ (0.5 ppm) mercury (Figure 1-A). Among rats fed a selenium-adequate diet (1.25 $\mu\text{mole Se/kg}$; 0.1 ppm), growth of the group fed the highest mercury

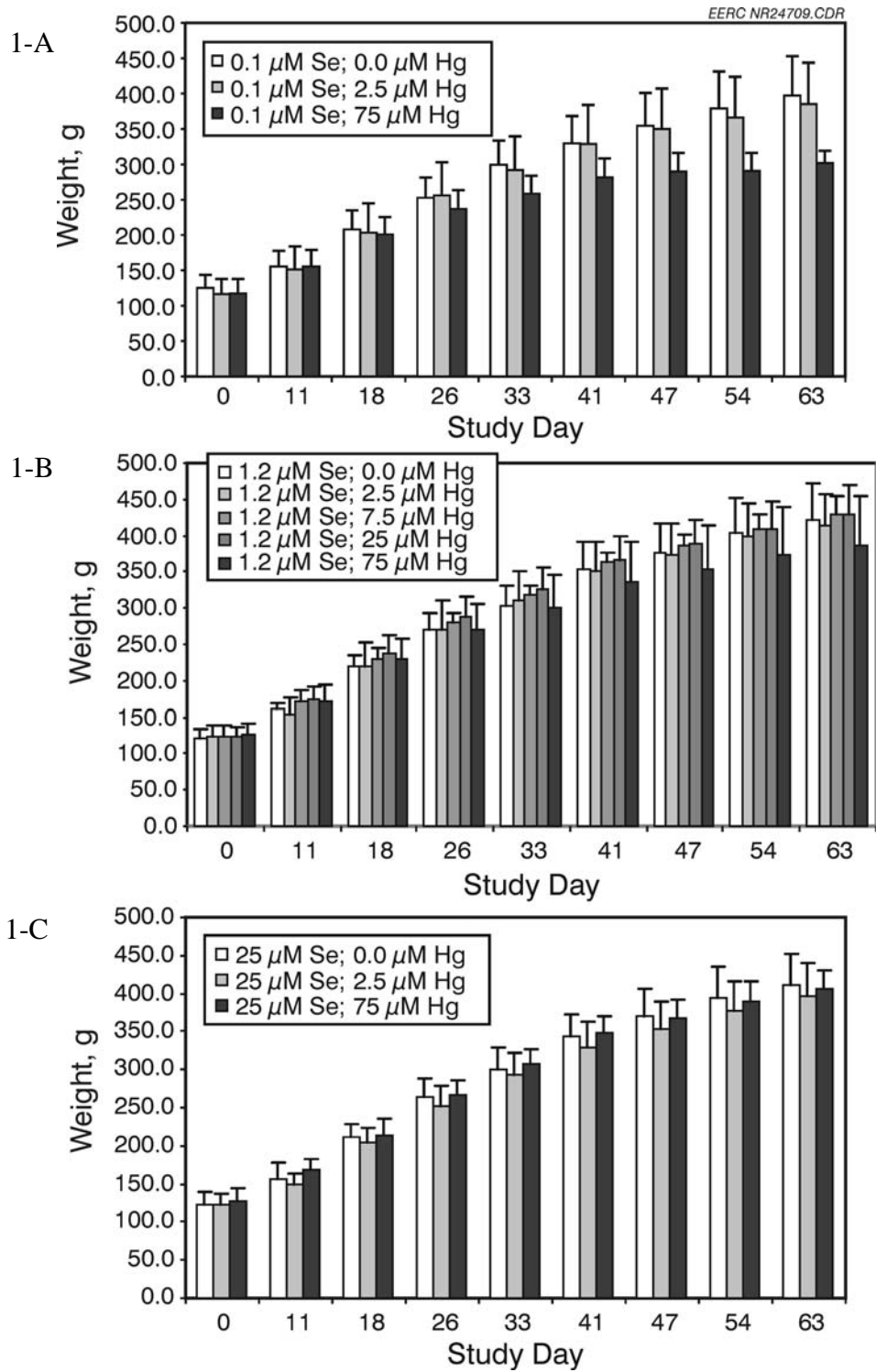


Figure 1. Effects of mercury and selenium on weight gain in rats.

concentration tended to be slightly depressed (Figure 1-B), but not nearly so much as was apparent in the selenium-deficient study group. The protective effect of selenium was even more apparent among rats fed selenium-rich diets (Figure 1-C) where there was no indication of mercury-dependent growth depression.

The hair samples collected at the end of Week 9 have been analyzed, and the results shown in Figure 2 depict their Hg and Se contents. The results showed a positive linear relationship between mercury consumption and hair mercury levels. Selenium status did not appear to have a major influence on hair mercury deposition, and there were no significant differences between white and black hair from Long Evans rats.

Ethnicity and genetic variables displayed in human hair composition and texture cannot be adequately studied in animals, but the results of this study suggest that differences reported between hair colors in humans are not likely to be directly related to hair color itself. Controlled human studies need to be performed to verify if compositional differences that accompany distinctions in hair color in humans have an influence on distribution of mercury into hair.

However, these data provide evidence that hair color is not a contributing factor that needs to be considered in future animal studies involving Long Evans rats.

The blood samples collected at the end of the study have been analyzed for mercury and selenium (Figure 3). The animals fed selenium-deficient and selenium-adequate diets displayed a decrease in blood selenium concentrations with the increase of blood mercury concentrations. This was not seen in the animals fed selenium-rich diets. Also, in the animals fed selenium adequate diets, there is no difference in blood selenium or blood mercury levels in the animals fed 2.5 or 7.5 $\mu\text{mol Hg/kg}$ diet. This may indicate previously unsuspected effects of selenium-dependent influences on mercury distribution into blood.

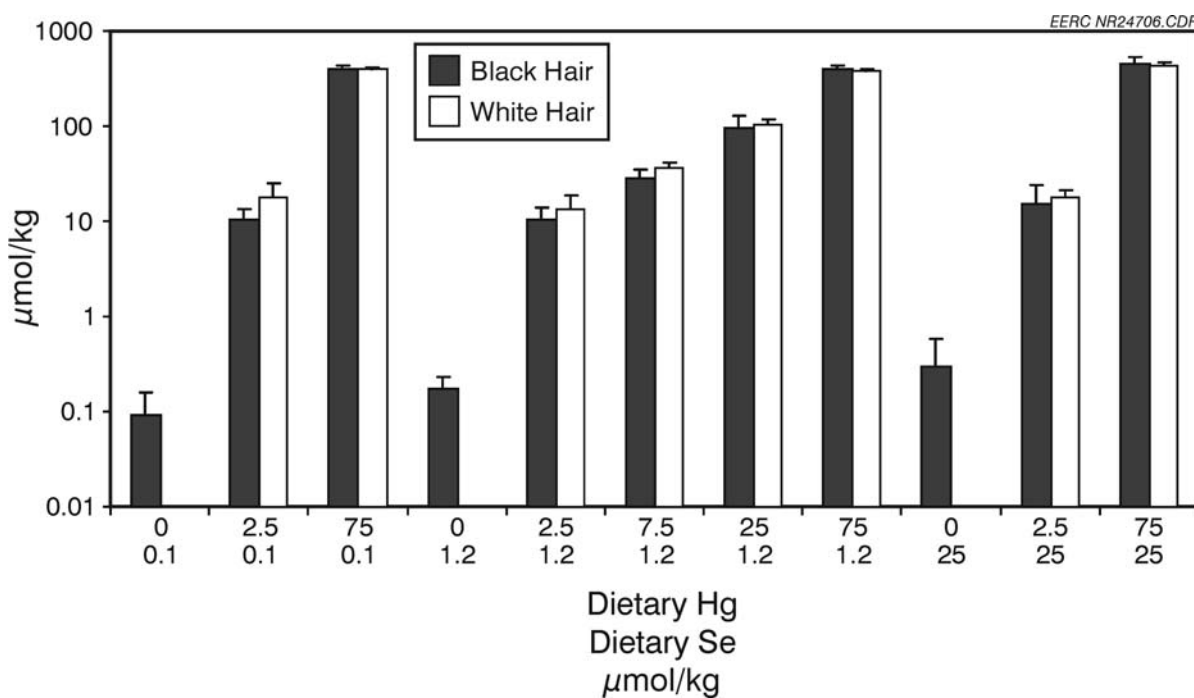


Figure 2. Effects of diet on mercury accumulation in black and white hair.

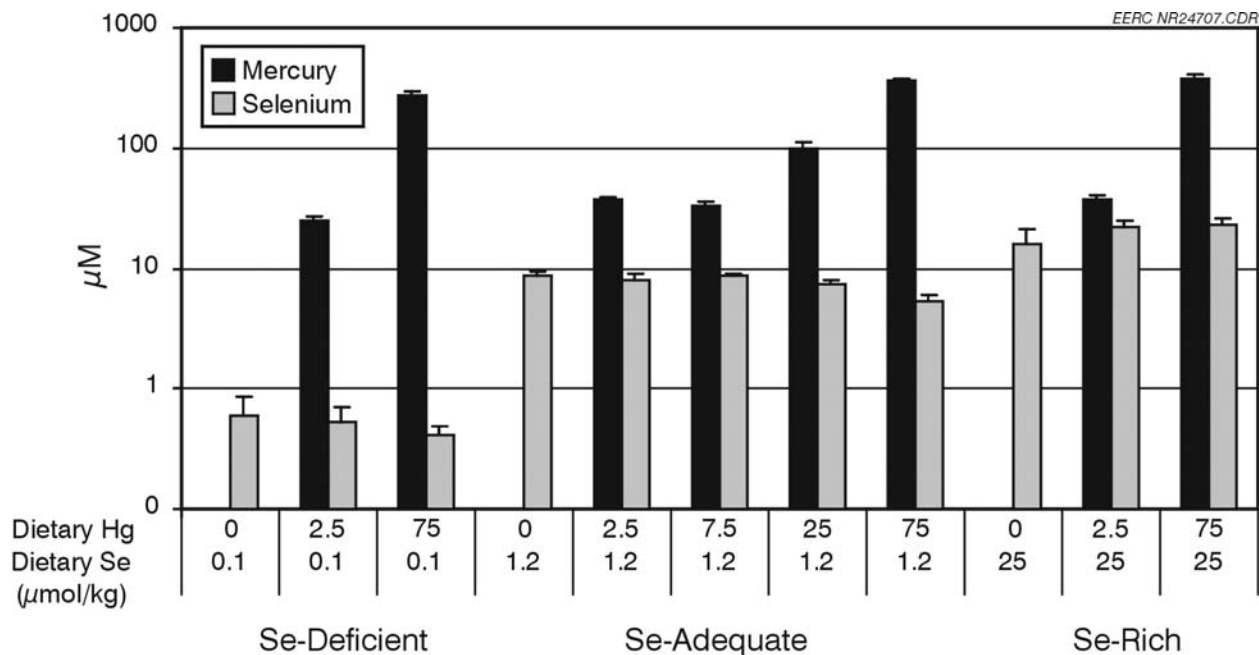


Figure 3. Effects of diet on mercury and selenium in blood.

Dietary selenium did not greatly influence the mercury distribution in blood. However, dietary mercury diminished the distribution of selenium into the blood of rats fed diets that were deficient or adequate in selenium, but did not have this effect in rats fed selenium-rich diets.

Selenium and mercury contents and selenoenzyme activities of brain and pituitary tissues are currently under way and will be evaluated in relation to dietary treatment as well as to hair and blood analytical results.

Seychelles Study of Selenium in Maternal and Fetal Blood Samples

Plasma and serum selenium concentrations are approximately equivalent and reflect recent dietary intakes and fluctuations in metabolic activities such as inflammation. Whole blood selenium concentrations are normally 10%–25% higher than plasma or serum concentrations and are a better indicator of long-term status. Whole blood selenium is nonlinear in relation to plasma/serum selenium, correlating better at low levels of intake. Thus whole blood selenium concentration is a more sensitive measure of selenium status, especially at moderate to abundant nutritional intakes.

Over 200 samples of maternal and fetal blood from the Seychelles 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 time of delivery. The blood selenium concentrations observed in the total data set and from the subset of maternal–fetal-paired samples are shown below.

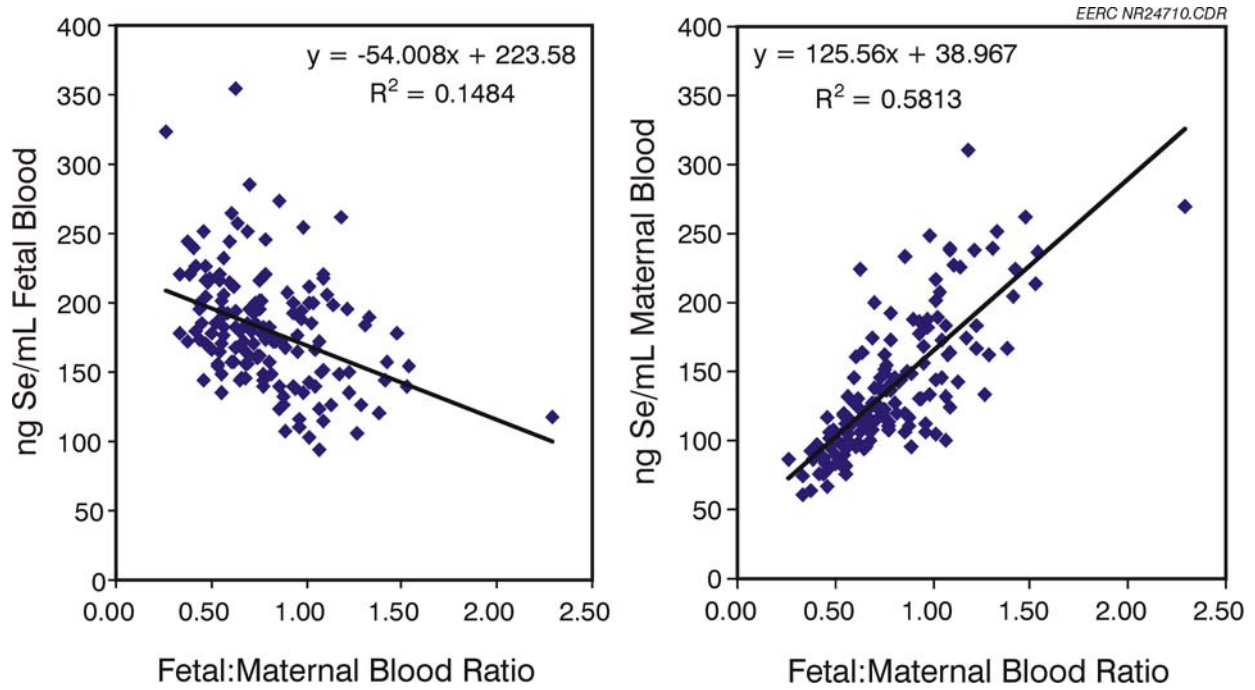


Figure 4. Maternal and fetal blood correlations.

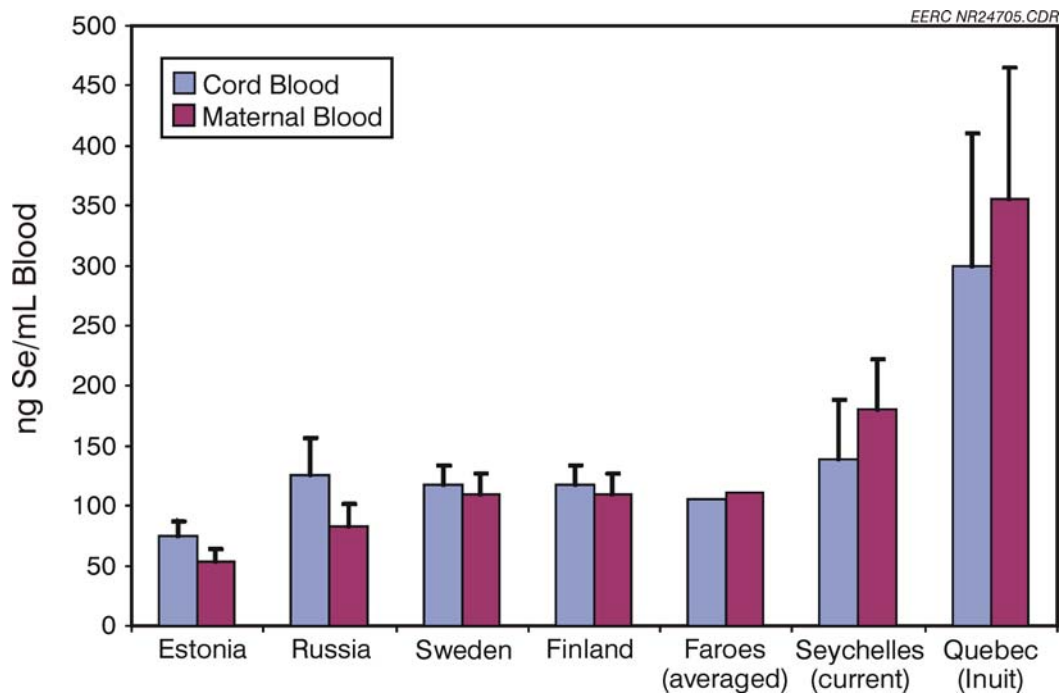


Figure 5: Comparison of maternal and fetal cord blood selenium.

Our results indicate that maternal whole blood selenium concentrations were higher than fetal (cord blood) selenium. As shown in Figures 4 and 5, cord blood selenium concentrations show signs of being highly regulated. This may relate to homeostatic regulatory mechanisms in the placenta.

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

Observing maternal–fetal selenium data sets from a series of studies (1–3) in comparison to our results (Figure 5) reveals maternal transport of Se to the fetus is a tightly regulated process. The whole blood selenium level required to maximize GPx activity is generally accepted to be ~ 120 ng/mL. The accompanying graph suggests that in countries where maternal blood Se was below ~ 120 ng/mL, Se was preferentially supplied to the fetus. When maternal blood Se was above ~ 120 ng/mL, cord blood levels remained lower than maternal levels.

The whole blood selenium data obtained in this study will be used in the multivariate analysis of fish consumption from food history, blood mercury, and neurofunctional end points. Effects of mercury exposure and the protective effects of dietary selenium will be assessed.

The Faroes study did not examine maternal blood selenium levels and did not report mean and standard deviation data. The Faroes data reflected in the graph above represent the averaged mean of the multiple medians in their blood selenium data sets.

The geologic distribution of selenium varies across the globe. This causes variation in selenium contents of foods which are reflected in differences in blood levels. To put the results from Figure 5 into perspective, Figure 6 shows the range of whole blood selenium concentrations observed in various countries around the world (1–11). As is apparent by the heterogeneity of blood selenium contents in various parts of North America, pronounced differences can be observed within different continental regions.

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 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.

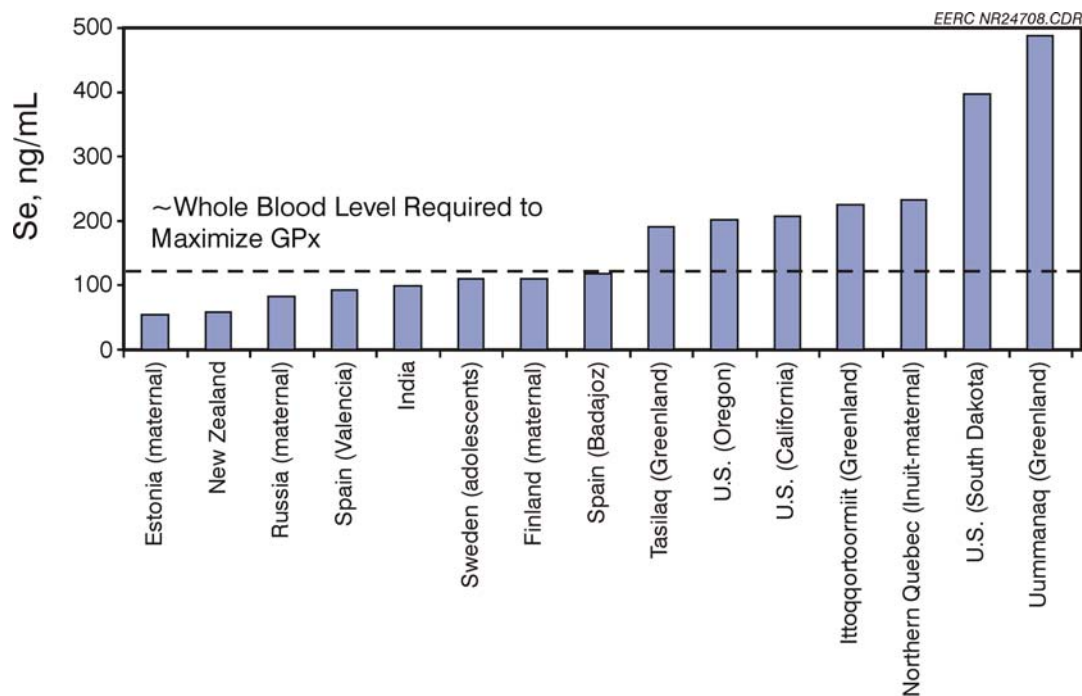


Figure 6. Global whole blood selenium concentrations.

Measurement /Data Acquisition

Rat weights 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 QC 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 QC sample analytical results were within the expected range. The analytical results from the animal study reflect expected trends in measured indices.

Potential Users/Technology Transfer

The findings of these studies provide important information for the U.S. Environmental Protection Agency, the U.S. Department of Energy, the U.S. Food and Drug Administration, and the World Health Organization. This information may assist these agencies in making regulatory policy decisions regarding mercury exposure. Recognizing the influence of molar relationships between mercury and selenium will help these agencies in assessing the risks of human mercury exposure and identifying populations at risk.

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